Chapter One

Introduction

1.1 Background to the Study

There is a need for the usage of therapeutic herbs and in recent years, there has been an upsurge in demand. According to the WHO Traditional Medicine (TM) Strategy 2014-2023, traditional therapies, traditional doctors, and herbal medicines represent the primary, if not the sole source of medical treatment for many millions of people[1]. It has also been estimated that over 80% of the population still relies on traditional medicine, for their basic health care, they rely mostly on medicinal plants[2]. Toxicity should be investigated for suitable and documented herbal medicines, just as it is for ordinary conventional medications that have undergone sufficient research and development; the toxicity of traditional medicinal plants is not adequately evaluated[3].

Medicinal plants have been utilized to treat a variety of ailments since prehistoric times, because they are extensively utilized by the local people and have tremendous relevance. Many individuals are involved in the global trade of vital medical plants. The locals, in particular, employ indigenous herbs as medicine[4]. Almost all communities across the world understand the medical potential of plants. Because synthetic pharmaceuticals are not available in distant areas, people have a strong grasp of the utilization of plants. They also use medicinal plants that grow around their settlements to survive. They found the medicinal agents of these herbs for some specific disorders based on positive or poor experiences.

These experiences are passed down from generation to generation. Herbal therapy and certain traditional medical systems are advancing in practically every country on the planet.

These ancient techniques are known as the Unani or Ayurvedic systems in the Indo-Pak subcontinent[4]. Ethnobotanical knowledge is required for plant life recording, particularly in rural and undiscovered areas[6]. Despite the advancement of modern treatment, many people continue to rely on herbal medicine[7]. Traditional remedies continue to be used by around 80% of the world's population[8]. Ethnobotanical knowledge is required for plant life recording, particularly in rural and undiscovered areas[6]. Despite the advancement of modern treatment, many people continue to rely on herbal medicine[7]. Medicinal plants dating back thousands of years have been used by mankind for their therapeutic value. An impressive number of modern drugs have been isolated from natural sources. Many of these isolated active principles were based on their uses in traditional medicine[9]. India has several traditional medical systems, such as Ayurveda and Unani, which have survived more than 3,000 years, mainly using herbal medicine. The medical equipment of these systems contains a rich heritage of indigenous herbal practices that have helped maintain the health of the majority of rural populations in India.

Croton zambesicus belongs to the *Euphorbiaceae* family and the Malpighiales order. Its common name is “Ajekobale” (Yoruba language, Nigeria) based on the dialect and Koriba in the Hausa language[11]. It is a 16m tall shrub or small tree found on the outskirts of forests and savannahs throughout Africa. The tree is very appealing, with scaly bark and silvery foliage with rusty scales below. It is commonly seen in cities and towns. It was formerly planted as a fetish tree. It is said to provide protection and is frequently planted at house entrances to fend off evil spirits. *Croton zambesicus* is widely used for therapeutic reasons in Africa. It is very vigilant regarding witches. Its Yoruba name, àjekòbàlé showing its therapeutic efficacy. To the Ejagham of southern Nigeria, it is a symbolic tree known as mfam, at least in religious

terms (properly the name of the Juju cult). *Croton zambesicus* is grown for therapeutic uses in several Nigerian communities[13].

It has the power to heal an infected person, or the leaves can be delicately drawn on the face of the dying to allow the spirit to depart without suffering. The wood is pale yellow, fine-grained, durable, and polishes well. In places in West Africa where there isn't any other wood, the stems are used for hut pillars and in Yoruba homes for beams. The bark stick has a pleasant scent, and an infusion of the bark is used to treat malaria in Nigeria. The leaves are said to be nourishing[14]. In southern Nigeria, a soup made from it is provided for dysentery patients, a decoction of the leaves is used as a wash in Nigeria and Sierra Leone and is used internally for dysentery, fever, convulsions, and so on, and in Nigeria for headaches and as an anthelmintic. In Sudan, the shoots and roots are used as a tonic, febrifuge, and to relieve menstruation discomfort. The root is eaten as an appetizer in Ghana. The fruits, like the bark, are edibleand are aromatic. They are used to season meals and make perfume in Nigeria's Adamawa area. In Togo, the seeds are thought to have therapeutic properties. They are used to flavour tea in Kordofan. In Zambia, an unnamed component of the plant is used to treat a sore nose[13]. The presence of trace levels of alkaloid in the stem and leaf of the Nigerian material was discovered[13].

Around 80% of people in underdeveloped nations rely largely on traditional medicine for their health care requirements, with plant extracts or active substances playing a key role. The lack of standardisation and quality control profiles is one of the accusations levelled towards phytotherapy. The species in question's accurate identification,whether fresh.

The phytochemistry of this valued medicinal plant is a distinct subject that exists midway between organic chemistry, plant biochemistry, and many other natural product-related disciplines. It is a collection of organic compounds found in plants. Not only are its chemical constituents like as carbs, proteins, and lipids used as food by humans, but it also contains a huge variety of chemicals such as glycosides, alkaloids, flavonoids, and so on. It is used as remedies in a variety of ways and by diverse approaches. The qualitative and quantitative evaluation of a medicinal plant's phytochemical components is regarded as a key stage in medicinal plant research[26].

Pharmacognosy knowledge is useful in this area of research activity. The term "pharmacognosy" is derived from two Greek words: "pharmakon" (drug) and "gnosis" (knowledge). Pharmacognosy, like many modern scientific subjects, has seen major changes in recent years and is now a highly multidisciplinary science that is one of the five great fields of pharmaceutical education. Its applications include the investigation of the physical, chemical, biochemical, and biological aspects of pharmaceuticals, prospective drugs, and medications of natural origin, as well as the search for novel drugs of natural origin.

Reverse pharmacognosy allows for the discovery of new biological targets for natural compounds by virtual or real-world screening, as well as the identification of natural resources containing active molecules[29]. With the merging of conventional knowledge, reverse pharmacognosy can alleviate different bottlenecks in the discovery of novel drugs[30]. As a result, this study focuses on *Croton zambesicuss* efficiency against pathogens such as *Staphylococcus aureus* and *Streptococcus species*, with the goal of discovering the bioactive components that boost its activity based on their phytochemical qualities.

1.2 Statement of Problem

It has been estimated that 80% of the population in developing countries cannot afford medicines and rely on traditional herbal medicines to meet their primary health care needs[22]. There are little or no controlled studies that have been conducted to test the use of *Croton zambesicus* leaf and stem to treat certain bacterial infections.

1.3 Justification of the Study

The World Health Organization estimates that about 80% of the world's population in developing countries rely on traditional medicinal plants for primary health care needs, a significant proportion of which are plant extracts or their ingredients assets[23]. The reasons for the use of traditional medicines may vary, apart from the fact that herbal preparations are relatively cheaper, adverse drug reactions are rarely observed compared to synthetically produced pharmaceuticals[24]. Several studies have established that herbal medicines are safe, effective and less expensive alternatives to synthetic medicines that treat certain bacterial infections[25]. Based on the aforementioned medicinal claim for *Croton zambesicus*, particularly its effects on certain infectious diseases, it is important to compare the inhibitory efficacy of certain solvent fractions of *Croton zambesicus* leaves and stem on some bacteria.

1.4 Aims and Objectives of the Study

This research work aims to determine the inhibitory effect of ethanol, acetone, chloroform and *Croton zambesicus* petroleum ether on *Eschericha coli, Samonella typhi, Enterobacter cloacae*, *Proteus mirabilis, Klebsiella pneumonia, Staphylococcus aureus* and to study their antioxidant properties *invitro*.

Aims

The specific objectives of the study are to;

i. determine the minimum inhibitory concentration of the solvent fraction of leaves and stems of *Croton zambesicus* on *Eschericha coli, Samonella typhi, Enterobacter cloacae, Proteus mirabilis, Klebsiella pneumonia, Staphylococcus aureus*
ii. determine the phytochemical constituents of the leaves and stems of *Croton zambesicus*
iii. determine the amount of phytochemical constituents in leaves and stems of *Croton zambesicus*

1.5 Significance of the Study

Herbal medicine research has received a lot of attention around the world, and there are a lot of interest in plants and their medical value[32]. Herbal therapies are the most popular type of traditional medicine, are extremely profitable in the international market, and have frequently retained their appeal for historical and cultural reasons. The WHO (World Health Organization) estimates that herbs are medicines for almost two-thirds of the original world population, or approximately 4 billion people[34].

In Africa, traditional medicine (TM) is used by more than 80% of the population to address their health-care needs[33]. People in Asia and Latin America continue to use TM owing to historical circumstances and cultural beliefs. In China, TM accounts for roughly 40% of all healthcare[34]. Meanwhile, Complementary and Alternative Medicine (CAM) is gaining popularity in many wealthy countries. According to the WHO Global Strategy for Traditional Medicine, the rising usage of medicinal plants around the world has made medicinal plant regulation a critical issue[35].

Multidrug resistance has emerged in recent years as a result of the misuse of existing antimicrobial medications in the treatment of infectious diseases. Antibiotics can occasionally cause side effects in the host, such as hypersensitivity. As a result, alternative

antimicrobial medications derived from other sources, such as plants, are required for the treatment of infectious diseases. Natural compounds derived from higher plants could be a new source of antibacterial medicines with novel modes of action[35].

All plants secrete chemicals as part of their normal metabolic activities[34]. These can be divided into primary metabolites, such as sugars, amino acids, nucleotides, and fats, present in all plants, and secondary metabolites that do not play a significant role in a plant's primary metabolism or growth. photosynthesis or other "primary" functions of the plant cell. They may play an ecological role, such as pollinator attractors, represent chemical adaptations to environmental stress, or be responsible for the chemical defense of the plant against microorganisms, insects, and higher predators[35].

1.6 Scope of the Study

This study investigates the medicinal efficacy of different solvent fractions from the stems and leaves of *Croton zambesicus* and the determination of their inhibitory effect on certain pathogenic bacteria. Certain phytochemical constituents of the requirement have also been determined.

1.7. Limitation to the Study

The leaf and stem of *croton zambesicus* was not easily accessible as it nearly grows in forest areas also it took a longer time to dry thereby leading to wastage of time. During milling the plant parts was so hard making the use of a specialized excella blender necessary. Careful precautions should also be taken to disallow contamination of the palnt parts used.

1.8 Operational Definition of Terms

Antioxidant: Antioxidants are man-made or natural substances that can prevent or delay certain types of cell damage. They are also compounds that inhibit oxidation, a chemical reaction that can produce free radicals and chain reactions that can damage the cells of organisms.

Bacteria: Bacteria are a large group of unicellular microorganisms that have cell walls but lack organelles and an organized nucleus, some of which can cause disease.

Minimum Inhibitory Concentrations: (MIC) are the lowest concentration of an antimicrobial that inhibits visible growth of a microorganism after 24 hours of incubation.

MDRM - multidrug-resistant microorganisms

K – Pneumonia

SXT - Septrin

Z - Zinnacef

AM - Amoxicillin

R - Rocephin

APX - Ampiclox

CPX – Ciprofloxacin

S - Streptomycin

CN – Gentamicin

PEF - Perfloxacin

E - Erythromycin

OFX - Tarivid

AU - Augmentin

SP - Sperfloxacin

Endnotes

[1] B. Li & T.J. Webster. *Bacteria Antibiotic Resistance: New Challenges and Opportunities for Implant-Associated Orthopedic Infections*. **J. Orthop. Res.** 36:2018 22–32. doi: 10.1002/jor.23656

[2] A. Shah, S.K., Marwat, F. Gohar; A. Khan,K.H. Bhatti, & M. Amin M.*Ethnobotanical Study of Medicinal Plants of Semi-Tribal Area of Makerwal & Gulla Khel (lying between Khyber Pakhtunkhwa and Punjab Provinces)*, **Pakistan. Am. J. Plant Sci**., 4: 2018, 98-116.

[3] C.C. Hammer, J. Brainard, & P.R. Hunter, P. R. *Risk Factors and Risk Factor Cascades for Communicable Disease Outbreaks in Complex Humanitarian Emergencies: A Qualitative Systematic Review*. **BMJ Glob. Health** 3:e000647:2018.

[4] B. Weniger, L. Lagnika, L.B. Djakou, C. VonthronSénéchaux, & A. Sanni, *L'ethnopharmacologie et la recherche de molécules antipaludéennes dans la biodiversité ivoirienne, béninoise et camerounaise.***Ethnopharmacologia** 41: 2020, 62- 70

[5] Z. Hamzaoui, A. Ocampo-Sosa, M.F. Martinez, S. Landolsi, S. Ferjani, & E. Maamar, *Role of Association Of Ompk35 And Ompk36 Alteration and Blaesbl and/or Blaampc Genes In Conferring Carbapenem Resistance Among Non-Carbapenemase-Producing Klebsiella pneumoniae*. **Int. J. Antimicrob. Agents** 52:2018 898–905. doi: 10.1016/j.ijantimicag.2018.03.020

[6] M. Haque, M. Sartelli, J. Mckimm, & M.A. Bakar. *Health Care Associated Infections–an Overview.* **Infect. Drug Resist**. 11:2018 2321–2333. doi: 10.2147/IDR.S177247

[7] N. Hegerle, M., Choi, J., Sinclair, M.N., Amin, M. Ollivault-Shiflett, & B. Curtis. *Development of a Broad Spectrum Glycoconjugate Vaccine to Prevent Wound and Disseminated Infections with Klebsiella pneumoniae and Pseudomonas aeruginosa*. **PLoS One** 13:e0203143:2018. doi: 10.1371/**journal. pone**.0203143

[8]A. Singh & P.K. Singh, *"An Ethnobotanical Study of Medicinal Plants in Chandauli District of Uttar Pradesh, India*," **Journal of Ethnopharmacolgy**, Vol. 121, No. 2, 2019, pp.324-329. doi:10.1016/j.jep.2008.10.018

[9]S. Boukhira, B. Mounyr M. Faisl,M. Stephane, T.M. Sghir, & B. Dalila, *Antimicrobial Activities of Essential Oil of Five Plant Species from Morocco against some Microbial Strain.* 2021.

[10]A. Manikandan, M. Durka, M. Selvi, S. Anthony. *Sesamum Indicum Plant Extracted Microwave Combustion Synthesis and Opto-magnetic Properties of Spinel Mn* *** Co1-* Al204 nano- catalysts; Journal of Nanoscience and Nanotechnology,** 16 (1), 2018,448-456.

[11] J.S. Kang, J. Yi, M.K. Ko, S.O. Lee, J.E. Lee, & K.H. Kim. *Prevalence and Risk Factors of Carbapenem-Resistant Enterobacteriaceae Acquisition in an Emergency Intensive Care Unit in a Tertiary Hospital in Korea: A Case-control Study.* **J. Korean Med. Sci.** 34:e140:2019 doi: 10.3346/ jkms.2019.34.e140

[12] J. Okokon, K. Davies, & A. Bassey; *Psycopharmacological study of Ethanol Leaf Extract of Solenostemon monostachyus*; **Journal of Applied Pharmaceutical Research,** 9(3): 2020, 59-63,

[13] A. Ogunbajo, I.A. Ogunwande, H. Gbadamosi, R. Giwa, & G. Flamini; *Chemical Composition of the Leaf Essential Oils of Croton zambesicus*; **European Journal of Medicinal Plants,** 4(12): 2019, 1524-1533.

[14] T.A. Russo, & C.M. Marr, *Hypervirulent Klebsiella pneumoniae.* **Clin. Microbiol. Rev**. 32, e00001–e00119:2019 doi: 10.1128/CMR.00001-19

[15] T.A. Russo, R. Olson, C.T. Fang, N. Stoesser, M. Miller, & U. Macdonald, *Identification of Biomarkers for Differentiation of Hypervirulent Klebsiella pneumoniae from Classical K. pneumoniae*. **J. Clin. Microbiol**. 56, e00776–e00818:2018 doi: 10.1128/JCM.00776-18

[16] O.A. Odukoya, M.O. Sofidiya, A.T. Samuel, I. Ajose, M. Onalo & B. Shuaib.*Documentation of Wound Healing Plants in Lagos-Nigeria: Inhibition of Lipid Peroxidation as In-vivoPrognostic Biomarkers of Activity,* **Annals of Biological Research,** 3(4): 2022, 1683-1789

[17] J.P. Metlay, G.W. Waterer, A.C. Long, A. Anzueto, J. Brozek, & K. Crothers. *Diagnosis and Treatment of Adults with Community-Acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases society of America.* **Am. J. Respir. Crit. Care Med.** 200, e45–e67:2019 doi: 10.1164/rccm.201908-1581ST

[18] L.R. Lima, & J.R. Pirani. *Taxonomic Revision of Croton sect*. *Lamprocroton* (Müll. Arg.) Pax (Euphorbiaceae s.s.) **Biota Neotrop**. 8: 2018; 177–231. [Google Scholar]

[19] A. Salatino, M.L.F. Salatino & G. Negri. *Traditional Uses, Chemistry and Pharmacology of Croton species (Euphorbiaceae)* **J. Braz. Chem. Soc**. 18: 2019; 11–33. [Google Scholar]

[20]https://www.prota4u.org/database/protav8.asp?g=pe&p=Croton+lobatus+L.#:~:text=Croton%20lobatus%20occurs%20in%20the,east%20to%20Eritrea%20and%20Ethiopia.

[21] I. Monye, & A.B. Adelowo, *Strengthening Immunity through Healthy Lifestyle Practices: Recommendations for Lifestyle Interventions in the Management of Covid-19*. **Lifesty. Med**. 1:e7. doi: 2020, 10.1002/lim2.7

[22] J. H. Sampson, J.D. Phillipson, N.G. Bowery, M.J. O'Neill, J.G. Houston, & J.A. Lewis. *Phytotherapy Research*, **J. Chem. Soc**. 14, 2020, 24.

[23] C.M. Chariandy, C.E. Seaforth, R.H. Phelps, G.V. Pollard, B.P. & Khambay. *Screening of Medicinal Plants from Trinidad and Tobago for Antimicrobial and Insecticidal Properties.* **Journal of Ethnopharmacology,** 64(3), 2019, 265-70.

[24] K. Vermani, & S. Garg. *Herbal Medicines for Sexually Transmitted Diseases and AIDS.***Journal of Ethnopharmacology,** 80(1), 2022, 49-66

[25] H. Yao, S. Qin, S. Chen, J. Shen, & X.D. Du. *Emergence of Carbapenem-Resistant Hypervirulent Klebsiella pneumoniae*. **Lancet Infect. Dis.** 18:2018, 25. doi: 10.1016/S1473-3099(17)30628-X

[26] J. D. Stanaway, R. C. Reiner, & B. F. Blacker. *"The Global Burden of Typhoid and Paratyphoid Fevers: A Systematic Analysis for the Global Burden Of Disease Study"* **The Lancet Infectious Diseases,** vol. 19, no. 4, 2019, pp. 369–381,

[27] L. M. Kaljee, A. Pach, D. Garrett, D. Bajracharya, K. Karki, & I. Khan, *"Social and Economic Burden Associated with Typhoid Fever in Kathmandu and Surrounding Areas: A Qualitative Study,"* **The Journal of Infectious Diseases,** vol. 218, 2018supplement_4, pp. S243–S249.

[28]K.Dhalwal, V.M Shinde, K.R Mahadik & A.G Namdeo. *Raoid Densitometric Method for Simultaneous Analysis of Umbelliferone, Psoralen and Eugenol in Herbal Raw Materials using HPTLC:* **Journal of Separation Science,** 30 (13) 2021, 2053-2058

[29]R.Verpoorte. *Pharmacognosy in the new Millennium: Leadfinding and Biotechnology.* **Journal of Pharmacy and Pharmacology,** 52, 2020, 253-262

[30] A. Radhakrishnan, D. Als, & E. D. Mintz. *"Introductory Article on Global Burden and Epidemiology of Typhoid Fever,"* **The American Journal of Tropical Medicine and Hygiene,** vol. 99:2018 3_Supplement, pp. 4–9.

[31] S. Malhotra & A.S. Pal. *A Review of Pharmacology of Phytochemicals from Indian Medicinal Plants.* The Internet. **Journal of Alternative Medicine**. 5(1): 2019; 23- 27.

[32] M. K. Rasheed, S. S. Hasan, Z. U. D. Babar, & S. I. Ahmed, "*Extensively Drug-Resistant Typhoid Fever in Pakistan*," **The Lancet Infectious Diseases**, vol. 19, no. 3, pp. 242-243, 2019.

[33] J. R. Andrews, F. N. Qamar, R. C. Charles, & E. T. Ryan, *"Extensively Drug-Resistant Typhoid—are Conjugate Vaccines Arriving Just in Time?,"* **New England Journal of Medicine,** vol. 379, 2018, no. 16, pp. 1493–1495.

[34] C. D. Britto, V. K. Wong, G. Dougan, & A. J. Pollard, *"A Systematic Review of Antimicrobial Resistance in Salmonella enterica Serovar Typhi, the Etiological Agent of Typhoid*," **PLoS Neglected Tropical Diseases**, Vol. 12, 2018, no. 10.

[35] A.A.Samah, Z. Aisha & B.M. NaserEldin. *Antimicrobial activity of Croton zambesicus, Acacia ehrenbergiana, and Fagoniacretica.* **National Journal of Advanced Research**. Vol 2; Issue 3; 2019, Pg No. 16-20

Chapter Two

Literature Review

Croton zambesicus is a medicinal plant commonly found in Africa. It has different names depending on where you are in Africa. It is known as "Ajekobale" in Ondo State, although its names differ in the southern part of Nigeria. The leaves and the branch of the plant are extracted for therapeutic purposes. A decoction of leaves is used in antihypertensive and antimicrobial treatment (urinary tract infections) in Benin and as antidiabetic and antimalarial treatment in parts of Nigeria[1]. Ibibios in the Niger Delta region of Nigeria use the roots as antimalarial, febrifuge, and antidiabetic medicine[3]. The root is used in Sudan for menstrual pain and as an aperitif. The composition of the essential oils extracted from the leaves, *Croton zambesicus* stems and roots includes three types of oils of comparable composition, with abundant monoterpenes in the leaves and stems, and sesquiterpenes in the root bark[5]. Spatunolol and linalool have been found to be essential components from the root bark and stem oils, which have been found to be rich in oxygen-containing chemicals. Ethanol leaf extract has been shown to have biological remedy for antiplasmodial, antidiabetic, anti-inflammatory, analgesic, and antipyretic, while root extract has antimalarial, anticonvulsant, and antiulcer activities[3]. Ent-trachylobanediterpene, derived from the dichloromethane extract of the leaves, shows the cytotoxic effect on Hela cells[2]. Ethanol leaf extract has been shown to have biological remedy for antiplasmodial, antidiabetic, anti-inflammatory, analgesic, and antipyretic, while root extract has antimalarial, anticonvulsant, and antiulcer activities[4]. Ent-trachylobanediterpene, derived from dichloromethane extract of leaves, shows the cytotoxic effect on hela cells[2]. Ethanol leaf extract has been shown to have biological remedy for antiplasmodial, antidiabetic, anti-inflammatory, analgesic, and

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Croton zambesicus phytochemistry research is a distinct discipline somewhere between organic chemistry, plant biochemistry and much more related to natural product derivatives.

It is a variety of organic substances deposited in plants[10]. The plant can be considered as a biosynthetic process[8]. Not only its chemical compounds such as carbohydrates, proteins, and lipids that are used as food by humans, but there are also numerous amounts of compounds such as glycosides, alkaloids, flavonoids, etc.[11]. The qualitative and quantitative estimation of the phytochemical constituents of a medicinal plant is considered an important step in medicinal plant research[9].

2.1 Scientific Classification

Kingdom: Plantae

Phylum: Tracheophytes

Class: Magnoliopsida

Order: Malpighiales

Family: *Euphorbiaceae*

Gender: *Croton*

Species: *zambesicus*

2.2 Morphology

Croton zambesicus is a shrub or small tree that grows to about 16m in height in forests, mainly along streams and savannahs. It is widely distributed in Gambia, southern Nigeria and parts of tropical Africa[12]. The tree has an attractive appearance, flaky bark and scaly silvery rusty leaves underneath. The leaves are greenish with a silvery appearance on top, while some leaves are orange. It is usually planted in parts of urban communities, but mostly in rural settlements. The plant has a pleasant smell. The wood is hard, pale yellow in color and fine-grained, giving it a soft tan. The stems are used in parts of East Africa[13].

Figure 2.1: *Croton zambesicus* plant[102]

2.3 Medicinal use of *Croton zambesicus*

Reports of *Croton zambesicus* root extracts include; antiulcer, antimalaria, anticonvulsant, anti-inflammatory, analgesic and antipyretic, antidiabetic and lipid-lowering activities[16]. *Croton zambesicus* root extract has been reported to have a protective remedy for the kidneys against gentimicin-induced kidney damage[14]. In addition, health benefits of immunostimulation, cytotoxicity against Hela cell line, and anti-leishmanic activities of the root extract have been reported[19]. The essential oil composition of *Croton zambesicus* roots has been performed and it has been revealed that the oil from the root the bark contained

sesquiterpenes, rich in oxygen compounds containing, with espatulenol and linalool as main components[17].

2.4 Chemical Components of *Croton zambesicus*

*Croton zambesicus*is widely used in traditional medicines in Africa and in other parts of the world[21]. Contains alkaloids, terpenes, flavonoids, glycosides, saponins, volatile oils including; sesquiterpenes, cardiac glycosides, monoterpenes and diterpenes and other chemicals[21]. The identified phytochemicals of this plant include diterpenes that have vascular and vasorelaxant activities, betulinic acid, betulin, diterpene and kaurane-3,16, lupenone, lupeol, 17-triol and vitexin; glucopyranoside 30-methyl ether, helichrysoside-30-methyl ether, quercetin-3-O-p-6-O (p-coumaroyl), as well as apigenin-6-C-glucoside, glucopyranoside, kaempferol-3-O-p-600 (pcumaroyl), tiliroside and isovitexin21 as antioxidant components of the plant leaf[23]. One report also showed that the volatile oil of *C. zambesicus* exhibited vasorelaxant activities[22].

Gas chromatography-mass spectrometry (GCMS) analysis of the hexane fraction of *Crotonzambesicus* root revealed the presence of hexadecanoic acid, 1-hexadecanol, 2-methyl, hexadecanoic acid, ethyl ester, linoleic acid, ethyl ester , 9-octadecanoic (Z), 2-hydroxyl-1-hydroxymethyl ethyl ester, hexadecanoic acid, 2-methyl-, methyl ester, trachylobane, androst-4-ene-3,17-dione, androst-4-ene-17 -one, 3-hydroxy -,(5ά)-, 2,4(1H,3H)-pyrimidinedione,5-nitro, retinol, myrcene, ɣ-terpinene, linalool, linalool acetate, lupeol, α-humulene, α- muurolene, stigmast-4-in-3-one, Lanost-7-in-3-one, 9ά, 13ά, 14ά, 17ά)[17].

2.5 Phytochemical Activities of *Croton Zambesicus*

The various phytochemicals detected in *Croton zambesicus* are known to have beneficial importance in industrial processes and medical sciences[24]. Plant phenolic compounds, in particular flavonoids, are attracting increasing interest due to their supposed health-promoting properties (antioxidants); Flavonoids have been shown to have anti-inflammatory, anti-allergenic, antiviral, anti-aging, and anti-cancer activity[25].

2.5.1 Flavonoids

The vast therapeutic effects of flavonoids can be entirely attributed to their antioxidant properties. Flavonoid compounds have been shown to exert protection against heart disease by inhibiting cyclooxygenase and lipoxygenase activities in platelets and macrophages[26].

2.5.2 Tannins

Tannins have been shown to possess physiological astringent and hemostatic properties, accelerating wound healing, improving inflamed mucous membrane, and also inhibiting the growth of microorganisms by precipitating microbial proteins and making nutritional proteins unavailable to them. Form irreversible complexes with proline-rich proteins, leading to inhibition of cellular protein synthesis[20]. They have important functions as powerful and stable antioxidants[25]. They act as binders and for the treatment of diarrhea and dysentery[27].

Tannin has also been reported to exhibit antibacterial, antiviral, and antitumor activities. It was also discovered that certain tannins could inhibit the selectivity of HIV replication and were also used as diuretics[25]. Vegetable tannins have been recognized for their pharmacological properties as well as for their insect repellent action[28].

2.5.3 Plant Steroids

Plant steroids are plant compounds with chemical structure and biological functions similar to cholesterol. Plant steroids contain an additional methyl, ethyl, or double bond group. The most abundant plant steroids are sitosterol, campesterol, and stigmasterol [103].

Plant steroids are known to be important for their cardiotonic, insecticidal and antimicrobial properties. They are also used in nutritional diets, phytotherapy, skin care[29]. Plant steroids help lower cholesterol levels by limiting the amount of cholesterol that enters the body. They are also effective in treating heart disease, colon cancer, stomach cancer, obesity, and heart disease. attack[104].

2.5.3 Saponins

Saponins are natural bioorganic compounds that have at least one glycosidic bond (CO-sugar bond) at C-3 between the aglycone and a sugar chain[30]. Hydrolysis of the saponin molecule produces two parts, the aglycone and a sugar moiety. Isolated solid amorphous saponins have a high molecular weight and contain 27 to 30 carbon atoms in the non-saccharide part [105].

Saponins have expectorant properties that work in managing inflammation of the upper respiratory tract. The saponins present in *Crotonzambesicus* plants are cardiotonic in nature and are said to have antidiabetic and antifungal properties[25]. Previous studies show that saponins exhibit biological role and medicinal properties as hemolytic factor with anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer, cytotoxic and molluscicidal action. In addition, saponins are said to have a hypocholesterolemic action in animals and humans[105].

2.6 Pathogenic Bacteria and their Characteristics

2.6.1 *Escherichia coli*

*Escherichia coli*is one of the most genetically multifaceted microorganisms and is capable of colonizing and adapting in various niches, both in the environment and in hosts. Commensal strains of *E. coli* colonize the human gastrointestinal tract within hours of birth, resulting in a symbiotic relationship between the microflora and its host[30]. However, the mechanisms by which *E. coli* ensures the effectiveness of the symbiosis are not well understood. This could be due to its great capacity to absorb nutrients in the colon[30]. Several studies have shown that competition for nutrients between microflora and pathogens limits the colonization of pathogens, leading to fierce competition between these microorganisms[31].

2.6.1.2 Morphology

Escherichia coli are gram-negative short rod bacilli of the *Enterobacteriaceae* family, they are facultative anaerobes and non-spore-forming microorganisms. *E. coli* strains with K1 capsular polysaccharide antigen cause approximately 40% of sepsis cases and 80% of meningitis cases[33].

2.6.1.3 Virulence Factor

Most strains of *E. coli* are harmless, but some strains acquire enterotoxins or invasion factors encoding bacteriophages or plasmids and become pathogenic[32]. These virulent strains are responsible for diarrheal infections worldwide, as well as neonatal meningitis, sepsis, and urinary tract infections (UTIs) [33].

There are pathogenic variants of *E. coli*, subgrouped into diarrheal and extraintestinal pathogens, with different pathotypes and several natural hybrid strains. These variants can be

facultative or obligate pathogens. Facultative bacteria colonize the intestinal tract and can act as opportunistic pathogens when outside their natural habitat, causing various types of extraintestinal infections. On the other hand, variants of obligate intestinal pathogens cause infections in different conditions, from mild diarrhea to more life-threatening cases, as a severe outcome[34].

Human pathotypes of diarrheal *E. coli* (DEC) are differentiated from nonpathogenic *E. coli* and extraintestinal pathogenic *E. coli* (ExPEC) based on the virulence factor of *E. coli* genetics. ExPECs are classified as uropathogenic *E. coli* (UPEC), septicemic *E. coli* (SEPEC), and neonatal meningitis-associated *E. coli* (NMEC) [32]. Recent virulent genes and phenotypic classification of E. pathotypes, proposed by their differential characteristics and the essential pathogens that define each subgroup, such as Shiga toxin-producing *E. coli* (STEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), adherent-invasive *E. coli. coli* (AIEC) and cell-detaching E. coli (CDEC)[35].

Table 2.1: Main Characteristics of the Classic *E. coli* Pathotypes: Extraintestinal (ExPEC) and Diarrheal[36]

Pathotype *E. coli* (DEC and ExPEC)	Main Virulence Traits	Clinical Manifestation	Antimicrobial Resistance (AMR) is Common	Determinants of Mobile Genetic Resistance
Shigatoxin Producers (STEC)	Shiga toxin	Not associated with human disease.	Streptomycin, Ampicillin, Tetracycline and Sulfonamides	DN [36]
Enterohemorrhagic (EHEC)	EscF, EscC, EspA, EspB, EspD, Intimin, Tir and Shiga-toxin	Foodborne bloody diarrhea and HUS	Streptomycin, Ampicillin, Tetracycline and Sulfonamides	Plasmid-mediated resistance (such as pO157, pO111-CRL115, pO26-CRL125, pO145-13514)[36]
Enteropathogens (EPEC)	Plasmid EscF, EscC, EspA, EspB, EspD, Intimin, Tir, EAF (tEPEC) and Bfp (tEPEC)	watery diarrhea	Streptomycin, ampicillin, tetracycline, trimethoprim, and sulfamethoxazole	Plasmid-mediated resistance (such as pEAF, MB80, pB171_90, pED208)[37]
Enteroaggregative (EAEC)	PAA Plasmid, Adhesion Aggregate Fimbriae (AAF), AggR Regulator and Dispersin	Acute and chronic diarrhea	Ampicillin, trimethoprim, sulfamethoxazole, nalidixic acid, and ciprofloxacin	Plasmid-mediated resistance (such as pAA), gyrB, and parC[37] chromosomal

Table 2.1 Cont'd				mutations
Enteroinvasive (EIEC)	Plasmid pINV and invasions	bacillary dysentery	Carbapenem, fosfomycintrome thanol, nitrofurantoin, chloramphenicol, beta-lactams, nalidixic acid, ampicillin, and fluoroquinolones	Plasmid-mediated resistance, chromosomal mutations in gyrB and parC[35]
Enterotoxigenic (ETEC	Heat-stable (ST) and heat-labile (LT) enterotoxins	Watery diarrhea, known as traveler's diarrhea.	Ampicillin, sulfamethoxazol e, tetracycline, and azithromycin	Plasmid-mediated resistance (distinct Inc-type conjugative plasmids)[35]
Diffuse Membrane (DAEC)	Adhesions Afa/Dr.	Acute diarrhea in asymptomatic cases	Ampicillin, trimethoprim, sulfamethoxazol e, fosfomycin, piperacillin, tetracycline, ciprofloxacin, co-trimoxazole, nitrofurantoin, oxacillin, bactericin, cloxacillin, chloramphenicol, and nalidixic acid	Plasmid resistance, chromosomal mutations in gyrB and parC[33]

Table 2.1 Cont'd

Adherent-invasive (AIEC	secretory system type VI, pili type I, long polar fimbriae	Chronic intestinal inflammation and Crohn's disease	Ampicillin and Ciprofloxacin	Plasmid-mediated resistance, chromosomal mutations in gyrB and parC[32]
Cellular Detachment (CDEC)	K-hemolysin, pyelonephritis-associated hairs, and cytotoxic necrosis factor 1 (CNF1)	Diarrhea in infants, shedding of cells and linked to cases of Crohn's disease	Amoxicillin-clavulanic acid, ampicillin, mezlocillin, piperacillin, tetracycline, trimethoprim, trimethoprim, sulfamethoxazole, spectinomycin, streptomycin, and sulfonamide	Plasmid-mediated resistance, integrons[38]
Uropathogen (UPEC)	fimbriae P, some other mannose-resistant adhesins and fimbriae type 1, capsule K, hemolysin, aerobactin	Urinary and blood infections	Fluoroquinolone, aminoglycosides, trimethoprim sulfamethoxazole, and carbapenems	Resistance mediated by plasmids, transposons, integrons, chromosome gyrA, gyrB, parE, parC and marA[34] mutations
Causing sepsis (SEPEC)	Fimbriae type 1, P and S, capsule	Bacteremia and sepsis	Carbapenem	Plasmid-mediated

Table 2.1 Cont'd

	K K1/K5, hemolysin, aerobactin, yersiniabactin, salmochelin, CNF1, autotransport secreted toxin, serum resistance, and colicin V			resistance, integrons[39]
Neonatal Associated Meningitis (NMEC)	ompTp, hlyF, cvaC, etsA, cvaA, etsB, cvaB, iss, iutA and tsh	Meningitis and bacteremia in newborns	Streptomycin sulfisoxazole, ampicillin, tetracycline, chloramphenicol, kanamycin, and trimethoprim sulfamethoxazole	Plasmid-mediated resistance[34]

Source: [36]

2.6.2 Morphology of *Klebsiella pneumonia*

Klebsiella pneumonia is a ubiquitous, nonmotile, encapsulation fermenting, rod-shaped, ubiquitous facultative anaerobic bacterium found as flora in the mouth, skin, intestines, and feces of approximately 5% of people. *Klebsiella pneumoniae* belongs to the *Enterobacteriaceae* family and is considered one of the opportunistic pathogens that cause a wide spectrum of diseases in humans[40].*K. pneumonia* accounts for approximately one-third of all gram-negative infections, including urinary tract infections, cystitis, pneumonia,

surgical wound infections, endocarditis, and sepsis[41]. It also causes necrotizing pneumonia, pyogenic liver abscess, and endogenous endophthalmitis[3]. High mortality and morbidity rates, causalities hospitalization, coupled with high cost are often associated with infections caused by this organism[41].

2.6.2.2 Subgroups of *Klebsiella pneumoniae*

Klebsiella pneumonia can be broadly classified into two subtypes; *Klebsiella pneumoniae* classical (cKp) and *Klebsiella pneumoniae* non-classical (ncKp). The antimicrobial resistance profile and virulence profiles of these strains vary[42]. Several clones of these ncKp have been reported to cause severe and difficult to manage infections due to their continuous mutation and acquisition of plasmids and transposons carrying resistant and virulent genes[42]. This genetic mutation has led to the appearance of hypervirulent strains such as Klebsiella pneumoniae (hvKp) or the hypermucoviscous Klebsiella pneumoniae (HMKP). This subtype is not resistant to the most commonly used antimicrobials, such as colistin and carbapenems. But recent reports of carbapenem-resistant hvKp strains belonging to sequence type 11 (ST11) strains, ST25 and ST65 pose a significant clinical concern[50]. HvKp strains can cause serious infections in immunocompetent, sick and healthy young people, preferably younger than 5 years. This hvKp is known to harbor (i) a siderophore; of which aerobactin predominates which is concomitant with hypermucoviscosity, (ii) virulent factors such as; Capsular types K1, K2, K20, mucoid regulatory genes rmpA and rmpA252. Horizontal transfer of these plasmids and transposons has led to the multidrug resistance (MDR) and extremely drug resistant (XDR) nature of most of these subtypes. The high prevalence rate of the MDR and XDR subtypes of *K. pneumoniae* reflects a multifactorial dissemination process that includes, but is not limited to: the propagation of a high-risk multi-resistant global genetic line;

acquisition of successful multiresistant plasmids; and the acquisition of resistant genes located on successful transposons. *Klebsiella* is a major source of carbapenem resistance worldwide through spread of its plasmids that is facilitated by high gene transfer (HGT) to other species. Propagation of these plasmids encoding extended-spectrum β-lactamase (ESBL) and carbapenemase poses a major threat, as acquisition of these plasmids transforms bacteria into MDR and XDR[40]. A high-risk global multi-resistant genetic line; acquisition of successful multiresistant plasmids; and the acquisition of resistant genes located on successful transposons[42]. *Klebsiella* is a major source of carbapenem resistance worldwide through spread of its plasmids that is facilitated by high gene transfer (HGT) to other species. Propagation of these plasmids encoding extended-spectrum β-lactamase (ESBL) and carbapenemase poses a great threat, as acquisition of these plasmids transforms bacteria into MDR and XDR[40]. A high-risk global multi-resistant genetic line; acquisition of successful multiresistant plasmids; and the acquisition of resistant genes located on successful transposons. *Klebsiella* is a major source of carbapenem resistance worldwide through spread of its plasmids that is facilitated by high gene transfer (HGT) to other species. Propagation of these plasmids encoding extended-spectrum β-lactamase (ESBL) and carbapenemase poses a major threat, as acquisition of these plasmids transforms bacteria into MDR and XDR[40]. *Klebsiella* is a major source of carbapenem resistance worldwide through spread of its plasmids that is facilitated by high gene transfer (HGT) to other species. Propagation of these plasmids encoding extended-spectrum β-lactamase (ESBL) and carbapenemase poses a major threat, as acquisition of these plasmids transforms bacteria into MDR and XDR[40]. *Klebsiella* is a major source of carbapenem resistance worldwide through spread of its plasmids that is facilitated by high gene transfer (HGT) to other species[43]. Propagation of

these plasmids encoding extended-spectrum β-lactamase (ESBL) and carbapenemase poses a major threat, as acquisition of these plasmids transforms bacteria into MDR and XDR40.

Resistance rates of *K. pneumoniae* to imipenem and meropenem were found to have increased more than eight-fold between 2005 and 2018 (from 3.0% to 25% and 2.9% to 26.3%, respectively), while those of ceftazidime and ciprofloxacin increased. 19.7% to 21.7% and 17.3% to 22.4%, respectively, from 2014 to 201946. The resistance mechanism of *K. pneumoniae* mainly includes the production of beta-lactamases, the absence of membrane porin proteins and the active output of antibacterial drugs. Extended-spectrum beta-lactamase (ESBL)-producing *K. pneumoniae* exhibit a high degree of drug resistance, which can exhibit multiple resistance mechanisms simultaneously, often leading to multidrug resistance[44].

Carbapenem antibiotics are commonly used clinically for ESBL-producing *K. pneumoniae.* However, its excessive use has led to significant increases in *K. pneumoniae* resistance rates in recent years. *K. pneumoniae* strains are naturally resistant to ampicillin, carbenicillin, and ticarcillin due to the production of a chromosomal variable sulfhydryl penicillinase (SHV-1)[48].

2.6.3 Morphology of *Proteus mirabilis*

The genus Proteus comprises Gram-negative rod-shaped bacteria belonging to the family Enterobacteriaceae[40]. Proteus mirabilis are Gram-negative, motile, facultatively anaerobic, rod-shaped bacteria. They are widely dispersed in the environment, mainly in water, soil, and the gastrointestinal tract. Humans and animals. Proteus has the ability to transform its rod-shaped vegetative cell into a strongly flagellated elongated cell[45].

*protea*they represent <0.05% of the normal flora of the human intestinal microbiota40. Proteus mirabilis is the most common cause of human infections among all Proteus species. It is an opportunistic pathogen that has caused several human infections of the respiratory tract, gastrointestinal tract, eyes, ears, and skin, among others[40].

2.6.3.2 *Proteus mirabilis* Virulence Factor

Proteus mirabilis expresses several virulence factors that caused infection in the host, such as adhesins, flagella, toxins, quorum sensing, enzymes, and immune invasion[52]. Bacterial attachment to the epithelial surfaces of the urinary tract plays an important role in urinary tract infections. Genomic analysis revealed that a total of 17 potential fimbrial adhesins from *Proteus mirabilis*, only 5 fimbriae were studied. Mannose resistant/Proteus-like (MR/P), Proteus mirabilis fimbriae (PMF), uroepithelial cell adhesion (UCA), room temperature fimbriae (ATF), and P. mirabilis P-type pili (PMP)[52].

2.6.3.3 Enzymes

Urease is an important enzyme in the pathogenesis of *Proteus mirabilis*. The secretion of the enzyme urease in the host has the capacity to form kidney and bladder stones and also to block the urinary tract[44]. The urease gene cluster (ureRDABCEFG) breaks down urea into ammonia and carbon dioxide and increases urine pH. The change in pH can facilitate the adhesion, colonization and biofilm formation of P. mirabilis in the host[49].

2.6.3.4 Toxins

Hemolysin

Hemolysin is a toxin that penetrates host cell membranes and creates pores in the membrane, which damages host cells[46]. This toxin facilitates *Proteus mirabilis* in the kidney and also

causes pyelonephritis in the urinary tract of the host[47]. *P. mirabilis* has two hemolysin genes hpmA and hpmB. HpmA is present in the periplasmic spaces, whereas HpmB is probably present in the outer membrane of the host cell where it is involved in the secretion process of HpmA. However, the virulence of hemolysin is not fully understood[49].

2.6.3.5 Proteus Toxic Agglutinin (PTA)

This toxic protein is active on the outer membrane of the host and facilitates cell-to-cell aggregation. The α domain of the toxic Proteus agglutinin has the ability to lyse kidney and bladder cells. The PTA gene of *P. mirabilis* has a less severe pathogenesis as well as invasion of the bladder, kidneys and colonization of the spleen[54].

2.6.3.6 Immune Evasion

The presence of bacteria in the host cell must have escaped the host's primary and secondary immune responses. Proteus mirabilis codes for ZapA, which breaks the chain of immunoglobulins A1 (IgA1), IgA2 and IgG in the host[42]. Mutation of the zapA genes can enhance the immune system to find Proteus mirabilis in a host cell. This bacterium has the capacity to express different types of fimbriae such as MR/P and flagellin, which increase the adherence of the bacterium to the epithelium of the host cell[53].

2.6.3.7 Swarm Capacity

The swarming behavior of *Proteus mirabilis* is controlled by the rsbA gene. RsbA functions as a sensor protein of environmental conditions against bacteria in difficult and favorable situations[55]. The RsbA gene is also responsible for the formation of biofilms and the formation of extracellular polysaccharides[56].

2.6.3.8 Biofilm Formation

Proteus mirabilis biofilms in the urinary tract can increase the duration of infection and slow antibiotic activity and the immune response[51]. During the attachment phase, the bacteria adhere to the uroepithelial surfaces (urethra) of the host cell, causing an accumulation of ammonia in the uroepithelial cells that becomes toxic and causes direct tissue damage[57]. The formation of *P. mirabilis*-infected stones in the urinary tract can block the flow of urine through the catheter, bladder, or kidneys and then cause serious problems, including the formation of pyelonephritis, sepsis, and shock. Diseases related to biofilm formation include chronic wound infection, endocarditis, urinary tract infection, cystic fibrosis, and periodontitis[61].

2.6.3.9 Serotyping

The important virulence factor of *Proteus mirabilis* is lipopolysaccharide. Lipopolysaccharides illustrate the role of serology in bacteria. *Proteus mirabilis* are grouped into 80 O-serogroups . OPS is acidic in all serogroups O, due to the presence of amino acids, uronic acids, phosphate, altruronic acid and other non-sugar acid components[59]. *Proteus mirabilis* has branched or linear polysaccharides consisting of repeating oligosaccharide units. However, *P. mirabilis* strains belong to different serogroups such as O3, O10, O11, O13, O23, O27 and O30[58].

2.6.4 *P. mirabilis* in Urinary Tract Infections

Proteus mirabilis is one of the microorganisms responsible for urinary tract infections in patients with anatomical or functional problems. This is especially common in patients undergoing long-term indwelling urinary catheterization who may develop catheter-associated urinary tract infections (CUTs). These infections are complicated by the unique

ability of *P. mirabilis* to form crystalline biofilms on uroepithelial cells, eventually leading to catheter encrustation and blockage[56]. Patients may then experience urinary retention and reflux, which are accompanied by painful bladder discharge and pyelonephritis. Fatal complications can then occur, such as sepsis and endotoxic shock[63]. Furthermore, this can lead to rupture of the urethra and bladder lining upon withdrawal of the catheter[62].

2.6.4.2 *Proteus mirabilis* in Wound Infection

Proteus mirabilisi is capable of causing wound infection, particularly in diabetic wounds[60]. *Proteus mirabilis* are found in nosocomial infections. *Proteus mirabilis* causes clinical infections, making it difficult to eliminate bacteria from the host cell in complicated wound infections, underlying diseases, and immune-compromised patients[66].

2.6.4.3 *Proteus mirabilis* in Meningitis

*Proteus mirabilis*it is also implicated in the formation of neonatal sepsis and central nervous system infections. *Proteus mirabilis* is reported to cause approximately 4% of cases of neonatal meningitis[49]. Brain abscess formation and pneumocephalus have also been identified to be associated with Proteus infections[64].

2.6.4.4 Antimicrobial Susceptibility

Proteus mirabilis are sensitive to most first- and second-line antibiotics, such as penicillin, cephalosporins, aminoglycosides, rifamycin, and fluoroquinolones, while they are resistant to amoxicillin, cefotaxime, and carbenicillin[71].

Proteus mirabilis are sensitive to most β-lactam-containing antibiotics because they do not mediate a chromosomally encoded AmpC cephalosporinase[60]. However, Proteus mirabilis

shows resistance against a broad spectrum of β-lactamases and AmpC enzymes when they have acquired β-lactamase genes[68].

Resistance to amoxicillin in *Proteus mirabilis* is mainly due to penicillinases TEM-1 and TEM-2. *Proteus mirabilis* increased the production of extended-spectrum β-lactamases (ESBL). The most predominant enzymes of Proteus mirabilis such as TEM, CTX-M, VEB- and PER are less common[76].

Plasmids, transposons and integrons play an important role in the transfer of antibiotic resistance genes in bacterial species[65]. Resistance to aminoglycoside antibiotics, chloramphenicol, β-lactams, trimethoprim, erythromycin, and rifampicin is due to integron gene transfer. Proteus mirabilis has been shown to be resistant to ampicillin, tetracycline, gentamicin, and kanamycin[73]. This resistance can only occur due to class 1 integrons that carry antibiotic resistance genes, such as aadA1, aadB, and aadA2. Class 2 integrons that are also involved in antibiotic resistance gene transfer include; dhfr1, sat1 and aadA1[49].

2.6.5 *Salmonella typhi*

Salmonella enterica Typhi serovars are facultative anaerobes, and gram-negative rod-shaped flagellates. *Salmonella* strains have evolved to infect a wide variety of reptiles, birds, and mammals, resulting in many different syndromes ranging from colonization and chronic carrier status to acute fatal disease. *Salmonella typhi* cells are rod-shaped, 2 to 3 µm long and 0.4 to 0.6 µm in diameter.[67]

2.6.5.2 Virulence Factor

These bacteria are serologically positive for the lipopolysaccharide antigens O9, O12 and positive for the distinct capsular polysaccharide antigen Vi. Vi-negative antigenic strains

appear to be less infectious and less virulent than Vi-positive antigenic strains. [70] Differences in lipopolysaccharides (LPS) generate antigenic variations, which also affect the virulence of the strains.

2.6.5.3 Typhoid

Salmonella typhi is the etiologic agent of typhoid fever. Typhoid fever is an infectious disease with worldwide distribution[72]. It is a protracted febrile illness and continues to be a health problem in developing countries with poor sanitation, low levels of personal hygiene, and prevalence of contaminated food. It is endemic in many parts of the developing world and, as global travel increases, the disease can and does occur throughout the world in one day[72].

Typhoid fever is prevalent in children and young adults and is associated with low-income areas where poor sanitation thrives. In 2000, it was estimated that typhoid fever caused 21.7 million maladies and 216,000 deaths in the world, and the International Vaccine Institute estimated 11.9 million typhoid fever cases and 129,000 deaths. Millions of decades in the pay for reliable income or intermediaries in 2010. Cependant, these children are more than likely one of their representatives of the veritable charge of morbidity is that a large proportion of patients are treated in outpatients or do not recognize any treatment No way. In the United States, approximately 200 to 300 cases of *Salmonella enterica* serotype Typhi are reported each year and about 80% of these cases come from travelers returning from an endemic region. In the era before antibiotics.

2.6.5.4 Transmission Mode

Salmonella enterica serotype Typhi is usually contracted by ingesting food or water contaminated with the bacteria by human carriers. The bacterium must resist the gastric pH of the host before adhering to the stomach wall in the small intestine. An infective dose of *Salmonella enterica* serotype typhi in a healthy individual ranges from 1,000 to 1 million organisms, compromising host immune defenses[68].

2.6.5.5 Pathogenesis

Salmonella enteric serotype Typhi enters the submucosal region of the small intestine by direct penetration into epithelial tissue mediated by the cystic fibrosis transmembrane conductance regulator (CFTR) or through the M cell, a specialized lymphoid epithelial cell. Once in the submucosa, the bacteria cause hypertrophy of Peyer's patches[68].

The spread of the organism from Peyer's patches is through the lymphatic system and the bloodstream. Cell replication within the reticuloendothelial system is a hallmark of the disease and ultimately causes the systemic symptoms that a physician will observe. After replication, the organisms will reside in macrophages in the liver, spleen, and bone marrow. *Salmonella enterica* serotype typhi can usually be cultured from bone marrow even after initiation of antimicrobial therapy[68].

2.6.5.6 Multidrug Resistant of Typhimurium

In recent years, the rate of antibiotic resistance in *S. typhimurium* has increased, becoming a growing global problem that could lead to more serious health problems[74]. Some studies have shown that the proportion of multiresistant bacteria in *S. typhimurium* is very high and the most frequently observed antibiotic resistance profiles are ASSuT (ampicillin, streptomycin,

sulfonamides and tetracycline) and ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline) tetra-resistant and penta-resistant regimens, respectively[75]. [76]Multi-resistant *S. typhimurium* has caused difficulties in clinical management, leading to increased morbidity and mortality. Outbreaks of *S. typhimurium* have occurred throughout the world.

2.6.6 *Staphylococcus aureus*

Staphylococcus aureus, also known as "*Staphylococcus aureus*", is a Gram-positive cocci belonging to the Bacilli class, Bacillales order, *Staphylococcaceae* family, and *Staphylococcus* genus[78]. It is a facultative anaerobe, often positive for catalase and nitrate reduction, and its coagulase is variable, that is, possibly cogulase positive or negative[79]. The bacterium is nonmotile, does not form spores, and appears like a bunch of grapes under the microscope. On blood agar, large, round, golden yellow colonies with a diameter of 1∼2 mm often with hemolysis[80].

2.6.6.2 Epidemiology

Staphylococcus aureus is known to be an important and versatile pathogen causing a wide range of clinical manifestations, including skin and soft tissue infections, pneumonia, sepsis, pleuropulmonary, osteomyelitis, endocarditis, arthritis, gastroenteritis, meningitis, urinary tract infections, urinary tract infections device- and toxin-related (such as food poisoning, septic shock, scalded skin syndrome, and toxic shock syndrome)[81].

Staphylococcus species are present all over the world. *S. aureus* is a ubiquitous organism that colonizes the upper respiratory, gastrointestinal, and urogenital tracts of approximately 20-

30% of humans, acting as long-term carriers[82]. However, certain groups of people are at increased risk of *S. aureus* colonization (up to 80%), including health care workers, people with diabetes, patients receiving intravenous medication, people with low immunity[83], hospitalized patients with surgical operations recipients, users of indwelling catheters, dialysis patients, people with chronic metabolic diseases - vulnerable people, subjects who have already been infected with methicillin-resistant *S.aureus* (MRSA) and people suffering from skin infections[26]. Carriers are sources of infection; Transmission of *S. aureus* can occur from person to person through close or direct contact, sharing of personal items, food contamination, and fomite contamination such as doorknobs[83]. Colonization by *S. aureus* in different parts of the body increases the risk of surgical site infection, as well as lower respiratory tract and bloodstream infections in the hospital[11]. These infections increase in health establishments because the microorganism adapts quickly and efficiently to the hospital environment[84]. For this reason, various measures are taken in hospitals to manage and reduce colonization and, subsequently, eliminate infections. Examples of these strategies include the use of different disinfectants, the local application of antibiotics (such as mupirocin), and the use of systemic antibiotics[20]. Some hospitals deliberately colonize the nasal passages of patients with the harmless 502A strain of *S. aureus* to reduce the colonization density of *S. aureus* through competition[86].

2.6.6.3 Emergence of MRSA

Overuse and addiction to antibiotics has led to the emergence of multidrug-resistant microorganisms (MDRM), such as methicillin-resistant *Staphylococcus aureus*, due to collection of the mecA86 resistance gene[85]. The discovery of antibiotics began with penicillin

in 1928 by Sir Alexander Fleming. The subsequent production and clinical use of pure penicillin to treat infections caused by *S. aureus* has been achieved[77].

The first case of penicillin-resistant *S. aureus* was reported later that year[90]. A decade after reporting penicillin resistance in *S. aureus*, most cases have been confined to hospital settings. The mechanism of resistance to penicillin by beta-lactam penicillinase was elucidated in 1981, followed by the identification of the mecA90 resistance gene. The clinical use of methicillin, a derivative of penicillin, was introduced in 1961 to eliminate the enzymatic degradation of penicillinase; its efficacy lasted less than a year before methicillin resistance was reported in *S. aureus*[90].

The incidence of MRSA increased worldwide over the next decade in the form of hospital-acquired MRSA (HA-MRSA), particularly in Europe[90]. In addition, the management of MRSA-associated infections has become more difficult due to the emergence of multidrug-resistant strains of *S. aureus* which have developed resistance to a variety of antibiotics such as methicillin, cephalosporins, nafcillin, and oxacillin due to its production of penicillin-binding protein (PBP)-2a[91]. Therefore, treatment options for life-threatening infections caused by multidrug-resistant strains of staphylococci were limited[88]. Thus, *S. aureus* was placed at the top of the list of bacteria-resistant microorganisms by the World Health Organization (WHO) in 2017[89].

2.6.7 *Enterobacter cloacae* Morphology and Taxonomy

Enterobacter cloacae belongs to the genus *Enterobacter*. It is now divided into two subspecies: *E. cloacae* subsp. *cloacae*, which was negative for esculin, and *E. cloacae* subsp. dissolves, with a negative reaction to esculin[93]. *Enterobacter cloacae* bacteria are nonmotile, gram-negative, facultative anaerobic rods belonging to the family *Enterobacteriaceae. E.*

cloacae are a flora of the human respiratory and gastrointestinal tract, but it can be an opportunistic pathogen in nosocomial infections, particularly in newborns, immune-compromised patients and hospitalized in intensive care units[93]. Reservoirs of *E. cloacae* infection in hospitals are thought to include equipment, cleaning solutions, and healthcare workers[93]. Also, *Enterobacter cloacae*they are associated with contamination of blood products, intravenous fluids, catheters, catheters, respiratory therapy equipment, and the colonized hands of healthcare workers[92]. Invasive procedures, such as catheterization and intubation, and prolonged hospital stay, frequently found in an intensive care unit (ICU), represent a major source of Enterobacter infection[97].

2.6.7.2 Virulence Factor

Some pathogenicity factors have been identified as hemolytic and leukotoxic membrane cell cytotoxins[94]. Regarding epidemiological spread, several studies have confirmed that *E.cloacae* colonization's/infections correspond to the spread of several clusters corresponding to known main multilocus sequence types and that there is no relationship with geographic origin[96].

2.6.7.3 Antibiotic Resistance

Antibiotic resistance is a growing problem in the treatment of Enterobacter infections. *Enterobacter species* (spp.) have multidrug resistance through loss of porin, activation of the efflux system, AmpC cephalosporinase, and metallo-beta-lactamase enzyme systems[87]. The main mechanism of antimicrobial resistance in Enterobacter species is the presence of beta-lactamases. They can hydrolyze the beta-lactam ring of penicillins and cephalosporins[87]. Carbapenems should be the most effective agent for the treatment of multidrug-resistant

Enterobacteriaceae infections[95]. However, species of carebapenem-resistant *Enterobacter* (CRE) and extended-spectrum betalactamase (ESBL) have been recorded to cause serious nosocomial outbreaks in different countries with a high mortality rate[100].

Enterobacter cloaca is naturally resistant to ampicillin, amoxicillin-clavulanic acid, cephalothin and cefoxitin due to low production of the natural inducible Bush group 1 cephalosporinase (class C). Ureidopenicillins and carboxypenicillins are active in at least half of the strains[96]. In the chromosomal cephalosporinase AmpC, depression and constitutive production by mutation can generate resistance to a large number of beta-lactams, especially third-generation cephalosporins, with the exception of cefepime[101]. This AmpC-type resistance represents 50% of the resistance to beta-lactams in clinical strains and frequently coexists with that due to the expression of extended-spectrum beta-lactamases (ESBLs)[96].

2.7 Antibiotic Review

2.7.1 Chloramphenicol

Chloramphenicol is an antibiotic first isolated from cultures of *Streptomyces venezuelae* in 1947, but now produced synthetically[98]. It has a relatively simple structure and was the first broad-spectrum antibiotic to be discovered. It works by interfering with bacterial protein synthesis and is primarily bacteriostatic. Chloramphenicol is an antibacterial of the amphenicol class. The chemical classification of chloramphenicol is Amfenicoles[104].

Chloramphenicol diffuses through the bacterial cell wall and reversibly binds to the bacterial 50S ribosome[99]. The bond interferes with the activity of peptidyltransferase, which prevents the transfer of amino acids to the growing peptide chain and thus blocks the formation of peptide bonds. As a result, the synthesis of bacterial proteins is blocked, which prevents the proliferation of bacterial cells[105].

2.7.2 Nitrofurantoin

Nitrofuratoin is a synthetic derivative of imidazolidinedione. Nitrofurantoin inhibits bacterial synthesis of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) and cell wall proteins. It increases bacterial resistance to antibiotics against other commonly used agents, such as fluoroquinolones and trimethoprim/sulfamethoxazole, which has generated increased interest in the use of nitrofurantoin. It is activated by bacterial flavoproteins that inactivate bacterial ribosomal proteins. Nitrofurantoin is used prophylactically as a urinary anti-infective agent against most gram-positive and gram-negative bacteria and for long-term suppression of infections[106].

Nitrofuratoin is an antibiotic used to prevent and treat urinary tract infections caused by susceptible strains of *Escherichia coli, Staphylococcus aureus, Enterococcus, Klebsiella,* and *Enterobacter*[107].

2.7.3 Tetracycline

Tetracycline is a broad-spectrum naphthacene antibiotic produced semi-synthetically from chlortetracycline, an antibiotic isolated from the bacterium *Streptomyces aureofaciens*. In bacteria, thetracycline binds to the 30s ribosomal subunit, interfering with the binding of aminoacyl-tRNA to the mRNA-ribosome complex, thus inhibiting protein synthesis. Tetracycline is an antibiotic used to treat a number of bacterial infections. It is commonly used to treat acne and rosacea. Historically, it has played an important role in reducing the number of deaths from cholera[108].

Tetracyclines have a broad spectrum of antibiotic action. They originally possessed some level of bacteriostatic activity against almost all genera of medically relevant aerobic and anaerobic bacteria, both gram-positive and gram-negative, with a few exceptions, such as Pseudomonas aeruginosa and *Proteus spp*., which exhibit intrinsic resistance. However, acquired resistance (as opposed to inherent resistance) has proliferated in many pathogenic organisms and has significantly eroded the once great versatility of this group of antibiotics. Tetracyclines remain particularly useful in the management of infections by certain obligately intracellular pathogenic bacteria such as Chlamydia, Mycoplasma, and Rickettsia[109].

2.7.4 Amoxicillin/Clavulanic

Amoxicillin/clavulanic acid or co-amoxiclav is a useful antibiotic for the treatment of various bacterial infections. It is a combination antibiotic composed of amoxicillin trihydrate, a β-lactam antibiotic, and potassium clavulanate, a β-lactamase inhibitor. This combination results in an antibiotic with a broader spectrum of action and restored efficacy against amoxicillin-resistant bacteria that produce β-lactamase[110].

Amoxicillin/clavulanic acid is widely used to treat or prevent many infections caused by susceptible bacteria, such as urinary tract infections, respiratory tract infections, skin and soft tissue infections, sinus infections, cat scratch, and cat scratch infections caused by bacterial flora in the mouth[111].

2.7.5 Cotrimoxazole (Septrin)

Cotrimoxazole (Septrin) is an approved antibiotic that consists of a mixture of two drugs. Both drugs prevent bacteria from reproducing by preventing the production of folic acid (vitamin B9). It is used to treat bacterial infections from the simplest to the most advanced[112].

The bacteria that are often killed by co-trimoxazole depend on dihydropteroic acid and tetrahydrofoil acid to produce folic acid for their survival. Folic acid is used by bacteria to create genetic material. Sulfamethoxazole is similar to para-aminobenzoic acid in that it prevents the production of dihydropteroic acid. Trimethoprim decreases the production of the bacterial enzyme dihydrofolate reductase which inhibits tetrahydrofolic acid.

Co-trimoxazole is prescribed for pneumocystic pneumonia, chronic bronchitis, urinary tract infection, acute ear infections in children, skin or wound infections, gastrointestinal tract infections, shigellosis, diarrhea of the traveler. Whipple's disease, cerebral toxoplasmosis in patients with HIV and melioidosis[113].

2.7.6 Nalidixic Acid

Nalidixic acid is the first of the synthetic quinolone antibiotics. In a technical sense, it is a naphthyridone and not a quinolone[114].

Nalidixic acid is effective primarily against gram-negative bacteria with less activity against gram-positive bacteria at lower concentrations. It acts bacteriostatically by inhibiting bacterial growth and reproduction. At higher concentrations, it is bactericidal[115]. Historically it has been used to treat urinary tract infections caused by *Escherichia coli* and species of *Proteus, Shigella, Enterobacter* and *Klebsiella*[116]. It selectively and reversibly blocks DNA gyrase and topoisomerase IV and induces the formation of cleavage complexes. It also inhibits the cleavage-closing activity at the bottom of DNA gyrase, which releases positive binding tension in supercoiled DNA[117].

*Aeromonashydrophila*and *Clostridium* and *Haemophilusspecies* are generally sensitive to nalidixic acid, while other bacteria such as Bifidobacteria, *Lactobacillus, Pseudomonas* and *Staphylococcus species* are resistant.

2.7.7 Ofloxacin

Ofloxacin is an antibiotic used to treat bacterial infections. It belongs to the fluoroquinoloneclass of antibiotics that includes levofloxacin, ciprofloxacin, gatifloxacin, norfloxacin, moxifloxacin, trovafloxacin, and others. Ofloxacin stops the multiplication of bacteria by inhibiting the reproduction and repair of their genetic material (DNA) [118].

Ofloxacin is used to treat pneumonia and bronchitis caused by Haemophilus influenza and *Streptococcus pneumoniae*. It is also used to treat skin infections caused by the bacteria *Staphylococcus aureus* and *Streptococcus pyogenes*. Ofloxacin is also used to treat sexually transmitted diseases such as gonorrhea and chlamydia, but it is not effective against syphilis. Ofloxacin is often used to treat urinary tract infections, cystitis, pelvic inflammatory disease, and prostate infections caused by *E. coli*. Certain strains of *Streptococcus*, *Enterococcus* and anaerobic bacteria are known to be resistant to ofloxacin[119].

2.7.8 Streptomycin

Streptomycin is an antibiotic, the first in a class of drugs called aminoglycosides to be discovered, and it was the first effective treatment for tuberculosis. It is derived from Actinobacterium Streptomyces griseus. Streptomycin is a bactericidal antibiotic[120].

It works by binding to the 30S ribosomal subunit of susceptible organisms and interrupting the initiation and elongation steps of protein synthesis. It is bactericidal due to effects that are not completely known[121].

2.7.9 Gentamicin

Gentamicin is a complex of closely related aminoglycosides obtained from Micromonospora purpurea and related species[122]. These are broad-spectrum antibiotics, but have been found to cause ear and kidney damage. They act by inhibiting protein biosynthesis[123].

It is active against a wide range of bacterial infections, primarily Gram-negative bacteria, including Gram-positive Pseudomonas, *Proteus*, *Escherichia coli*, *Klebsiella pneumoniae,* Enterobateraerogenes, Serratia, and Staphylococcus. Gentamicin is also used in the treatment of respiratory tract infections, urinary tract infections, blood, bone, and soft tissue infections caused by these susceptible bacteria. However, gentamicin is not used for bacterial infections with *Neisseria gonorrhoeae*, *Neisseria meningtidis*, or *Legionella pneumophilia* because of the risk of shock from the lipid A endotoxin present in some gram-negative organisms. Gentamicin is also useful against *Yersinia pestis* certain *Enterobacteriaceae*[124].

2.7.10 Amoxicillin

Anhydrous amoxicillin is the anhydrous form of a broad-spectrum, semi-synthetic aminopenicillin antibiotic with bactericidal activity. Amoxicillin binds to and inactivates penicillin-binding proteins (PBPs) located on the inner membrane of the bacterial cell wall. Inactivation of PBPs interferes with the crosslinking of peptidoglycan chains necessary for the strength and rigidity of the bacterial cell wall. This disrupts bacterial cell wall synthesis and results in bacterial cell wall weakening and cell lysis.

Amoxicillin, also called amoxicillin or amox, is an antibiotic useful for treating a number of bacterial infections. It is the first line treatment for middle ear infections. It can also be used to treat pneumonia, skin and urinary tract infections, among others. Amoxicillin is also used in the treatment of streptococcal pharyngitis and Salmonella infection[126].

2.7.11 **Erythromycin**

Erythromycin belongs to a group of medicines called macrolide antibiotics. Macrolide antibiotics slow the growth of susceptible bacteria, or sometimes kill them, by reducing the production of important proteins needed for the bacteria to survive. Erythromycin is an antibiotic used to treat certain infections caused by bacteria, including bronchitis, diphtheria, Legionnaires' disease, whooping cough (whooping cough), pneumonia, rheumatic fever; venereal diseases (VD), ear, intestine, lung, urinary tract and skin infections. It is also used before surgery or dental work to prevent infection. Antibiotics don't work for colds, the flu, or other viral infections.

2.7.12 Cephalexin

Cephalexin is an antibiotic that can treat a number of bacterial infections. It kills gram-positive bacteria and some gram-negative bacteria by disrupting the growth of the bacterial cell wall. Cefalexin is a beta-lactam antibiotic of the first-generation cephalosporin class. It works the same as other agents in its class, including intravenous cefazolin, but can be taken orally. Cephalexin can treat certain bacterial infections, including those of the middle ear, bones and joints, skin, and urinary tract. It can also be used for certain types of pneumonia, strep throat, and to prevent bacterial endocarditis Cephalexin is not effective against infections caused by methicillin resistant *Staphylococcus aureus* (MRSA) *Enterococcus* or *Pseudomonas*[128].

Like other antibiotics, cephalexin cannot treat viral infections, such as the flu, colds, or acute bronchitis. Cephalexin can be used in people with a mild to moderate allergy to penicillin. However, it is not recommended for people with severe allergies to penicillin. Common side

effects include stomach upset and diarrhea. An allergic reaction and Clostridium difficile infection, a type of diarrhea, are also possible.

2.7.13 Ampicillin

Ampicillin is a broad-spectrum beta-lactam semi-synthetic penicillin antibiotic with bactericidal activity. Ampicillin binds to and inactivates penicillin-binding proteins (PBPs) located on the inner membrane of the bacterial cell wall. The inactivation of PBPs interferes with the crosslinking of peptiodoglycan chains necessary for the strength and rigidity of the bacterial cell wall[125]. This disrupts the synthesis of the bacterial cell wall and results in the weakening of the bacterial cell wall and causes cell lysis. Ampicillin is stable against hydrolysis by a variety of beta-lactamases, so it can be used in a wide variety of gram-positive and gram-negative infections, meningitis, Salmonella, and endocarditis. It can also be used to prevent group B strep infections in newborns. Common side effects include rash, nausea, and diarrhea. It should not be used in people allergic to penicillin. Serious side effects may include Clostridium difficile colitis or anaphylaxis. Although it can be used in people with kidney problems, the dose may need to be reduced[128].

2.7.14 Cefuroxime

Cefuroxime is a semi-synthetic cephalosporin antibiotic, chemically similar to penicillin. Cephalosporins stop or slow the growth of bacterial cells by preventing bacteria from forming the cell wall that surrounds each cell[131]. The cell wall protects the bacteria from the outside environment and holds the cell contents together. Without a cell wall, bacteria cannot survive. Cefuroxime is effective against a wide variety of bacteria including *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenza, Escherichia coli, Neisseria*

gonorrhoeae, and many others. Cefuroxime is effective against susceptible bacteria that cause middle ear infections (otitis media), tonsillitis, throat infections, laryngitis, bronchitis, sinusitis, and pneumonia[127]. It is also used to treat urinary tract infections, skin infections, and gonorrhea. Furthermore, it is useful in the treatment of acute bacterial bronchitis in patients with chronic obstructive pulmonary disease (COPD). Lyme disease and impetigo are also treated with cefuroxime[129].

Cefuroxime is generally well tolerated, and its side effects are usually transient[132]. If taken after a meal, this antibiotic is better absorbed and less likely to cause the more common side effects of diarrhea, nausea, vomiting, headache/migraine, dizziness, and abdominal pain, compared to most antibiotics of his class[130]

Endnotes

[1] K. Antri, M. Akkou, & C. Bouchiat. *High Levels of Staphylococcus aureus and MRSA Carriage in Healthy Population of Algiers Revealed by Additional Enrichment and Multisite Screening*. **Eur J Clin Microbiol Infect Dis**.37 (8):2018, 1521–1529. doi:10.1007/s10096-018-3279-6

[2] T. Kazimoto, S. Abdulla, & L. Bategereza. *Causative Agents and Antimicrobial Resistance Patterns of Human Skin and Soft Tissue Infections in Bagamoyo, Tanzania.* **Acta Trop.** 186:2018, 102–106. doi:10.1016/j.actatropica.2018.07.007

[3] H. Deguchi, K. Kitazawa, & K. Kayukawa. *The trend of resistance to antibiotics for ocular Infection of Staphylococcus aureus, Coagulase-Negative Staphylococci, and Corynebacterium Compared with 10-years Previous: A Retrospective Observational Study.* **PLoS One.** 13(9):2018, e0203705. doi:10.1371/journal.pone.0203705

[4] A. A. Olajide, D. M. Goshen, & C. Blessing. *Antimicrobial Activity Of Chitosan, Moringa Oleifera, Croton Zambesicus And Their Synergistic Activity As Immune Booster.***Int. J. Adv. Res**. 9(10), 2021, 495-502

[5] Y. Belyhun, F. Moges, & M. Endris. *Ocular Bacterial Infections And Antibiotic Resistance Patterns in Patients Attending Gondar Teaching Hospital, Northwest Ethiopia***. BMC Res Notes.** 11:2018, 597. doi:10.1186/s13104-018-3705-y

[6] F.F. Boyom, F. Keumdjio, P.M. Dongmo, B.T. Ngadjui, P.H. Amvam-Zollo, C. Menut & J.M. Bessiere.*Essential oil from Croton zambesicus Muell. Arg. growing in Cameroun.***Flavour and Fragrance Journal,** 17: 2022, 215-217.

[7] F. Keihanian, A. Saeidinia, K. Abbasi, & F. Keihanian. *Epidemiology of Antibiotic Resistance of Blood Culture in Educational Hospitals in Rasht, North of Iran.* **Infect Drug Resist.** 11:2018, 1723–1728. doi:10.2147/IDR.S169176

[8] N.S. Yadav, S. Sharma, & D.K. Chaudhary. *Bacteriological Profile of Neonatal Sepsis and Antibiotic Susceptibility Pattern of Isolates Admitted at Kanti Children's Hospital, Kathmandu, Nepal.* **BMC Res Notes**. 11:2018, 301. doi:10.1186/s13104-018-3394-6

[9] A. Banso & S.O. Adeyemo. *Phytochemical and antimicrobial evaluation of ethanolic extract of Dra-caena manni Bark.***Nig. J. Biotech.** 18: 2018, (1-2):

[10]A.O. Ajayi & I.E. Omomagiowawi. *Antimicrobial Activity of Croton zambesicus on Staphylococcus aureus and Streptococcus s*pecies. *Sylwan*., 160: 2018, (10)

[11] J.C. Onwusonye, I.K. Muritala, D.A. Odu, N.I. Uzomba., M.I. gbonnaya & C.C. Duruamaku.*Oral acute toxicity study of methanol leaf extracts of Croton Zambesicus in mice*.

Merit Research Journal of Pharmacy and Pharmaceutical Sciences Vol. 2(1) 2019, pp. 001-005.

[12] J.E. Okokon & P.A. Nwafor. *Antiplasmodial Activity of Root extract and Fractions of Croton zambesicus*. **J. Ethnopharm**. 121: 2019; 74-78.

[13] J.E. Okokon, & P.A. Nwafor. *Antiulcer and anticonvulsant activities of Croton zambesicus root extract*. **Pak J Pharmaceut Sci.** 22: 2019; 384- 390.

[14] N. Buetti, E. Lo-Priore, & A. Atkinson. *Low Incidence of Subsequent Bacteraemia or Fungaemia after Removal of a Colonized Intravascular Catheter Tip.* **Clin Microbiol Infect.** 24:2018, 548e1–548e3. doi:10.1016/j.cmi.2017.09.009

[15] J.E. Okokon, P.A. Nwafor, E. Ekpo, P. Okokon & J. Udobang. *Antidiabetic and hypolipidemic activities of ethanolic root extract of Croton zambesicus on alloxan induced diabetic rats.***Asian J Pharmaceut Res.** 1: 2021; 493 – 499.

[16] J.E. Okokon, P.A. Nwafor & K. Noah. *Nephroprotective effect of Croton zambesicus root extract against gentimicin-induced kidney injury.***Asian Pac J Trop Med.** 4: 2021; 969-972.

[17] J.E. Okokon, A. Dar & M.I. Choudhary. *Immunomodulatory, Cytotoxic and Antileishmanial activities of Phytoconstituents of Croton zambesicus Phytopharmacol.* **J. Ethnopharm** 4(1): 2018; 31 – 40.

[18] F.F. Boyom, F. Keumdjio, P.M. Dongmo,B.T. Ngadjui, P.H. Amvam-Zollo, C. Menut C, & J.M. Bessiere.*Essential oil from Croton zambesicus Muell. Arg. growing in Cameroun.* **Flav Fragr J.** 17: 2022; 215– 217.

[19] J.E. Okokon, I.L. Augustine, E. Bassey,E. Nyong A. & Utibe. *Edem. Croton zambesicusRoot Extract Exert Laxative Effect in Rats*. **Asian Journal of Biology.** 10(2): 2020; 22-26, Article no AJOB.61412 ISSN: 2456-7124

[20] M. Walaszek, A. Różańska, M.Z. Walaszek, & J. Wójkowska-Mach. *The Polish society of Hospital Infections Team. Epidemiology of Ventilator-Associated Pneumonia, Microbiological Diagnostics and the Length of Antimicrobial Treatment in the Polish Intensive Care Units in the years 2013–2015*. **BMC Infect Dis**. 18:2018, 308. doi:10.1186/s12879-018-3109-6

[21] C. Baccelli, A. Martinsen, N. Morel, & J. Quetin-Leclercq.*Vasorelaxant activity of essential oils from Croton zambesicus and some of their constituents.* **Planta Med.** 76(14): 2020; 1506-11.

[22] A. Martinsen, B. Chiar, N. Ismae, A. Antonio, J. Quetin-Leclercq, & M. Nicole.*Vascular activity of a natural diterpene isolated from Croton zambesicus and of a structurally similar synthetic trachylobane*. **Vasc Pharmacol**. 52(1/2): 2020; 63-69.

[23] Y. Huang, Y. Jiao, & J. Zhang. *Microbial Etiology and Prognostic Factors of Ventilator-Associated Pneumonia: A Multicenter Retrospective Study in Shanghai.* **Clin Infect Dis.** z7(S2): 2018, S146–S152. doi:10.1093/cid/ciy686

[24] N. M. N Abdalaziz, A. Amna & A. S. Kabbashi. *In vitro antioxidant activity and phytochemical screening of Croton zambesicus.* **Journal of Pharmacognosy and Phytochemistry;** 5(6): 2020, 12-16

[25] A. Bonell, R. Azarrafiy, & V.T. Huong, *A Systematic Review and Meta-Analysis of Ventilator-Associated Pneumonia In Adults in Asia: An Analysis of National Income Level on Incidence and Etiology.* **Clin Infect Dis.** 68(3):2018, 511–518. doi:10.1093/cid/ciy543

[26] S. Dharmananda. *Gallnuts and the uses of tannins in Chinese medicine.* **A Paper Delivered at the Institute for Traditional Medicine, Portland, Oregon**, 2020.

[27] O. Aiyelaagbe, & P.M. Osamudiamen.*Phytochemical Screening for Active Compounds in Mangifera indica Leaves from Ibadan, Oyo State,***Plant Sciences Research.** 1(2): 2019; 11-13

[28] P.M. Denwick. *Natural Products A Biosynthetic Approach.* 2nd Edn. John Wiley and Sons, Ltd., England. **Journal of University of Anbar for Pure Science** 2022, 241243.

[29] Q.R. Ducarmon, R.D. Zwittink, B.V.H. Hornung, W. Van-Schaik, V.B. Young, & E.J. Kuijper.*Gut Microbiota and Colonization Resistance against Bacterial Enteric Infection*. **Microbiol. Mol. Biol. Rev.** 83 2019, (3), e00007–e00019. doi: 10.1128/MMBR.00007-19

[30] B.C. Lustri, V. Sperandio, & C.G. Moreira. *Bacterial Chat: Intestinal Metabolites and Signals in Host-Microbiota-Pathogen Interactions*. Infec. Immun. **Journal of University of Anbar for Pure Science** 85: 2017, (12), e00476. doi: 10.1128/IAI.00476-17

[31] E. Barrios-Villa, G. Cortés-Cortés, P. Lozano-Zaraın, A. Arenas-Herna ndez, M., Martınez de la C.F. Peña & Y. Mart ınez-Laguna. *Adherent/ invasive Escherichia coli (AIEC) Isolates from Asymptomatic People: New E. coli* ST131 O25:H4/H30-Rx virotypes. **J. Ann. Clin. Mic. Antim**. 17: 2018, (1), 42. doi: 10.1186/s12941-018-0295-4

[32] W. Jan, H. Sebastian, & Q. Chantal. *Healthcare-Associated Pneumonia in Acute Care Hospitals in European Union/European Economic Area Countries: An Analysis of Data from a Point Prevalence Survey, 2011 to 2012.* **Euro Surveill.** 23 (32):2018, 1700843.

[33]B. V. Santos, K. Melchior & C. G. Moreira. *Escherichia coli as a multifaceted Pathogenic and Versatile Bacterium*. FCIMB. **J. Antimicrob. Chemother.** Vol 10, 2020

[34] B. Pawłowska, & B.M. Sobieszczańska. *Intestinal Epithelial Barrier: The target for pathogenic Escherichia coli. Adv. Clin. Exp. Med*. 26 (9), 2017, 1437–1445. doi: 10.17219/acem/64883

[35] M. Day, M. Doumith, C. Jenkins, T.J. Dallman, K.L. Hopkins, & R. Elson, .*Antimicrobial Resistance in Shiga Toxin-producing Escherichia coli Serogroups O157 and O26 Isolated from Human Cases of Diarrhoeal Disease in Englan*. **J. Antimicrob. Chemother.** 72(1): 2017, 145–152. doi: 10.1093/ jac/dkw371

[36] D.J. Ingle, M.M. Levine, K.L. Kotloff, K.E. Holt, & R.M. Robins-Browne. *Dynamics of Antimicrobial Resistance in Intestinal Escherichia coli from Children in Community Settings in South Asia and Sub-Saharan Africa.* **Nat. Microbiol.** 3 (9): 2018, 1063–1073. doi: 10.1038/s41564-018-0217

[37] D.V. Rakitina, A.I. Manolov, A.V. Kanygina, S.K. Garushyants, J.P. Baikova, & D. G. Alexeev, *Genome Analysis of E. coli Isolated from Crohn's Disease Patients.* **BMC Genomics,** 18 (1): 2017, 544. doi: 10.1186/s12864-017-3917-x

[38] D. Nagarjuna, G. Mittal, R.S. Dhanda, R. Gaind, & M. Yadav. *Alarming Levels of Antimicrobial Resistance among Sepsis Patients Admitted to ICU in a Tertiary Care Hospital in India - A Case Control Retrospective Study.* **Antimicrob. Resist. Infect. Control** 7: 2018, 150. doi: 10.1186/s13756-018-0444-8

[39] T. J. Saif & A. S. Farhan. *Klebsiella Pneumonia: Epidemiology, Virulence Factors and Treatment.* **Journal of University of Anbar for Pure Science** (JUAPS). 14 (2): 2020, 5 – 10

[40] S. Navon-Venezia, K. Kondratyeva, & A. Carattoli. *Klebsiella pneumoniae: A Major Worldwide Source and Shuttle for Antibiotic Resistance*. **FEMS Microbiol Rev.** 41(3): 2017; 252–75.

[41]C.Y. Efah, T. Sun, S. Liu & Y. Wu. *Klebsiella pneumoniae: An Increasing Threat to public Health*. **Annals of Clinical Microbiology and Antimicrobials.** 19: 2020, 1

[42] D.X. Gu, Y.L. Huang, J.H. Ma, H.W. Zhou, Y. Fang, J.C. Cai, Y.Y. Hu, & R. Zhang.*Detection of Colistin Resistance Gene mcr-1 in Hypervirulent Klebsiella pneumoniae and Escherichia coli Isolates from an Infant with Diarrhea in China.* **Antimicrob Agents Chemother.** 60(8): 2019; 5099–100

[43] B. Yao, X. Xiao, F. Wang, L. Zhou, X. Zhang & J. Zhang. *Clinical and Molecular Characteristics of Multi-clone Carbapenem-Resistant Hypervirulent (Hypermucoviscous) Klebsiella pneumoniae Isolates in a Tertiary Hospital in Beijing, China.* **Int J Infect Dis**. 1(37): 2019; 107–12.

[44] R.H. El-Sokkary, R.A. Ramadan, & M. El-Shabrawy. *Community Acquired Pneumonia among Adult Patients at an Egyptian University Hospital: Bacterial Etiology, Susceptibility Profile and Evaluation of the Response to Initial Empiric Antibiotic Therapy*. **Infect Drug Resist**.11:2018, 2141–2150. doi:10.2147/IDR.S182777

[45]China Antimicrobial Resistance Surveillance System (CARSS). *Antimicrobial Resistance of Bacteria: Surveillance Report from China Antimicrobial Resistance Surveillance System in* 2014–2019. **Chin J Infect Control** 20: 2021; 15–31. (in Chinese).

[46] Y. Chong, S. Shimoda & N. Shimono N. *Current Epidemiology, Genetic Evolution and Clinical Impact of Extended-Spectrum b-lactamase-producing Escherichia coli and Klebsiella pneumoniae*. **Infect Genet Evol** 61: 2018; 185–188.

[47]Z. Lin, J. Yu, S. Liu & M. Zhu. *Prevalence and Antibiotic Resistance of Klebsiella pneumoniae in a Tertiary Hospital in Hangzhou, China, 2006–2020* **Journal of International Medical Research,** 50(2): 2020, 1–10

[48]U. Zafar, M. T. Kamran, & I. Nawaz, *Proteus mirabilis as a Pathogenic Organism.* **International Journal of Biosciences** | IJB | Vol. 14, No. 3, 2019, p. 443-450.

[49]R. Wasfi, M. H. Samira, A.A. Mai & L. I. Fahmy. *Proteus mirabilis Biofilm: Development and Therapeutic Strategies.***Frontier in Cellular and Infection Microbiology**. Vol 10. 2020, Art 414

[50]D. Drzewiecka. *Significance and Roles of Proteus spp. Bacteria in Natural Environments.* **Microb. Ecol**. 72: 2019, 741–758. doi: 10.1007/s00248-015-0720-6

[51]U.Zafar, M. K. Taj, & I. Nawaz, *Proteus mirabilis as a Pathogenic Organism.* **International Journal of Biosciences** | IJB | Vol. 14, No. 3, 2019: p. 443-450.

[52] L. Tian, Z. Sun, & Z. Zhang. *Antimicrobial Resistance of Pathogens Causing Nosocomial Bloodstream Infection in Hubei Province, China, from 2014 to 2016: a Multicenter Retrospective Study.* **BMC Public Health**. 18:2018, 1121. doi:10.1186/s12889-018-6013-5

[53] P. Alamuri, & H.L.T. Mobley. *A Novel Autotransporter of Uropathogenic Proteus mirabilis is both a Cytotoxin and an Agglutinin.* **J. Molecular Microbiology** 68: 2018, 997–1017. http://dx.doi.org/10.1111/j.1365-2958.2008.06199.x

[54] W.W. Lim, P. Wu, & H.S. Bond. *Determinants of MRSA Prevalence in the Asia Pacific Region: a Systematic Review and Meta-Analysis.* **J Global Antimicrobial Resist.** 16:2018, 17–27. doi:10.1016/j.jgar.2018.08.014

[55] S.J. Liaw, H.C. Lai, & W.B. Wang. *Modulation of Swarming and Virulence by Fatty Acids through the RsbA Protein in Proteus mirabilis Infection and Immunity*. 72(12): 2019, 6836–6845. http://dx.doi.org/10.1128/IAI.72.12.6836-6845.2004

[56] M.S. Sara. *Importance of Biofilms in Urinary Tract Infections: New Therapeutic Approaches.* **Advance Human Biology**, 2019, 1–13. http://dx.doi.org/10.1155/2014/543974.

[57] W. Kaca, J. Glenska, L. Lechowicz, S. Grabowski, A. Brauner, & M. Kwinkowski. *Serotyping of Proteus mirabilis Clinical Strains Based on Lipopolysaccharide O Polysaccharide and Core Oligosaccharide Structures.* **Published in Russian in Biokhimiya** 76(7): 2019, 1039-1050.

[58] Y.A. Knirel. *Structure of the O-antigenic Polysaccharides of Proteus bacteria.* **Polish Journal of Chemother** 73: 2019, 895–9071. http://dx.doi.org/10.1007/s00005-006-0031-1

[59] R.T. Jamil, L.A. Foris, & J. Snowden.*"Proteus mirabilis Infections," in StatPearls.* **J. Antimicrob. Chemother.** *(Treasure Island, FL: StatPearls Publishing)*. 2020, Available online at: https://www.ncbi.nlm.nih.gov/books/NBK442017/

[60] S.M. Jacobsen, D.J. Stickler, H.L. Mobley, & M.E. Shirtliff.*Complicated Catheter-Associated Urinary Tract Infections due to Escherichia coli and Proteus mirabilis.* **Clin. Microbiol. Rev.** 21: 2018, 26–59. doi: 10.1128/CMR.00019-07

[61] A.J. Sabat, M. Tinelli, & H. Grundmann. *Daptomycin Resistant Staphylococcus aureus Clinical Strain with Novel Non-Synonymous Mutations in the mprF and vraS Genes: A New Insight into Daptomycin Resistance.* **Front Microbiol.** 9:2018, 2705. doi:10.3389/ fmicb.2018.02705

[62] C.Y. Chen, Y.H. Chen, P.L. Lu, W.R. Lin, T.C. Chen, & C.Y. Lin. *Proteus mirabilis Urinary Tract Infection and Bacteremia: Risk Factors, Clinical Presentation, and Outcomes.* **J. Microbiol. Immunol. Infect**. 45: 2022, 228–236. doi: 10.1016/j.jmii.2011.11.007

[63] H. Phan, & D. Lehman. *Cerebral Abscess Complicating Proteus mirabilis Meningitis in a Newborn Infant.* **Journal of Child Neurology** 27(3): 2022, 405-407. https://doi.org/10.1177/0883073811422271

[64] K.L. Hansson, M. Sundstro, A. Pelletier, & P.H. Roy. *Intl2 Integron Integrase in Tn7.* **Journal of Bacteriology** 184: 2022, 1712–1721

[65] S.R. Patridge, C.M. Collis & R.M. Hall. *Class 1 Integron Containing a New Gene Cassette, aadA10, associated with Tn1404 from R151.* **Antimicrobe Agents Chemother** 46: 2022, 2400–2408. http://dx.doi.org/10.1128/AAC.46.8.2400- 2408.2002

[66]K.H.Khan, Deepak Ganjewala & K.V. Bhaskara Rao. *Recent Advancement in Typhoid Research - a Review*. **Advanced Biotech.** 2018, pp. 36

[67] D.F. Khamash, A. Voskertchian, & P.D. Tamma. *Increasing Clindamycin and Trimethoprim-Sulfamethoxazole Resistance in Pediatric Staphylococcus aureus Infections***. J Pediatric Infect Dis Soc.** 2018;XX(XX):1–3. (Ahead of print).

[68] J.A. Crump, M. Sjolund-Karlsson, M.A. Gordon, & C.M. Parry. *Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections.***Clin Microbiol Rev**. (4): 2021, 901-37

[69] C.M. Parry, T.T. Hien, G. Dougan, N.J. White, & J.J. Farrar. *Typhoid Fever.* **N Engl J Med.** 28: 2022, 347(22):1770-82

[70] J. Fierer, & D.G. Guiney.*Diverse Virulence Traits Underlying Different Clinical Outcomes of Salmonella infection*.**J. Clin Invest**.107 (7): 2021, 775-80.

[71] M. George, J.S. Iramiot, R. Muhindo, P. Olupot-Olupot, & A. Nanteza. *Bacterial Aetiology and Antibiotic Susceptibility Profile of Post-Operative Sepsis among Surgical Patients in a Tertiary Hospital in Rural Eastern Uganda.* **Microbiol Res J Int.** 24(2):2018, 1–8. doi:10.9734/MRJI

[72] M.Z. David, & R.S. Daum. *Treatment of Staphylococcus aureus Infections*. **Curr Top Microbiol Immunol**. 409:2017, 325–383. doi:10.1007/82_2017_42

[73] Y. Jiu, X. Meng, X. Hong, Q. Huang, C. Wang, & Z. Chenl. *Prevalence and Characterization of Salmonella in three Typical Commercial Pig Abattoirs in Wuhan, China.* **Foodborne Pathog**. 2019, Dis. doi: 10.1089/fpd.2019.2737 [Epub ahead of print].

[74]X. Wang, S. Biswas, N. Paudyal, H. Pan, X. Li, & W.Fang, W. *Antibiotic Resistance in Typhimurium Isolates Recovered from the Food Chain through National Antimicrobial Resistance Monitoring System between 1996 and 2016.* 2019, **Front. Microbiol.** 10:2019, 985. doi: 10.3389/fmicb.2019.00985

[75]K. Mellor, L. Petrovska, N. Thomson, K. Harris, S. Reid, & A. Mather. *Antimicrobial Resistance Diversity Suggestive of Distinct Typhimurium Sources or Selective Pressures in Food-Production Animals.* **Front. Microbiol**. 10: 2019, 708. doi: 10.3389/fmicb.2019.00708

[76]M. Martínez, P. Retamal, J. Rojas-Aedo, J. Fernández, A. Fernández, A. & L. Lapierre.*Multidrug-Resistant Outbreak-Associated Salmonella Strains in Irrigation Water from the Metropolitan Region, Chile*. **Zoonoses Public Health** 64: 2017, 299–304. doi: 10.1111/zph.12311

[77]M. Masalha , I. Borovok, R. Schreiber, Y. Aharonowitz, & G. Cohen.*Analysis of Transcription of Staphylococcus aureus Aerobic Class 1b and Anaerobic Class III Ribonucleotide Reductase Genes in Response to Oxygen.* **Journal of Bacteriology** 183: 2021, 7260-7272. Link: https://goo.gl/ChuYAJ

[78] G. Malihar & A. Zehra. *Staphylococcus aureus: A Brief Review*. **International Journal of Veterinary Science and Research.** 4(1): 2018, 020-022. DOI: http://dx.doi.org/10.17352/ijvsr.000031

[79] C.J. Gonzalez-Perez, J. Tanori-Cordova, E. Aispuro-Hernandez, I. VargasArispuro, I. & M.A. Martinez-Tellez. *Morphometric Parameters of Foodborne Related-Pathogens Estimated by Transmission Electron Microscopy and their Relation to Optical Density and Colony Forming Units.* **J. Microbiol.** Methods 165:2019, 105691 doi: 10.1016/j.mimet.2019.105691

[80] T.A. Taylor, & C.G. Unakal. *"Staphylococcus aureus," In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*; **J**. **Adv. Urol.** 2019.

[81]Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. *"Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management,"* **Clinical Microbiology Reviews**, vol. 28, no. 3, 2019, pp. 603-661.

[82]CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement.* CLSI document M100- S25. Wayne, PA: **Clinical and Laboratory Standards Institute;** 2019.

[83] U. Abubakar, & B. Tangiisuran. *"Nationwide Survey of Pharmacist's Involvement in Antimicrobial Stewardship Program in Nigerian Tertiary Hospitals,*" **Journal of Global Antimicrobial Resistance,** 2019.

[84]European Centre for Disease Prevention and Control. *Surveillance of Antimicrobial Resistance in Europe 2018*. 2019; https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe2018. Accessed 11th May, 2020.

[85]P. O. Olorunfemi, J. A. Onaolapo & Y. K.E. Ibrahim. *Prevalence and Antibiotic Susceptibility of Community acquired Methicillin Resistant Staphylococcus aureus from Healthy Students of University of Jos.* **J. Pharmacy & Bioresources** . Vol. 17 no. 2, 2019, pp. 131-141 (September 2020)

[86] A. Onanuga A. & G.O. Awhowho. *"Antimicrobial Resistance of Staphylococcus aureus Strains from Patients with Urinary Tract Infections in Yenagoa, Nigeria,"***Journal of Pharmacy & Bioallied Sciences**, vol. 4, no. 3, 2021, pp. 226,

[87] E. Tacconelli E. & N. Magrini. *Global Priority List of Antibiotic-Resistant Bacteria to guide Research, Discovery, and Development of New Antibiotics.* **World Health Organization,** 2017.

[88] A. R. Narin, & R.H. Nawfal. *Staphylococcus aureus: An Overview of Discovery, Characteristics, Epidemiology, Virulence Factors and Antimicrobial Sensitivity.***European Journal of Molecular & Clinical Medicine.** Volume 08, Issue 03, 2021

[89] M.F. Khan. *Brief History of Staphylococcus aureus: A Focus to Antibiotic Resistance.* **EC Microbiology.** 5: 2017; 36-9.

[90]A. Davin-Regli, L. Jean-Philippe, P. Jean-Marie.*Enterobacter spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance.* **Clinical Microbiology Reviews.** Volume 32 Issue 4, 2019

[91]D. Moradigaravand, S. Reuter, V. Martin, S. J. Peacock, & J. Parkhill. *The Dissemination of Multidrug Resistant Enterobacter cloacae throughout the UK and Ireland*. **J Antimicrob Chemother** 2019

[92] H.N. Leong, A. Kurup, & M.Y. Tan. *Management of Complicated Skin and Soft Tissue Infections with a special Focus on the Role of Newer Antibiotics.* **Infect Drug Resist.** 11:2018, 1959–1974. doi:10.2147/IDR.S172366

[93] R.K. Salimiyan, K. Ghazvini and H. Farsiani. *Clinical and Pathogenesis Overview of Enterobacter Infections*. **Rev Clin Med.** 6(4): 2020; 146-54.

[94] A. Davin-Regli, J.P. Lavigne, & J.M. Pagès.*Enterobacter spp. Update on Taxonomy, Clinical aspects, and emerging Antimicrobial Resistance*. **Clin Microbiol Rev.** 32(4): 2019; e00002-19.

[95] D. Ramirez, M. & Giron.*Enterobacter Infections*. In: StatPearls (Internet). Treasure Island (FL): **StatPearls Publishing**; 2020.

[96] E.M. Eichenberger, & J.T. Thaden *Epidemiology and Mechanisms of Resistance of Extensively Drug Resistant Gram-negative bacteria*. **J Antimicrob Chemother** 8(2): 2019; 37.

[97] C. Meryem, & A. Nergis. *Antimicrobial Resistance Profiles of Enterobacter cloacae and Klebsiella aerogenes a Tertiary Hospital in Turkey:* A five-years study. ***Medicine Science Discovery***. 8(3): 2019; 161-166

[98] A. Noël, C. Vastrade, S. Dupont, M. de Barsy, T.D. Huang, & T. Van Maerken, *Nosocomial Outbreak of extended-Spectrum β-Lactamase Producing Enterobacter cloacae among Cardiothoracic Surgical Patients causes and Consequences.* **J Hosp Infect.** 102(1): 2019; 54-60

[99] A. Ito, T. Nishikawa, M. Ota, T. Ito-Horiyama, N. Ishibashi, T. Sato, M. Tsuji, & Y. Yamano. *Stability and low Induction Propensity of Cefiderocol against Chromosomal AmpC -lactamases of Pseudomonas aeruginosa and Enterobacter cloacae*. **J Antimicrob Chemother,**201873: 2018, 3049 –3052.

[100]https://www.webmd.com/vitamins/ai/ingredientmono-1537/plant-sterols

[101]S. Malhotra, & A.S. Pal. *A Review of Pharmacology of Phytochemicals from Indian Medicinal Plants. The Internet*. **Journal of Alternative Medicine.** 5(1): 2017; 23- 27.

[102] M. Miklasinska-Majdanik, M. Kepa, & R.D. Wojtyczka. *Phenolic Compounds Diminish Antibiotic Resistance of Staphylococcus aureus Clinical Strains.* **Int J Environ Res Public Health** 15:2018, E2321. doi:10.3390/ijerph15061188

[103] S. Malhotra, & A.S. Pal. *A review of pharmacology of phytochemicals from Indian medicinal plants.* **Journal of Alternative Medicine.**5(1): 2017; 23- 27.

[104]E.I.Woods, & L.R. Adrienne. *The American Socirty of Health-System Pharmacists*.*Delmar*. **J Antimicrob Chemother** 2018, p.296. ISBN 9781428361065

[105] C.B. Creech, R.J. Frenck & E.A. Sheldon. *Safety, Tolerability, and Immunogenicity of a Single Dose 4-antigen or 3-antigen Staphylococcus aureus Vaccine in Healthy Older Adults: Results of a Randomised trial.* **J. of Vaccine**. 35(2):2017, 385–394. doi:10.1016/j. vaccine.2016.11.032

[106]R. Huttner, P. Angela, E.M. Verhaegh, O. Harbarth, M. Stephan, K.I. Muller, E.T. Anouk & W.I. Johan, Nitrofurantoin Revisited: A Systemic Review and Meta-Analysis of Controlled Trials: **Journal of Antimicrobial Chemotherapy** (9): 2021, 2456-2464 doi: 10.1093/jac/dkv147. ISSN 0305-7453. PMID 26066581

[107]J.A. McKinnell, J.A. Stollenwerk, C.W. Jung & L.G. Miller, *Nitrofurantoin Compares "Favourably to Recommended Agents as Empirical Treatment of Uncomplicated Urinary Tract Infections in a Decision and Cost Analysis*. ***Mayo Clinic Proceedings*** 86(6): 2021, 480-8. doi:10.4065/mcp.2010.0800. PMC 3104907. PMID 21576512

[108]I. Chopra, M. Roberts & G.J. Anderson. *"Tetracycline Antibiotics: Mode of Action, Application, Molecular Biology and Epidemiology of Bacterial Resistance"*. **Microbiol. Mol. Biol. Rev.** 65(2): 2001, 232-260. doi: 10.1128/MMBR.65.2.232-260.2021. PMC 99026 PMID 11381101

[109] P. Surwade, C. Ghildyal, & C. Weikel. *Augmented antibacterial Activity of Ampicillin with Silver Nanoparticles against Methicillin-resistant Staphylococcus aureus (MRSA).* **J Antibiotics.** 2(1):2019, 50–53. doi:10.1038/s41429-018-0111-6

[110]D. Gordon, *Amoxicillin-Clavulanic Acid (Co-Amoxiclav). In Grayson M.L (eds). Kucers' the Use of Antibiotics: a Clinical Review of Antibacterial, Antifungal, Antiparasitic and Antiviral Drugs.* London: **Journal of Antimicrobial Chemotherapy** Hodder Arnold/ASM Press. 2020, Pp.193-4, ISBN 0-340-92767-4

[111]M. Gillies, A. Ranakusuma, T. Hofffman, S. Thorning, T. McGuire, & P. Glasziou, *Common Harms from Amoxicillin: A Systematic Review and Meta-Analysis of Randomized Placebo-Controlled Trials for any Indication. CMAJ:* **Canadian Medical Association / Journal del'Associationmedicale Canadienne** 187: 2021, E21-31. doi:10.1503/cmaj.140848. PMID 25404399.

[112] Z. Xu, H.M. Manukumar, & K.P. Rakesh. *Role of Bap-Dependent Multicellular Behavior of Clinically Important Methicillin-Resistant Staphylococcus aureus (MRSA) Biofilm adherence: A Key Virulence Study.* **Microb Pathog.** 123:2018, 275–284. doi:10.1016/j.micpath.2018.07.025

[113]A. Canessa, V. Del Bono, P. De Leo, N. Piersantelli & A. Terragna *Cotrimoxazole Therapy of Toxoplasma Gondii encephalitis in AIDS Patients*: **European Journal of ClinicalMicrobiology and Infectious Diseases**11(2): 2022, 125-130 doi:10.1007/BF01967063. PMID 1396726

[114]A. M. Emmerson, & A.W. Jones *"The Quinolones: Decades of Development and Use"* **The Journal of Antimicrobial Chemotherapy**. 51: 2020, 13-20 doi:10.1093/jac/dkg208. PMID 12702699

[115] E.C. O'Brien, & R.M. McLoughlin. *Considering the 'Alternatives' for Next-Generation Anti-Staphylococcus aureus Vaccine Development*. **Trends in Mol Med.** 25(3):2019, S1471–4914.

[116] L. Kremer, M. Walton, & E.N. Wardle. *Nalidixic Acid and Intracranial Hypertension***. British Medical Journal** 4(5577): 2019, 488 doi:10.1136/bmj.4.5577.488-a PMC 1748506. PMID 6055749

[117]Y.I. Pommier, G.L. Mandell, J.E. Bennett, & R.P. Dolin, *DNA Topoisomerases and their Poisoning by Anticancer and Antibacterial Drugs.* **Chem. Biol.** 17: 2020, 421-433 doi:10.1016/j.chembiol.2010.04.012. PMID 20534341

[118]K. Drlica, X. Zhao, A. De Sari & O.R. Zaleznik, *"DNA Gyrase, Topoisomerases IV and the 4-quinolones"*.**Microbiol. Mol. Biol. Rev.**61 (3): 2017, 377-392 PMC 232616 PMID 9293187

[119]E.N. Kawahara, *Ofloxacin, Resistant Bacteria Strains:* **Microbiol. Mol. Biol.Rev**. 52(2): 2018, 237-239 PMC 232514 PMID 9392185

[120] M. Qian, T. Zhao, & R. Li. *Targeting the R domain of coagulase by active vaccination protects mice against lethal Staphylococcus aureus infection*. **Microbes Infect.** S1286–4579. (18): 2018, 30177-1.

[121]J.H. Comroe, *"The Story of Streptomycin. Part I: from Waksman to Waksman* **American Review of Respiratory Diseases** 117 (4): 2018, 773-781 PMID 417651

[122]O.J. Weinstein & J.T. Marvi *Gentamycin, A New Antimicrobial Complex from Micromonospora* **J.Med. Chem**. 6: 2017, 463-464. doi:10.1021/jm00340a034

[123]N. Lopeze, M. Jose, P. Quiros, Z. Yaremi, T. Vicente, L. Laura, S. Morales, T.Ana, N. Lopez & and J. Francisco *New Insights into the Mechanism of Aminoglycoside: An Integrative Point of View Kidney International* **American Review of Respiratory Diseases**79; 2021, 33 PMID 20861826

[124]A.M. Geddes, *Introduction: Historical Perspective and Development of Amoxicillin/Clavulanate.* **Int. J. Antimicrob. Agents** 30(Suppl 2): 2017, S109-12. doi:10.1016/j.ijantimicag.2007.07.015. PMID 17900874

[125]A. Ahovua-Soloranta, U.M. Rautakorpi, O.V. Borisenko, H. Liira, J.W. Williams & M. Makela *Antibiotics for Acute Maxillary Sinusitis* **The Cochrane Library**. 2021, pp 120-234

[126]C. Mims, H.M. Dockrell & R.V. Goering, *Attacking the Enemy: Antimicrobial Agents and Chemotherapy: Macrolids.* **In: Medical Microbiology 3rd Edition, LondonMobsy Ltd**: 2018, p489

[127]S.S. Bothara, K.R. Kadam & K.G. Mahadik, *Antibiotics, Principles of Medicinal Chemistry* **Journal of Antimicrobial Chemotherapy** 1(14thed.) 2020, p81 ISBN 8185790043

[128]J.P. Barrio-Lera, A.I. Alvarez & J.G. Prieto, *"Effects of Ethanol on the Pharmacokinetics of Cephalexin and Cefadroxil in the Rat".* **Journal of Pharmaceutical Sciences** 80(6): 2021, 511-516. doi:10.1002/jps.2600800602

[129]A.R. Hauser, *"Antibiotics Basics for Clinicians" 2nd ed. Lippincott, Williams and Wikins*. **Int. J. Antimicrob. Agents** 2018, pp.25-26

[130]G.R. Suleyman & M.J. Zervos "Safety and Efficacy of Commonly used Antimicrobial Agents in the Treatment of Enterococcal Infections: A Review.".Expert Opinion on Drug Safety**. Int. J. Antimicrob. Agents** 15 (2): 2019, 153-67 doi: 10.1517/14740338.2016.1127349 PMID 26629598

[131]M.E. Pichichero, *"Cephalosporins can be prescribed safely for penicillin-allergic patients"* **The Journal of Family Practice**. 55 (2): 2021, 106-12 PMID 16451776

[132]S. Walter, *"Drug Discovery: History*" **Int. J. Antimicrob. Agents** 23 (3): 2020, 101-104 PMID 23892644

Chapter Three

Methodology

3.1 Equipment, Glassware, Media, Reagents, Plant parts and Other Materials

Glassware

Beakers (100ml, 250ml, 500ml, and 1000ml), graduated cylinders (10ml, 50ml, and 100ml), Buchner funnel, micropipette (200 µL), Petri dishes, McCartney flasks, and glass stirrer.

Growth Medium

Nutrient Agar (NA) (HIMEDIA)

Reagents

Ethanol, petroleum ether, acetone, chloroform and water

Leaves and stem bark of *Croton zambesicus*

Other Materials

Cotton, aluminum foil, inoculating wire loop, forceps, tape, marker, Bunsen burner, filter paper (Whatman NO 1), and antibiotic discs (gram-positive and gram-negative).

Equipment

Autoclave (Model YM50), Electronic Balance (Mettler Toledo FA2104A), Incubator (Gallenhamp Model No: - DNP-9022A), Oven (SKY –B40), Water Bath (Uniscope SM101 Surgifriend Medical England), Alcohol Lamp, Bunsen Burner , a 5 ml syringe and needle, an 8 mm diameter corkscrew, a pH meter and a transparent ruler (30 mm).

3.2 Collection and Processing of Plant Material

*Croton zambesicus*leaves and stem were collected at Molete Market in Ibadan and identified at Ibadan Oyo State University Department of Botany with voucher number UIH-23099. The leaves and stem of the plant were thoroughly washed with distilled water. They were air dried at room temperature and ground to a fine powder with an Excella mixer grinder model number Cm/L.7962804 and stored in airtight sample bottles for testing purposes.

3.3 Collection of Test Organisms

The Medical Microbiology Laboratory, University Hospital, Ibadan, provided pure stock cultures of the pathogenic bacterial isolates. The pathogenic bacterial were *Staphylococcus aureus, Klebsiella Pneumoniae, Escherichia coli*, *Enterobacter cloacae, Proteus mirabilis* and *Salmonella typhi.*

3.4 Sterilization of Glassware and Non-glassware

All glass and non-glass material was thoroughly washed with liquid detergent, rinsed with distilled water, drained, dried, then wrapped in aluminum foil, and then sterilized in a hot air oven at 160°C for 2 hours. The inoculating wire loop and cork borer (8 mm) were resterilized by soaking in alcohol (ethanol) and then red-flashed using an alcohol lamp before and after use[1].

3.5 Preparation of Nutrient Media

According to the manufacturer's specifications, 28 g of nutrient agar powder was weighed using the sensitive weighing balance (Mettler Toledo FA2104A). In a 1000 ml conical flask, the agar powder was dissolved in 1000 ml of distilled water. The mouth of the conical flask was sealed with a cotton plug coated in aluminium foil. The solution was properly mixed before being homogenised in an 80°C water bath for 15 minutes. After removing the conical

flask containing the homogenised nutritional agar from the water bath, it was wrapped in aluminium foil and autoclaved for 15 minutes at 121°C at 15 psi pressure. The mixture was allowed to cool to around 47-50°C before being poured into sterile petri dishes and allowed to solidify[2].

3.6 Validity of Test Organisms

Organisms were cultured in fresh Petri dishes using the streak plate method after 48 hours on nutrient agar slants in a refrigerator at about 40°C. To demonstrate the validity and viability of the bacteria tested, the Gram staining process and other relevant biochemical tests such as catalase, starch hydrolysis, oxidase, urease, indole, and citrate were performed on each isolate[3].

3.2.4 Antimicrobial Susceptibility Tests

3.2.4.1 Preparation of the Extract Dilution

Using a balance, 200g of *Croton zambesicus* leaf and stem powder were weighed separately and separately dissolved in 1000 ml of extracts; Ethanol, petroleum ether, acetone, chloroform and water. The solution was left for 48 hours but stirred at regular intervals. The solutions were then filtered using Whatman No. 1 filter paper to obtain the extracts. Each of the concentrated filtrates was diluted to various concentrations ranging from 100 mg/ml to 66.7 mg/ml, 50 mg/ml, 40 mg/ml and 33.3 mg/ml. The extracts were then tested for their antimicrobial power on the following bacterial isolates: *Klebsiella pneumonia, Proteus mirabilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Enterobacter cloacae.*

Concentrations of extracts for antimicrobial analysis were determined using the following concentration formula:

$C_1V_1 = C_2V_2$

Where C_1 = Initial concentration

C_2 = Final concentration

V_1 = Initial Volume

V_2 = Final Volume

a) 10g of undiluted extract converted to milligrams = 10 g x 1,000 = 10,000 mg

Initial concentration = 10000mg

100ml

100 mg/ml = initial concentration

b) C_1 = 100mg/ml

C_2 =?

V_1 = 10ml

V_2 = 15ml

$$C_2 = \frac{C_1V_1}{V_2}$$

$$C_2 = \frac{100mg/ml \times 10ml}{15ml}$$

$$C_2 = \frac{100mg/ml}{15}$$

C_2= 66.7mg/ml = concentration of 1st dilution

c) C_1 = 100mg/ml

C_2= ?

$V_1 = 10ml$

$V_2 = 20ml$

$C_2 = \frac{C_1V_I}{V_2}$

$C_2 = \frac{100mg/ml \times 10ml}{20ml}$

$C_2 = \frac{1000mg}{20ml}$

$C_2 = 50mg/ml$ = concentration of 2nd dilution

d) $C_1 = 100mg/ml$

$C_2 = ?$

$V_1 = 10ml$

$V_2 = 25ml$

$C_2 = \frac{C_1V_1}{V_2}$

$C_2 = \frac{100 \times 10}{25ml}$

$C_2 = \frac{1000mg}{25ml}$

$C_2 = 40mg/ml$ = concentration of 3rd dilution

3.2.4.2 Diffusion Test in Agar Wells

The antibacterial activity of the extracts was evaluated using an agar well diffusion test. A sterile 6 mm corkscrew was used to make five wells in the agar plate, with the fifth serving as a control well in the centre of the plate. The extracts were distributed into the wells with a

syringe and needle, except for the centre, which was filled with solvent only. This permits the extract in the gel cavity to spread into the solid medium, limiting the growth of the inoculation microorganisms to the extract constituent's efficiency. This was demonstrated by a visible circular area surrounding the extract well.

3.2.4.3 Inhibitory Tests

Test organisms were distributed onto the surface of hardened nutrient agar plates using an inoculating wire loop. To produce a homogeneous deep well in the agar gel, a sterile 6 mm corkscrew was utilised. Following that, each well was filled with 1 ml of the extracts made in different solvents. The Petri dishes were left at room temperature for 30 minutes to allow the extract to diffuse properly. Controls were then made without the extract, using only the solvents. As a control for the aqueous sample, sterilised distilled water was employed. After incubating the plates at 37°C for 24 hours, the zone of inhibition was measured using a 30 mm transparent measuring ruler[4].

3.2.4.4 Determination of the Minimum Inhibitory Concentration (MIC)

The agar streak technique was used to determine the lowest inhibitory concentration. The minimum inhibitory concentration is the lowest concentration of extract required to completely inhibit the test organism for up to 48 hours of incubation or the lowest concentration that can produce a significant decrease in inoculum viability of more than 90%.[5].

3.2.4.5 Antibiotic Susceptibility Testing

The disc diffusion method was used to test antibiotic susceptibility using antibiotic discs (gram-positive and gram-negative). Each nutrient agar slant's pathogenic bacterial isolate was streaked onto the nutrient agar plates. Using sterile forceps, the antibiotic discs were then

aseptically put in each Petri dish. The plates were then incubated at 37°C for 24 hours. After that, the zones of inhibition of the bacterial isolates on each plate were analysed. Using the transparent measuring ruler, each zone of inhibition around each antibiotic disc was measured in millimetres. The diameter of the antibiotic's zone of inhibition was measured to determine antibiotic susceptibility. Antibiograms are patterns of antibiotic susceptibility caused by zones of inhibition found on agar plates.

3.3 Phytochemical Detection of Extracts from Leaves and Stems of *Croton zambesicus*

Phytochemical screening was performed to identify phytochemicals in the leaf and stem of *Croton zambesicus*. This analysis was performed according to standard procedures. Phytochemicals that have been investigated are saponins, tannins, steroids, terphenoids, anthraquinones, flavonoids, alkaloids, and flabotanins[5].

3.3.1 Phytochemical Qualitative Test

Chemical tests were performed on the extract using the standard procedure to identify the constituents[8].

3.3.1.1 Tannin Test

In one test, one gramme of extract was cooked in 20 ml of water and then filtered. After adding a few drops of 0.1 percent ferric chloride, a green or blue-black tint was seen, demonstrating the presence of tannin.

3.3.1.2Phlobatannin Test

The deposition of a red precipitate when 2 ml of extract from each plant sample was boiled with 1% aqueous hydrochloric acid was used to prove the presence of phlobatannins.

3.3.1.3 Saponin Test

In a water bath, 5 ml of the extract was cooked in 20 ml of distilled water and filtered. To create a stable and persistent foam, 10 ml of the filtrate was mixed with 5 ml of distilled water and forcefully agitated. After rapidly shaking the froth with 3 drops of olive oil, the creation of an emulsion was observed, demonstrating the presence of saponin.

3.3.1.4 Flavonoid Test

Three milliliters of a 1% aluminum chloride solution was added to 5 ml of each extract. A yellow color was observed indicating the presence of flavonoids. 5 ml of a dilute ammonia solution was added to the above mixture followed by the addition of concentrated H_2SO_4. A yellow color disappeared on standing. Yellow color that disappears upon standing indicates a positive test for flavonoids.

3.3.1.5 Steroid Test

Two millilitres of acetic anhydride were added to two millilitres of extract from each sample, followed by two millilitres of H2SO4. The presence of steroids is indicated by a shift in colour from purple to blue or green.

3.3.1.6 Terpenoid Test (Salkowski test)

Five millilitres of each extract were carefully combined with two millilitres of chloroform and three millilitres of pure H_2SO_4. A reddish-brown colouring of the interface was generated to indicate the presence of terpenoids.

3.3.1.7 Cardenolides and Cardiac Glycosides Test (Keller-Kilani Test)

Five millilitres of each extract were treated with two millilitres of glacial acetic acid containing one millilitre of ferric chloride solution. This was reduced by using 1ml of pure

sulfuric acid. A brown ring at the interface shows a cardenolide deoxysugar, indicating the existence of cardenolides. A green-violet ring appearing below the brown ring, in the acetic acid layer, indicates the positive presence of glucoside.

3.3.1.8Alkanoids

On a steam bath, one millilitre of the extract was mixed with 5ml of 1 percent aqueous HCl and filtered hot. 1 ml of the filtrate was treated with a few drops of Mayer's reagent (potassium mercuric iodide solution), Wagner's reagent (iodine solution in potassium iodide), or Dragendorff's reagent (potassium iodide solution). Bismuth and potassium iodide). A positive test for alkaloids is the production of a cream hue with Mayer's reagent and a reddish-brown precipitate with Wagner and Dragendorff's reagent.

3.3.1.9 Anthraquinone

Five millilitres of extract were combined with ten millilitres of benzene, filtered, and 5 millilitres of 10% NH3 solution were added to the filtrate. The mixture was mixed, and the presence of anthraquinones was indicated by the presence of a pink, red, or purple colour in the ammoniacal (lower) phase.

3.3.1.10 Chalcones

Ammonia solution (2 mL) was added to 5 mL of plant extract from each portion.The formation of a reddish color confirmed the presence of chalcones.

3.3.1.11 Phenol

Five millilitres of the extract were pipetted into a 30 ml test tube, followed by ten millilitres of distilled water. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl

alcohol were added as well, and the mixture was allowed to react for 30 minutes. The appearance of a bluish-green tint was seen as positive for phenol.

3.4 Quantitative Determinations

3.4.1 Tannin

A 0.20g sample was placed in a 50 ml beaker. 20 ml of 50% methanol was added, wrapped in parafilm, and placed in a 77-80°C water bath for 1 hour. It was carefully stirring to achieve a uniform mixture. The extract was quantitatively filtered through Whatman No. 41 double layer filter paper into a 100 mL volumetric flask, followed by 20 ml water, 2.5 ml Folin-Denis reagent, and 10 ml 17 percent Na_2CO_3. The marking mixture has been created with well-mixed water and set aside for 20 minutes; the bluish-green hue will appear after 20 minutesThe 0-10 ppm tannin working standard solutions were processed in the same manner as the previous 1 ml sample. The absorbance of Tannic acid standard solutions and samples was measured using a Spectronic 21D spectrophotometer at 760nm after colour development. Tannin percentage was computed as follows:

Tannin percentage = Sample Absorbance X Average Gradient Factor X Dilution Factor

10.000 x sample weight[11]

3.4.2 Alkaloid Procedure

To make a homogenous paste, a finely powdered 2g sample was weighed into a 100 ml beaker and 20 ml of 80 percent pure alcohol was added. The liquid was transferred to a 250ml flask, and 100ml of alcohol and 1g of magnesium oxide were added. The mixture was digested in a boiling water bath for 1.5 hours with intermittent stirring under an air reflux condenser. A tiny Büchner funnel was used to filter the heated mixture. The residue was

reintroduced to the flask and digested for 30 minutes with 50 cc of alcohol before being evaporated and hot water added to restore the lost alcohol.After removing all of the alcohol, 3 drops of 10% HCL were added.

After a few minutes, the flask was filtered through dry filter paper, and 10 ml of the filtrate was transferred to a separatory funnel, where the alkaloids were violently agitated out with five successive amounts of chloroform. The resulting residue was diluted in 10 ml of hot distilled water and transferred to a Kjeldahl tube with 0.20 g of sucrose, 10 ml of H2SO4 Conc., and 0.02 g of selenium for digestion in a colourless solution to calculate percent N using the Kjeldahl distillation method. The resulting nitrogen percentage is transformed into a percentage of total alkaloid by multiplying it by 3.26.Total alkaloid as a percentage = NX 3.26

Alkaloids percentage = percent NX 3.26[13, 14]

3.4.3 Determination of Flavonoids

To prevent lumping, 0.5g of finely ground sample was weighed into a 100 mL beaker and 80 mL of 95 percent ethanol was added and agitated with a glass rod. Whatman No. 1 was used to filter the mixture. Filter into a 100 mL volumetric flask and top up with ethanol to volume. 1 ml of the extract was pipetted into a 50 ml volumetric flask, four drops of concentrated HCL were pipetted in using a dropper pipet, and 0.5 g of magnesium shavings were added to create a magenta red tint. From a 100 ppm stock solution, a 0-5 ppm flavonoid standard solution was generated and similarly treated with magnesium chips and HCL as a sample.A Jenway V6300 digital spectrophotometer was used to measure the red-magenta colour absorbance of the sample and standard solutions at 520 nm. The formula is used to calculate the proportion of flavonoids.

$$\frac{\text{Sample absorbance X mean gradient factor X dilution factor}}{\text{Sample weight X } 10{,}000}$$ [15]

3.4.4 Saponin

A finely pulverised sample (1g) was weighed into a 250 ml beaker, followed by 100 ml of isobutyl alcohol. To achieve equal mixing, the slurry was shook for 5 hours using a UDY shaker. The mixture was then filtered through Whatman No. 1 filter paper into a 100 ml beaker before being mixed with 20 ml of 40% saturated magnesium carbonate solution. The resulting saturated MgCO3 combination was filtered again through Whatman No. 1 filter paper to achieve a clear colourless solution. 1ml of the colourless solution was pipetted into a 50ml volumetric flask, followed by 2ml of 5% $FeCl_3$ solution brought up to volume with distilled water. It was let to stand for 30 minutes to acquire the blood red colour. A saponin stock solution was used to make standard saponin solutions ranging from 0 to 10 ppm. Standard solutions were similarly handled with 2 ml of 5% $FeCl_3$ solution as the prior sample (1ml). After colour development, the absorbance of the sample and saponin reference solutions was measured on a Jenway V6300 spectrophotometer at wavelength

$$= \frac{\text{Sample absorbance X gradient factor X dilution factor.}}{\text{Sample weight X } 10000}$$ [16]

3.4.5 Glycosides

Ten milliliters of extract was pipetted into a 250 ml conical flask. 50 ml of chloroform was added and stirred on a Vortex mixer for 1 hour. The mixture was filtered into a 100 ml conical flask and added 10 ml of pyridine, 2 ml of 2% sodium nitroprusside, stirred vigorously for 10 minutes. Then 3 ml of 20% NaOH was added to develop a brownish-yellow color. Glycoside standards of concentrations ranging from 0 to 5 mg/ml were

prepared from a 100 mg/ml glycoside standard stock. The 0-5 mg/ml standard pool was treated in the same way as the previous sample. The absorbances of the sample as well as the standards were read on a Spectronic 21D digital spectrophotometer at a wavelength of 510 nm.

$$\frac{\text{Sample absorbance X gradient factor X dilution factor}}{\text{Sample weight X10000}}$$ [17]

3.4.6 Steroids

In a dry bottle, six millilitres of Liebermann Burchard's reagent was applied, and the absorbance at 620 nm was measured using a Spectronic 21D digital spectrophotometer. Standard steroids with concentrations ranging from 0 to 4 mg/ml were produced from a 100 mg/ml steroid stock solution and treated in the same manner as the preceding sample. The percentage of steroids was calculated using the following formula:

The percentage of steroids was calculated using the following formula:

$$\frac{\text{Sample absorbance X Gradient = Dilution factor}}{\text{Sample weight X 10000}}$$ [18]

3.4.7 Determination of Phlobatanin

In a 50 ml beaker, the sample extract (0.5 g) was weighed. 20 ml of 50% methanol was added, wrapped in parafilm, and placed in a 77-80°C water bath for 1 hour. The mixture was thoroughly mixed before being filtered through Whatman No. 1 filter paper into a 50 ml volumetric flask with aqueous methanol to rinse and make up to volume with distilled water. 1 mL of the sample extract was pipetted into a 50 ml volumetric flask, followed by 20 ml of

water, 2.5 ml of Folin-Dennis reagent, and 10 ml of sodium carbonate. 17% of the total for 20 minutes, this combination was completely homogeneous.

$$\frac{\text{Sample absorbance X gradient factor X dilution factor}}{\text{Sample weigh X 10000}}$$ [18]

3.4.8Anthraquinones Determination

Sample (0.5 g) was weighed into a 250 ml beaker and 60 ml of benzene was added and stirred with a glass rod to avoid lumps. Filtered via Whatman No. 1 filter paper into a 100 ml volumetric flask. 10 ml of filter was pipetted into another 100 ml volumetric flask with 0.2 percent zinc dust, then 50 ml of hot 5 percent NaOH solution was added. The mixture was heated for five minutes to just below boiling point, then filtered and rinsed once with water. To generate a red hue, the filtrate was boiled again with an additional 50 mL of 5% NaOH.

From a 100 mg/l anthraquinone stock, a 0-5 mg/l anthraquinone standard solution was generated and similarly treated with 0.2 percent zinc dust and NaoH type sample. A computerised equipment was used to measure the absorbance of the sample as well as the absorbance of the standard concentrations.Spectrophotometer with a 640nm wavelength. The proportion of anthraquinone is computed as follows:

$$\frac{\text{Sample absorbance X gradient factor X dilution factor}}{\text{Sample weight X 10000}}$$ [18]

3.4.9 Terpenes

The sample (0.5 g) was placed in a 50 ml conical flask, and 20 ml of a 2:1 chloroform-methanol mixture was added, agitated thoroughly, and allowed to stand for 15 minutes. After

that, the mixture was centrifuged for another 15 minutes. The resulting supernatant was discarded, and the precipitate was rinsed with another 20 ml of the chloroform-methanol combination before centrifugation.

The precipitate formed was dissolved in 40 ml of 10% sodium deodocyl sulphate solution. Sigma-Aldrich Chemicals, USA, provided a 100 mg/l terpene stock solution.

$$\frac{\text{Sample absorbance X gradient factor X dilution factor}}{\text{Sample weight X 10000}}$$ [18]

3.4.10Phenol Determination

To avoid the formation of lumps, the sample (0.20 g) was weighed into a 50 ml beaker, 20 ml of acetone was added, and the mixture was homogenised for 1 hour. The mixture was filtered through Whatman No. 1 filter paper into a 100 ml volumetric flask, rinsed with acetone, and filled to volume with distilled water, thoroughly mixed. 1 ml of the sample extract was pipetted into a 50 ml volumetric flask, 20 mL of water was added, 3 ml of phosphomolybdic acid was added, 5 ml of Na_2CO_3 at 23% was added and completely mixed, it was completed until the mark with distilled water and allowed to stand for 10 minutes until it developed a bluish-green colour.The phenol standard was made from a 100 mg/l phenol solution obtained from Sigma-Aldrich in the United States. The absorbance of the sample and the amounts of phenol standard were measured using a digital spectrophotometer at 510 nm. The proportion of phenol is computed as follows:

$$\frac{\text{Absorbance of the sample X dilution factor X gradient factor}}{\text{Sample weight X 10,000}}$$ [18]

Endnotes

[1]H. Girish, & S. Satish, *Antibacterial activity of important medicinal plants on phytopathogenic Xanthomonas compestris Pathovars*, 2018, ***Lett Microbiol*** 28: 145-147.

[2] B. Weniger, L. Lagnika, L.B. Djakou, C. VonthronSénéchaux, & A. Sanni, *L'ethnopharmacologie et la recherche de molécules antipaludéennes dans la biodiversité ivoirienne, béninoise et camerounaise.***Ethnopharmacologia** 41: 2020, 62- 70

[3] Z. Hamzaoui, A. Ocampo-Sosa, M.F. Martinez, S. Landolsi, S. Ferjani, & E. Maamar, *Role of Association Of Ompk35 And Ompk36 Alteration and Blaesbl and/or Blaampc Genes In Conferring Carbapenem Resistance Among Non-Carbapenemase-Producing Klebsiella pneumoniae*. **Int. J. Antimicrob. Agents** 52:2018 898–905. doi: 10.1016/j.ijantimicag.2018.03.020

[4]M. Prescott, P. Harley, & D. Klen, *General Microbiology. McGrawHill Publishing Company* **Int. J. Antimicrob. Agents**, New Delhi 7th edition, 2018, 163-850.

[5]F. Akinnibosun, H. Akinnibosun, I. Ibeh, & F. Osaghae, *Antibacterial activity of Phyllanthus amarus schum and thonn on fivevegetative organisms,* ***Plant Archives*** 8(2): 2018, 563-568

[6]A. Ramasamy, S. Das, V. Mani, S. Sengottuvelu, & V. Prabhu, *Evaluation of Anti-diarrheal Potential of Hydroalcoholic Extracts of Leaves of Murraya koenigii in Experimental Animals,* **Journal of Dietary Supplements,** 13: 2016, 393–401.

[7]H. Bernstein, C. Bernstein, & R. Michod, *Sex in microbial pathogens, Infection, Genetics and Evolution,* **Int. J. Antimicrob. Agents.** Volume 57, 2016, 8-25.

[8] E. J. Klemm, S. Shakoor, & A. J. Page. *"Emergence of an Extensively Drug-Resistant Salmonella enterica Serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third-Generation Cephalosporins,"* mBio, vol. 9, 2018, no. 1.

[9] T.A. Russo, & C.M. Marr. *Hypervirulent Klebsiella pneumoniae.* **Clin. Microbiol. Rev.** 32, e00001–e00119. 2019, doi: 10.1128/CMR.00001-19

[10] C.C. Hammer, J. Brainard, & P.R. Hunter. *Risk Factors and Risk Factor Cascades for Communicable Disease Outbreaks in Complex Humanitarian Emergencies: A Qualitative Systematic Review*. **BMJ Glob. Health** 3:e000647. 2018 doi: 10.1136/bmjgh-2017-000647

[11]A.O.A.C 14th Edition. **Tropical Journal of Pharmaceutical Research** Pg 187-188. INC IIII, North Nineteenth Street, Suite 210 Arlington VA 222-114 2020.

[12] J.S. Kang, J. Yi, M.K. Ko, S.O. Lee, J.E. Lee, & K.H. Kim. *Prevalence and Risk Factors of Carbapenem-Resistant Enterobacteriaceae Acquisition in an Emergency Intensive Care Unit in A Tertiary Hospital in Korea: a case-control study*. **J. Korean Med. Sci.** 34:e140. 2019 doi: 10.3346/jkms.2019.34.e140

[13]T.A Henry – *A textbook titled The plant Alkaloids* **Int. J. Antimicrob. Agents.** 2019: pg 462-466

[14] K. Chatham-Stephens, F. Medalla, & M. Hughes. "*Emergence of Extensively Drug-Resistant Salmonella Typhi Infections Among Travelers to or from Pakistan — United States*," **Morbidity and Mortality Weekly Report** (MMWR), vol. 68, no. 1,2019, pp. 11–13.

[15]Allen's Commercial Organic Analysis, **Int. J. Antimicrob. Agents.** Vol. IX, 2019, Pg. 156-189.

[16]J.H. Brunner.*Direct Spectrophotometer determination of Saponin.* **Anal. Chem.** 34, 2021, pg 1314 - 1326.

[17]Methods of Analysis of Analytical Methods Committee of Royal Society of Chemistry . **Int. J. Antimicrob. Agents.** AMC-RSC. 2020, pp222-229

[18] W.V. Kern, & S. Rieg. *Burden of Bacterial Bloodstream Infection-a Brief Update on Epidemiology and Significance of Multidrug-Resistant Pathogens*. **Clin Microbiol Infect**.26(2):2020, 151–157.

Chapter Four

Results and Discussions of Findings

4.1 Results

In this research work, the antimicrobial activity of the leaf and stem extracts of the *Croton zambesicus* plant was tested against certain human pathogenic bacteria, which are *Eschericlia coli, Salmonella typhi, Enterobacter cloacae, Proteus mirabilis, Klebsiela pneumonia* and *Staphylococcus aureus*. The photochemical constituents of the extract were analyzed.

The results of the zone of inhibition of *C. zambesicus* leaf extract against human pathogenic bacteria are shown in Table 4.1. The chloroform and aqueous extracts were not reactive against any of the bacterial isolates. This finding is in line with reported research that the antimicrobial activity of *Crotonzambesicus* aqueous extract on *Staphylococcus aureus* and *Streptococcus species* was observed to be weak, respectively.

Ethanoid leaf extract reacted against *Eschericia coli* at 10.3 ± 1.53, *Enterobacter cloaca* at 10.0 ± 1.0, *Klebsiella pneumonia* at 11.0 ± 1.0 while *Salmonella typhi, Protens mirabilis* and *Staphylococcus aureus* They do not have a minimum inhibitory concentration. This result is consistent with the findings that *Croton zambesicus* ethanoic extract has the highest antimicrobial effect on *Staphylococcus aureus* with a concentration of 0.6 g/mL providing the highest zone of inhibition.

The leaf petroleum ether extract reacted against *Escharichea coli* at 13.7 ± 16, *Salmonella typhi* at 12.3 ± 1.5, *Enterobacter cloacae* at 13.0 ± 1.0, *Protens mirabilis* at 12.0 ± 1, 0, *Klebriellapneumonia* at 19.7 ± 0.59 and *Staphylococcusaureus* with no minimal growth inhibition. This result is consistent with the findings indicating that *S. aureus, S. pyogenes,*

Escharichia coli and *P. aeruginosa* are sensitive to traces of petroleum ether and methanol but resistant to ethyl acetate extract.

The acetone leaf extract reacted against*Escherichia coli* at 13.3 ± 2.9, *Enterobacter cloacae* at 12.3 ± 2.08, *Proteus mirabilis* at 12.0 ± 1.73, *Klebsiella pneumonia* at 10.3 ± 0.59 but no minimum activity concentration inhibitory on *Salmonella typhi* and *Staphylococcus aureus.*

The ethanoic stem extract reacted with *Escherichia coli* at 10.7 ± 1.53, *Salmonella typhi* at 9.3 ± 1.53, *Enterobacter cloacae* at 10.0 ± 2.0, *Protens mirabilis* at 14.3 ± 1.53 ,*Klebsiela pneumonia* at 12.0 ± 1.73 and *Staphylococcus aureus* at 9.3 ± 0.39 and there is a finding that crude ethanoic extract and chloroform extract were only active against *B.subtilis.*

Petroleum ether stem extract in *Esherichiacoli* reacted at 0, *Salmonella typhi* at 11.0 ± 10, *Enterobactercloacae* at 28.7 ± 1.53, *Proteusmirabilis* at 10.0 ± 0.71, *Klebsiellapneumonia* at 10 0.3 ± 1.16 and *Staphylococcusaureus* at 14.7 ± 0.39. The result shows that the sensitivity to petroleum ether.

The extract concentration with the lowest inhibitory concentration is the lowest concentration of extract.at which a viable inoculum will be inhibited by approximately 75% and the result represented in vitro by a diameter of 10 mm. There are results of the inhibition zone of the *C. zambesicus* leaf extract against humanpathogenic bacteria. It was observed that the aqueous and chloroform extracts were not reactive against any of the bacterial isolates.

Using ethanol as the extraction solvent, it was found to have 50% potency against the isolates used, particularly some of the gram negative bacteria. Furthermore, it has been found to only have antiseptic but not bactericidal properties, i.e. a narrow spectrum of activity. This is

consistent with the finding that the crude ethanoic extract, chloroform, was active against most bacterial isolates except the broad-spectrum ethyl acetate extract.

Using petroleum ether and acetone as the extraction solvent, both were found to have 83% potency against isolated bacteria, making petroleum ether a broad gram-positive and gram-negative bacterium, while acetone it had a narrow spectrum, as it was only effective against gram-negative bacteria. This result is in agreement with the results found previously.

Leaf and stem MICs were found not to vary greatly, thus both had the same effect on the test organisms.

The diameter of the zone of inhibition demonstrated that the antibiotics listed below were effective against some of the microbiological isolates: Rocephin was effective against Staphylococcus aureus (17 mm) and Proteus mirabilis (13 mm), *Samonella* (17 mm), *Escherchia coli* (14 mm), *Enterobactercloaca* (16 mm) and *Klebsiellapneumonia* (14 mm) However, Ampliclox had no effect against all organisms tested while Ciprofloxacillin had an effect on *Escherichiacoli* (19 mm), *Samonella* (16 mm), *Salmonella typhi* (20 mm) , *Staphylococcus aureus* (15 mm), *Proteus mirabilis* (15 mm), *E. cloacal* (20 mm) and *K. pneumonia* (19 mm) Amoxacillin also had no effect on Staphylococcus aureus.and *Proteus mirabilis, Escherichia coli, Enterobacter cloaca* and *Klebsiella pneumonia* (10 mm), *Samonella typhi* (15) also Zinnacef had no effect on all organisms tested except Samonella (11 mm) while gentamicin had effect on *Staphylococcus aureus* (16 mm), *Proteus mirabilis* no effect, *Samonella* (14 mm), *Escherchiacoli* (12 mm), *Enterobactercloaca* (14 mm) and *Klebsiella pneumonia* (7 mm) also pefloxacin had an effect on *Staphylococcus aureus* (10 mm), *Proteus mirabilis* (16 mm), *Samonella* (18), *Escherichia coli* (17 mm), *Enterobacter cloaca* (17 mm) and *Klebsiella pneumonia* (15 mm) also erythromycin had an effect on

Staphylococcus aureus (15 mm), *Proteus mirabilis* (8 mm), *Samonellatyphi*without effect, *Escherchia coli* (12 mm), *Enterobacter cloaca* (14 mm) and *Klebsiella pneumonia* (16 mm) and finally, streptomycin had an effect on *Staphylococcus aureus* (14mm), *Proteus mirabilis* (11mm), *Samonella* (15mm), *Escherchia coli* without effect,*Enterobacter cloaca* and *Klebsiella pneumonia* ineffective (11 mm)

Septrin had no effect on all bacterial isolates except *Staphylococcus aureus* (13) and *Samonella* (12). Chloramphenicol, on the other hand, had an effect on *Staphylococcus aureus* (14 mm), *Samonella* (15 mm), *Klebsiella pneumonia* (12 mm), *Proteus mirabilis* with no effect, *Enterobacter cloacae* (11 mm), and *Escherichia coli* (11 mm).

Esperfloxacin had an effect on *Staphylococcus aureus* (19 mm), *Samonella* (17 mm), *Klebsiella pneumonia* (14 mm), *Proteus mirabilis* (16 mm), *Enterobacter cloaca* (19 mm), and *Escherichiacoli* (15 mm). On the contrary, all the isolates showed resistance to amoxicillin. Augmentin and streptomycin and pefloxacin except *Staphylococcus aureus* (30mm) which was controlled by the effect of antibiotics.

4.2 Discussion of Findings

Medicinal plant dated for year has been a vital source of medicinal and therapeutic purposes for African especially in the developing countries. A plethora of modern medications have been isolated from natural sources. Many of these active compounds were identified based on their traditional medical application[1]. There is a limited number of medications available to treat high resistance germs, and bacteria can be resistant to numerous drugs at the same time [2]. Bacteria change quickly, thus the mechanism for generating resistance evolves quickly. In recent years, antibiotic consumption has increased in low- and middle-income countries[3].

Antibiotic resistance has been identified as one of the three most serious public health issues of the twenty-first century by the World Health Organization[2].

The qualitative and quantitative analysis of the phytochemical screening of *Croton zambesicus* shows*Croton zambesicus* has all phytochemical constituents which includes Alkaloid, Saponin, Triterpenes, Steroid, flavous, glycosides, terpenoid, except phenolic and tannins. This study contradicts a researchwhich indicates absent of alkanoid and tannins[3].

Previous report has shown *Croton zambesicus* antimicrobial activities by past researcher who demonstrated the medicinal with pathogenic bacteria. Plants are rich reservoir of antimicrobials. It is observed that a single plant is known to contain several bioactive principles of biological significance[4]

Petroleum ether is an excellent solvent extractor in minimum inhibitor concentrates on pathogenic bacteria or otherwise treatment of pathogenic infection as it was documented to has the highest MIC on pathogenic bacteria. This result confirms petroleum Ether as excellent solvent in other medicinal plants past studies from *Parkia biglobosa*[3] and *Alstonia congensis,*[3] and in *Sphenocentrum jollyanum*[4].

The minimum inhibitory concentrates of *Croton zambesicus* on pathogenic bacteria has proven *Croton zambesicus* is an excellent herbal drug to use in treatment of both gram negative and gram positive bacterial infection. This result tally with other medicinal antimicrobial analysis of *Sphenocentrum jollyanum*[2], *Azadirachta indica* antimicrobial activities[1]. This result confirms the plant extract are more susceptible to gram positive bacteria than gram negative from past finding fromTulukeru and Delmar[4]. The aqueous and chloroform *e*xtract of *Croton zambesicus*has the lowest minimum inhibitory concentration.

Table 4.1:The Minimum Inhibitory Concentration (MIC) of *Croton zambesicus*of the Leaf Extract on some Harmful Microorganisms in Humans

	Isolates of Bacteria	Extraction Solvent	Stock	10ml	15ml	20ml	Control
1	*Escherichia coli*	Ethanol	10.3±1.53	-	-	-	-
		Petroleum ether	18.7 ± 1.16	15.7±1.53	13.7 ± 1.16	-	-
		Acetone	13.3±2.9	-	-	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
2	*Salmonella typhi*	Ethanol	-	-	-	-	-
		Petroleum ether	17.7±1.53	14.0 ± 1.0	13.0 ± 1.0	12.3±1.5	-
		Acetone	-	-	-	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
3	*Enterobacter cloacae*	Ethanol	10.0±1.0	-	-	-	-
		Petroleum ether	19.0 ± 1.6	16.3 ± 1.16	14.7±1.53	13.0 ± 1.0	-
		Acetone	12.3±2.08	-	-	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
4	*Proteus mirabilis*	Ethanol	-	-	-	-	-
		Petroleum ether	18.0 ± 1.0	14.0 ± 1.0	12.0±1.0	-	-
		Acetone	13.0 ± 2.0	12.0±1.73	-	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
5	*Klebsiella pneumonia*	Ethanol	11.0 ± 1.0	-	-	-	-
		Petroleum ether	29.7 ± 0.59	24.0 ± 1.0	19.7 ± 0.59	-	-
		Acetone	13.3±2.09	11.0 ± 1.0	10.3 ± 0.59	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
6	*Staphylococcus aureus*	Ethanol	-	-	-	-	-
		Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-

Source: Authors Finding, 2022

Table 4.2: *Croton zambesicus* Stem Extract's Minimum Inhibitory Concentration (MIC) against various Human Pathogenic Microorganisms

	Isolatesof Bacteria	Extraction Solvent	Stock	10ml	15ml	20ml	Control
1	*Escherichia coli*	Ethanol	10.7±1.53	-	-	-	-
		Petroleum ether	-	-	-	-	-
		Acetone	-	-	-	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
2	*Salmonella typhi*	Ethanol	-	-	-	-	-
		Petroleum ether	19.7±1.07	16.0 ± 1.0	11.0 ± 1.0	-	-
		Acetone	18.0 ± 1.0	14.0 ± 1.0	13.0 ± 1.0	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
3	*Enterobacter cloacae*	Ethanol	10.0±2.0	-	-	-	-
		Petroleum ether	28.7±1.53	-	-	-	-
		Acetone	16.3±0.59	-	-	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
4	*Proteus mirabilis*	Ethanol	-	-	-	-	-
		Petroleum ether	20.0±1.0	14.3±1.53	-	-	-
		Acetone	11.0 ± 1.0	11.0 ± 1.0	10.0 ± 0.71	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
5	*Klebsiella pneumonia*	Ethanol	12.0±1.73	-	-	-	-
		Petroleum ether	12.3 ± 1.16	11.7±1.53	10.3 ± 1.16	-	-
		Acetone	13.7 ± 1.16	-	-	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-
6	*Staphylococcus aureus*	Ethanol	-	-	-	-	-
		Petroleum ether	26.5±0.59	23.0 ± 1.73	21.3 ± 1.53	14.7±0.5	-
		Acetone	14.3±2.31	-	-	-	-
		Chloroform	-	-	-	-	-
		Aqueous	-	-	-	-	-

Source: Authors Finding, 2022

Table 4.3 Antibiotic Susceptibility Testing using a Standard Antibiotic Susceptibility Disk on the Isolates

				Isolates						
S/N	**Antibiotics**	**Code**	**Conc. (MG)**	***E. coli***	***S. aureus***	***E. cloacae***	***S. typhii***	***K. pneumoniae***	***P. aeruginosa***	***P. mirabilis***
1	Perfloxacin	PEF	30	1.1	1.8	1.5	1.6	1.7	1.6	0.7
2	Gentamycin	CN	30	0	0.3	1.6	1.6	0	0.3	0
3	Ampiclox	APX	30	0	0	1.6	1.3	0	0	0
4	Zinnacef	Z	20	0	0	1.2	1.6	0	0.4	0
5	Amoxacilin	AM	30	0	0.3	2.2	2.0	0	0	0
6	Roceptin	R	25	1.0	0.6	2.0	1.8	1.8	1.7	0
7	Cirpoflaxin	CPX	30	1.0	1.6	1.6	1.8	1.6	1.8	1.4
8	Streptomycin	S	30	1.5	0	1.6	1.5	0	0	0.5
9	Septrin	SXT	30	0.7	0	1.6	1.7	1.7	0	0
10	Erythromycin	E	10	1.4	0	1.5	1.5	1.6	1.6	0
11	Chloranphenicol	CH	30	0	0	1.7	1.6	0	0	0
12	Sparfloxacin	SP	30	1.4	1.5	1.4	1.6	1.8	1.7	1.0
13	Augmentin	AU	10	0	0	1.5	1.6	0	0	0
14	Tarivid	OFX	30	1.3	0.3	1.6	1.6	0	0.5	0

Source: Authors Finding, 2022

Table 4.4: Phytochemical Stem Screening

Samples Code	Configurations			
	Chloroform Stem	**Ethanol Stem**	**Pet Ether Stem**	**Aceone Stem**
Alkaloid	++	-	++	+
Tannins	-	-	-	-
Saponnis	++	++	++	++
Terperoid	++	-	++	-
Steroid	++	-	++	-
Flavoroid	-	++	++	++
Glycosides	+	+	+	-
Triterpenes	+	-	+	+
Phenolic	-	-	-	-

Source: Authors Finding, 2022

Table 4.5: Phytochemical Screening of Leaves

Samples Code	Parameters				
	Chloroform Leaf	**Ethanol Leaf**	**Water Leaf**	**Pet Ether Leaf**	**Aceone Leaf**
Alkaloid	++	++	++	+	+
Tannins	-	-	-	-	-
Saponnis	+++	+	+++	+++	++
Terperoid	+	-	+	++	-
Steroid	++	++	+	-	-
Flavoroid	-	-	++	++	++
Glycosides	+	+	+	-	-
Triterpenes	+	-	+	-	+
Phenolic	-	-	-	-	-

Source: Authors Finding, 2022

Table 4.6: Quantitative Phytochemical for Stem

Parameters/ Sample	Parameters			
	Chloroform Stem	**Ethanol Stem**	**Pet Ether Stem**	**Aceone Stem**
Alkaloids	0.267	0.000	0.286	0.135
Tannins	0.00	0.00	0.00	0.00
Saponins	0.279	0.273	0.292	0.348
Terpenoids	0.0068	0.0000	0.0056	0.0073
Steroids	0.0093	0.0000	0.0065	0.0000
Flavovoids	0.0000	0.0017	0.0025	0.0024
Glycosides	0.123	0.114	0.178	0.000
Triterpenes	0.0029	0.000	0.0023	0.0000
Phenolics	0.000	0.000	0.000	0.0000

Source: Authors Finding, 2022

Table 4.7: Quantitative Phytochemical for the Leaf

Parameters	Parameters				
	Chloroform	**Ethanol**	**Water**	**Ether**	**Acetone**
Alkaloids	0.195	0.307	0.168	0.321	0.154
Tannins	0.000	0.000	0.000	0.000	0.000
Saponins	0.315	0.235	0.308	0.265	0.294
Terpenoids	0.0027	0.0000	0.0015	0.0000	0.0000
Steroids	0.0076	0.0085	0.0059	0.0079	0.0000
Flavovoids	0.000	0.0000	0.0042	0.0036	0.0039
Glycosides	0.108	0.103	0.115	0.221	0.000
Triterpenes	0.0016	0.000	0.0019	0.0012	0.0000
Phenolics	0.0000	0.000	0.000	0.000	0.0000

Source: Authors Finding, 2022

Endnotes

[1]A.A. Olajide & I.E. Omomagiowawi. *Antimicrobial Activity of Croton zambesicuson Staphylococcus aureusand Streptococcus species*.**Research Gate Journal of Science.**2018, 4511-19

[2]K.D. Reuben,F.I. Abdulrahman, J.C. Akpan & B. A. Shyranda. *Comparison of the Chemical Constituents and Invitro Antibacterial Efficiencies of the Extracts of Croton zambesicus Stem Bark:* **Journal of Medicinal Plant Research** 2021, 4(19).

[3]E.O. Jude & A.N. Paul *Antimicrobial Activity of Root Extract and Crude Fractions of Croton zambesicus* : **Pakistan Journal of Pharmaceutical Sciences** 23(1): 2020, 114-8.

[4]A.N. Paul & E.O. Jude.*Antimicrobial Activity of Root Extract and Crude Fractions of Croton zambesicus* : **Pakistan Journal of Pharmaceutical Sciences** 23(1): 2021b, 114-8.

Chapter Five

Conclusion

5.1 Summary of Findings

Croton zambesicus leaf and stem has proven effective in inhibiting the growth of certain bacteria organisms thereby necessary for treating diseases caused by infectious bacteria.

5.2 Conclusion

In vitro evaluation of *Croton zambesicus* leaves and stem extracted with ethanol, acetone, chloroform, petroleum ether, and aqueous solution were tested on *Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi, Escherichia coli, Staphylococcus aureus*, and *Enterobacter cloacae*. The ethanolic and acetonic extracts of the leaf and stem were quite effective against all of the bacterial isolates tested. Acetone extract demonstrated a greater zone of inhibition in each of the bacteria studied and is thus regarded as the best solvent for extraction. *Croton zambesicus* leaf and stem extracts have inhibitory properties similar to conventional antibiotics. This confirms the antibacterial properties of plant extracts. The antibacterial action justifies the traditional use of *Croton zambesicus* in the treatment of various bacterial infections. The results also showed that acetone and ethanol are the best extraction solvents for the plant parts used.

Phytochemical analysis of *Croton zambesicus* leaf and stem revealed the presence of alkanoids, saponins, tannins, phlobatannins, steroids, and terpenoids. It can also be concluded that these phytochemicals seem to be responsible for the antimicrobial effects of the extracts of this plant.The plant exhibits different pharmacological activities such as antileishmanial, antidiabetic, anti-inflammatory, immunosuppressive, anticancer activity. Examination of this plant shows that the plants have different medicinal properties.

Croton zambesicus leaves and stem have antibacterial characteristics and can thus be used to make antimicrobial medicines. The indiscriminate use of drugs, particularly antibiotics, should be discouraged; sick patients should be urged to see a doctor in a hospital/clinic, take certain tests, and medicines should be completed in accordance with the test result and the medicine's dose. Massive public awareness efforts are needed to raise public knowledge of the developing trend of antibiotic resistance, its causes, and prevention in order to limit its incidence.Investigate better methods for extracting active antimicrobial compounds from plants and incorporating them into the production of newer, safer, and possibly less expensive medications. As a result, it is widely assumed that the benefits of the aforementioned technique would undoubtedly support potential health outcomes while also collectively reducing the global trend of multidrug resistance concerns.

5.2 Recommendations

1. The medicinal properties of *Croton zambesicus* need to be further investigated, especially the antimicrobial activities of the plant's stem and leaf extract, as well as the identification of its phytochemical components.
2. More study in the fields of pharmacy and medicine should be conducted to isolate, define, and purify the bioactive components of the plant in order to determine the range of antibacterial activity of plant extracts. This could result in the introduction of new antimicrobial compounds that can be used to tackle antibiotic-resistant bacteria, which has become a global problem.

5.3 Contribution to Knowledge

From the result obtained from this work, it has shown that acetone and ethanol extract are the most effective solvent of extraction for *Croton zambesicus* leaf and stem that was collected from Molete market.

5.4 Areas of Further Study

Further research should be done on other aprts of *Croton zabesicus* because the leaf and stem has proven to be effective against some bacteria pathogen.

Bibliography
Journals

A.O.A.C 14th Edition. **Tropical Journal of Pharmaceutical Research** Pg 187-188. INC IIII, North Nineteenth Street, Suite 210 Arlington VA 222-114 2020.

Abdalaziz, N.M.N. Amna, A. & Kabbashi, A.S. *In vitro antioxidant activity and phytochemical screening of Croton zambesicus.* **Journal of Pharmacognosy and Phytochemistry;** 5(6): 2020, 12-16

Abubakar, U. & Tangiisuran, B. *"Nationwide Survey of Pharmacist's Involvement in Antimicrobial Stewardship Program in Nigerian Tertiary Hospitals,"* **Journal of Global Antimicrobial Resistance,** 2019.

Ahovua-Soloranta, A. Rautakorpi, U.M. Borisenko, O.V. Liira, H. Williams & J.W. Makela, M. *Antibiotics for Acute Maxillary Sinusitis* **The Cochrane Library**. 2021, pp 120-234

Aiyelaagbe, O. & Osamudiamen, P.M. *Phytochemical Screening for Active Compounds in Mangifera indica Leaves from Ibadan, Oyo State,* **Plant Sciences Research.** 1(2): 2019; 11-13

Ajayi, A.O. & Omomagiowawi, I.E. *Antimicrobial Activity of Croton zambesicus on Staphylococcus aureus and Streptococcus s*pecies. *Sylwan*., 160: 2018, (10)
Akinnibosun, F. Akinnibosun, H. Ibeh, & Osaghae, I. F. *Antibacterial activity of Phyllanthus amarus schum and thonn on fivevegetative organisms, **Plant Archives*** 8(2): 2018, 563-568

Alamuri, P. & Mobley, H.L.T. *A Novel Autotransporter of Uropathogenic Proteus mirabilis is both a Cytotoxin and an Agglutinin.* **J. Molecular Microbiology** 68: 2018, 997–1017. http://dx.doi.org/10.1111/j.1365-2958.2008.06199.x

Allen's Commercial Organic Analysis, **Int. J. Antimicrob. Agents.** Vol. IX, 2019, Pg. 156-189.

Andrews, J.R. Qamar, F.N. Charles, R.C. & Ryan, E.T. *"Extensively Drug-Resistant Typhoid—are Conjugate Vaccines Arriving Just in Time?,"* **New England Journal of Medicine,** vol. 379, 2018, no. 16, pp. 1493–1495.

Baccelli, C. Martinsen, A. Morel, N. & Quetin-Leclercq, J. *Vasorelaxant activity of essential oils from Croton zambesicus and some of their constituents.* **Planta Med.** 76(14): 2020; 1506-11.

Banso, A. & Adeyemo, S.O. *Phytochemical and Antimicrobial Evaluation of Ethanolic Extract of Dra-caena Manni Bark.***Nig. J. Biotech.** 18: 2018, (1-2):

Barrios-Villa, E. Cortés-Cortés, G. Lozano-Zaraın, P. Arenas-Herna ndez, A. Martınez de la, M. Peña, C.F. & Mart ınez-Laguna, Y. *Adherent/ invasive Escherichia coli (AIEC) Isolates from Asymptomatic People: New E. coli* ST131 O25:H4/H30-Rx virotypes. **J. Ann. Clin. Mic. Antim**. 17: 2018, (1), 42. doi: 10.1186/s12941-018-0295-4

Belyhun, Y. Moges, F. & Endris, M. *Ocular Bacterial Infections And Antibiotic Resistance Patterns in Patients Attending Gondar Teaching Hospital, Northwest Ethiopia***. BMC Res Notes.** 11:2018, 597. doi:10.1186/s13104-018-3705-y

Bernstein, H. Bernstein, C. & Michod, R. *Sex in microbial pathogens, Infection, Genetics and Evolution,* **Int. J. Antimicrob. Agents.** Volume 57, 2016, 8-25.

Bonell, A. Azarrafiy, R. & Huong, V.T. *A Systematic Review and Meta-Analysis of Ventilator-Associated Pneumonia In Adults in Asia: An Analysis of National Income Level on Incidence and Etiology.* **Clin Infect Dis.** 68(3):2018, 511–518. doi:10.1093/cid/ciy543

Bothara, S.S. Kadam, K.R. & Mahadik, K.G. *Antibiotics, Principles of Medicinal Chemistry* **Journal of Antimicrobial Chemotherapy** 1(14thed.) 2020, p81 ISBN 8185790043

Boukhira, S. Mounyr B. Faisl, M. Stephane, M. Sghir, T.M. & Dalila, B. *Antimicrobial Activities of Essential Oil of Five Plant Species from Morocco against some Microbial Strain.* 2021.

Boyom, F.F. Keumdjio, F. Dongmo, P.M. Ngadjui, B.T. Amvam-Zollo, P.H. Menut C, & Bessiere, J.M. *Essential oil from Croton zambesicus Muell. Arg. growing in Cameroun.* **Flav Fragr J.** 17: 2022; 215– 217.

Brunner, J.H. *Direct Spectrophotometer determination of Saponin.* **Anal. Chem.** 34, 2021, pg 1314 - 1326.

Buetti, N. Lo-Priore, E. & Atkinson, A. *Low Incidence of Subsequent Bacteraemia or Fungaemia after Removal of a Colonized Intravascular Catheter Tip.* **Clin Microbiol Infect.** 24:2018, 548e1–548e3. doi:10.1016/j.cmi.2017.09.009

Canessa, A. Del Bono, V. De Leo, P. Piersantelli, N. & Terragna, A. *Cotrimoxazole Therapy of Toxoplasma Gondii encephalitis in AIDS Patients*: **European Journal of Clinical Microbiology and Infectious Diseases**11(2): 2022, 125-130 doi:10.1007/BF01967063. PMID 1396726

Chariandy, C.M. Seaforth, C.E. Phelps, G.V. B. Pollard, & Khambay, B.P. *Screening of Medicinal Plants from Trinidad and Tobago for Antimicrobial and Insecticidal Properties.* **Journal of Ethnopharmacology,** 64(3), 2019, 265-70.

Chatham-Stephens, K. Medalla, F. & Hughes. M. "*Emergence of Extensively Drug-Resistant Salmonella Typhi Infections Among Travelers to or from Pakistan — United States*," **Morbidity and Mortality Weekly Report** (MMWR), vol. 68, no. 1,2019, pp. 11–13.

Chen, C.Y. Chen, Y.H. Lu, P.L. Lin, W.R. Chen, T.C. & Lin, C.Y. *Proteus mirabilis Urinary Tract Infection and Bacteremia: Risk Factors, Clinical Presentation, and Outcomes.* **J. Microbiol. Immunol. Infect**. 45: 2022, 228–236. doi: 10.1016/j.jmii.2011.11.007

China Antimicrobial Resistance Surveillance System (CARSS). *Antimicrobial Resistance of Bacteria: Surveillance Report from China Antimicrobial Resistance Surveillance System in* 2014–2019. **Chin J Infect Control** 20: 2021; 15–31. (in Chinese).

Chong, Y. Shimoda, S. & Shimono N. *Current Epidemiology, Genetic Evolution and Clinical Impact of Extended-Spectrum b-lactamase-producing Escherichia coli and Klebsiella pneumoniae*. **Infect Genet Evol** 61: 2018; 185–188.

CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement.* CLSI document M100- S25. Wayne, PA: **Clinical and Laboratory Standards Institute;** 2019.

Comroe, J.H.*"The Story of Streptomycin. Part I: from Waksman to Waksman* **American Review of Respiratory Diseases** 117 (4): 2018, 773-781 PMID 417651

Creech, C.B. Frenck, R.J. & Sheldon, E.A. *Safety, Tolerability, and Immunogenicity of a Single Dose 4-antigen or 3-antigen Staphylococcus aureus Vaccine in Healthy Older Adults: Results of a Randomised trial.* **J. of Vaccine**. 35(2):2017, 385–394. doi:10.1016/j.vaccine.2016.11.032

Crump, J.A. Sjolund-Karlsson, M. Gordon, M.A. & Parry, C.M. *Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections.***Clin Microbiol Rev**. (4): 2021, 901-37

David, M.Z. & Daum, R.S. *Treatment of Staphylococcus aureus Infections*. **Curr Top Microbiol Immunol**. 409:2017, 325–383. doi:10.1007/82_2017_42

Davin-Regli, A. Jean-Philippe, L. Jean-Marie, P. *Enterobacter spp.: Update on Taxonomy, Clinical Aspects, and Emerging Antimicrobial Resistance.* **Clinical Microbiology Reviews.** Volume 32 Issue 4, 2019

Day, M. Doumith, M. Jenkins, C. Dallman, T.J. Hopkins, K.L. & Elson, R. *.Antimicrobial Resistance in Shiga Toxin-producing Escherichia coli Serogroups O157 and O26 Isolated from Human Cases of Diarrhoeal Disease in Englan*. **J. Antimicrob. Chemother.** 72(1): 2017, 145–152. doi: 10.1093/ jac/dkw371

Deguchi, H. Kitazawa, K. & Kayukawa, K. *The trend of resistance to antibiotics for ocular Infection of Staphylococcus aureus, Coagulase-Negative Staphylococci, and Corynebacterium Compared with 10-years Previous: A Retrospective Observational Study.* **PLoS One.** 13(9):2018, e0203705. doi:10.1371/journal.pone.0203705

Denwick, P.M. *Natural Products A Biosynthetic Approach.* 2nd Edn. John Wiley and Sons, Ltd., England. **Journal of University of Anbar for Pure Science** 2022, 241243.
Dhalwal, K. Shinde, V.M. Mahadik K.R. & Namdeo. A.G. *Raoid Densitometric Method for Simultaneous Analysis of Umbelliferone, Psoralen and Eugenol in Herbal Raw Materials using HPTLC:* **Journal of Separation Science,** 30 (13) 2021, 2053-2058

Dharmananda, S. *Gallnuts and the uses of tannins in Chinese medicine.* **A Paper Delivered at the Institute for Traditional Medicine, Portland, Oregon**, 2020.
Drlica, K. Zhao, X. De Sari, A. & Zaleznik, O.R. *"DNA Gyrase, Topoisomerases IV and the 4-quinolones"*.**Microbiol. Mol. Biol. Rev.**61 (3): 2017, 377-392 PMC 232616 PMID 9293187

Drzewiecka, D. *Significance and Roles of Proteus spp. Bacteria in Natural Environments.* **Microb. Ecol**. 72: 2019, 741–758. doi: 10.1007/s00248-015-0720-6

Ducarmon, Q.R. Zwittink, R.D. Hornung, B.V.H. Van-Schaik, W. Young, V.B. & Kuijper, E.J. *Gut Microbiota and Colonization Resistance against Bacterial Enteric Infection*. **Microbiol. Mol. Biol. Rev.** 83 2019, (3), e00007–e00019. doi: 10.1128/MMBR.00007-19

Efah, C.Y. Sun, T. Liu, S. & Wu, Y. *Klebsiella pneumoniae: An Increasing Threat to public Health*. **Annals of Clinical Microbiology and Antimicrobials.** 19: 2020, 1
Eichenberger, E.M. & Thaden, J.T. *Epidemiology and Mechanisms of Resistance of Extensively Drug Resistant Gram-negative bacteria*. **J Antimicrob Chemother** 8(2): 2019; 37.

Emmerson, A.M. & Jones, A.W. *"The Quinolones: Decades of Development and Use"* **The Journal of Antimicrobial Chemotherapy**. 51: 2020, 13-20 doi:10.1093/jac/dkg208. PMID 12702699

European Centre for Disease Prevention and Control. *Surveillance of Antimicrobial Resistance in Europe 2018*. 2019; https://www.ecdc.europa.eu/sites/default/files/docum ents/surveillance-antimicrobial-resistance-Europe2018. Accessed 11th May, 2020.

Fierer, J. & Guiney, D.G. *Diverse Virulence Traits Underlying Different Clinical Outcomes of Salmonella infection*.**J. Clin Invest**.107 (7): 2021, 775-80.

Geddes, A.M. *Introduction: Historical Perspective and Development of Amoxicillin/Clavulanate.* **Int. J. Antimicrob. Agents** 30(Suppl 2): 2017, S109-12. doi:10.1016/j.ijantimicag.2007.07.015. PMID 17900874

George, M. Iramiot, J.S. Muhindo, Olupot-Olupot, P. & Nanteza, A. *Bacterial Aetiology and Antibiotic Susceptibility Profile of Post-Operative Sepsis among Surgical Patients in a Tertiary Hospital in Rural Eastern Uganda.* **Microbiol Res J Int.** 24(2):2018, 1–8. doi:10.9734/MRJI

Gillies, M. Ranakusuma, A. Hofffman, T. Thorning, S. McGuire, T. & Glasziou, P. *Common Harms from Amoxicillin: A Systematic Review and Meta-Analysis of Randomized Placebo-Controlled Trials for any Indication. CMAJ:* **Canadian Medical Association / Journal del'Associationmedicale Canadienne** 187: 2021, E21-31. doi:10.1503/cmaj.140848. PMID 25404399.

Girish, H. & Satish, S. *Antibacterial Activity of Important Medicinal Plants on Phytopathogenic Xanthomonas Compestris Pathovars*, 2018, ***Lett Microbiol*** 28: 145-147.

Gonzalez-Perez, C.J. Tanori-Cordova, J. Aispuro-Hernandez, E. VargasArispuro, I. & Martinez-Tellez, M.A. *Morphometric Parameters of Foodborne Related-Pathogens Estimated by Transmission Electron Microscopy and their Relation to Optical Density and Colony Forming Units.* **J. Microbiol.** Methods 165:2019, 105691 doi: 10.1016/j.mimet.2019.105691

Gu, D.X. Huang, Y.L. Ma, J.H. Zhou, H.W. Fang, Y. Cai, Y.C. Hu, Y.Y. & Zhang, Y. *Detection of Colistin Resistance Gene mcr-1 in Hypervirulent Klebsiella pneumoniae and Escherichia coli Isolates from an Infant with Diarrhea in China.* **Antimicrob Agents Chemother.** 60(8): 2019; 5099–100

Hammer, C.C. Brainard, J. & Hunter, P. R. *Risk Factors and Risk Factor Cascades for Communicable Disease Outbreaks in Complex Humanitarian Emergencies: A Qualitative Systematic Review*. **BMJ Glob. Health** 3:e000647:2018.

Hansson, K.L. Sundstro, M. Pelletier, A. & Roy, P.H. *Intl2 Integron Integrase in Tn7.* **Journal of Bacteriology** 184: 2022, 1712–1721

Haque, M. Sartelli, M. Mckimm, J. & Bakar, M.A. *Health Care Associated Infections–an Overview.* **Infect. Drug Resist**. 11:2018 2321–2333. doi: 10.2147/IDR.S177247

Hauser, A.R. *"Antibiotics Basics for Clinicians" 2nd ed. Lippincott, Williams and Wikins*. **Int. J. Antimicrob. Agents** 2018, pp.25-26

Hegerle, N. Choi, M. Sinclair, J. Amin, M.N. Ollivault-Shiflett, M. & Curtis, B. *Development of a Broad Spectrum Glycoconjugate Vaccine to Prevent Wound and Disseminated Infections with Klebsiella pneumoniae and Pseudomonas aeruginosa*. **PLoS One** 13:e0203143:2018. doi: 10.1371/**journal. pone**.0203143
http://dx.doi.org/10.17352/ijvsr.000031

Huang, Y. Jiao, Y. & Zhang, J. *Microbial Etiology and Prognostic Factors of Ventilator-Associated Pneumonia: A Multicenter Retrospective Study in Shanghai.* **Clin Infect Dis.** z7(S2): 2018, S146–S152. doi:10.1093/cid/ciy686

Huttner, R. Angela, P. Verhaegh, E.M. Harbarth, O. Stephan, M. Muller, K.I. Anouk E.T. & Johan, W.I. Nitrofurantoin Revisited: A Systemic Review and Meta-Analysis of Controlled Trials: **Journal of Antimicrobial Chemotherapy** (9): 2021, 2456-2464 doi: 10.1093/jac/dkv147. ISSN 0305-7453. PMID 26066581

Ito, A. Nishikawa, T. Ota, M. Ito-Horiyama, T. Ishibashi, N. Sato, T. Tsuji, M. & Yamano, Y. *Stability and low Induction Propensity of Cefiderocol against Chromosomal AmpC -lactamases of Pseudomonas aeruginosa and Enterobacter cloacae*. **J Antimicrob Chemother,**201873: 2018, 3049 –3052.

Jacobsen, S.M. Stickler, D.J. Mobley, H.L. & Shirtliff, M.E. *Complicated Catheter-Associated Urinary Tract Infections due to Escherichia coli and Proteus mirabilis.* **Clin. Microbiol. Rev.** 21: 2018, 26–59. doi: 10.1128/CMR.00019-07

Jamil, R.T. Foris, L.A. & Snowden, J.*"Proteus mirabilis Infections," in StatPearls.* **J. Antimicrob. Chemother.** *(Treasure Island, FL: StatPearls Publishing)*. 2020, Available online at: https://www.ncbi.nlm.nih.gov/books/NBK442017/

Jan, W. Sebastian, H. & Chantal, Q. *Healthcare-Associated Pneumonia in Acute Care Hospitals in European Union/European Economic Area Countries: An Analysis of Data from a Point Prevalence Survey, 2011 to 2012.* **Euro Surveill.** 23 (32):2018, 1700843.

Jiu, Y. Meng, X. Hong, Q. Huang, C. Wang, & Z. Chenl. *Prevalence and Characterization of Salmonella in three Typical Commercial Pig Abattoirs in Wuhan, China.* **Foodborne Pathog**. 2019, Dis. doi: 10.1089/fpd.2019.2737 [Epub ahead of print].

Jude E.O. & Paul, A.N. *Antimicrobial Activity of Root Extract and Crude Fractions of Croton zambesicus* : **Pakistan Journal of Pharmaceutical Sciences** 23(1): 2020, 114-8.

Kaca, W. Glenska, J. Lechowicz, S. Grabowski, A. Brauner, M. & Kwinkowski, M. *Serotyping of Proteus mirabilis Clinical Strains Based on Lipopolysaccharide O Polysaccharide and Core Oligosaccharide Structures.* **Published in Russian in Biokhimiya** 76(7): 2019, 1039-1050.

Kang, J.S. Yi, J. Ko, M.K. Lee, S.O. Lee, J.E. & Kim, K.H. *Prevalence and Risk Factors of Carbapenem-Resistant Enterobacteriaceae Acquisition in an Emergency Intensive Care Unit in a Tertiary Hospital in Korea: A Case-control Study.* **J. Korean Med. Sci.** 34:e140:2019 doi: 10.3346/ jkms.2019.34.e140

Kawahara, E.N. *Ofloxacin, Resistant Bacteria Strains:* **Microbiol. Mol. Biol.Rev**. 52(2): 2018, 237-239 PMC 232514 PMID 9392185

Kern, W.V. & Rieg. *S. Burden of Bacterial Bloodstream Infection-a Brief Update on Epidemiology and Significance of Multidrug-Resistant Pathogens*. **Clin Microbiol Infect**.26(2):2020, 151–157.

Khamash, D.F. Voskertchian, A. & Tamma, P.D. *Increasing Clindamycin and Trimethoprim-Sulfamethoxazole Resistance in Pediatric Staphylococcus aureus Infections***. J Pediatric Infect Dis Soc.** 2018;XX(XX):1–3. (Ahead of print).

Khan, K.H. Deepak G. & Bhaskara, K.V. *Recent Advancement in Typhoid Research - a Review*. **Advanced Biotech.** 2018, pp. 36

Khan, M.F. *Brief History of Staphylococcus aureus: A Focus to Antibiotic Resistance.* **EC Microbiology.** 5: 2017; 36-9.

Klemm, E.J. Shakoor, S. & Page. A.J. *"Emergence of an Extensively Drug-Resistant Salmonella enterica Serovar Typhi Clone Harboring a Promiscuous Plasmid Encoding Resistance to Fluoroquinolones and Third-Generation Cephalosporins,"* mBio, vol. 9, 2018, no. 1.

Knirel, Y.A. *Structure of the O-antigenic Polysaccharides of Proteus bacteria.* **Polish Journal of Chemother** 73: 2019, 895–9071. http://dx.doi.org/10.1007/s00005-006-0031-1

Kremer, L. Walton, M. & Wardle, E.N. *Nalidixic Acid and Intracranial Hypertension***. British Medical Journal** 4(5577): 2019, 488 doi:10.1136/bmj.4.5577.488-a PMC 1748506. PMID 6055749

Leong, H.N. Kurup, A. & Tan, M.Y. *Management of Complicated Skin and Soft Tissue Infections with a special Focus on the Role of Newer Antibiotics.* **Infect Drug Resist.** 11:2018, 1959–1974. doi:10.2147/IDR.S172366

Liaw, S.J. Lai, H.C. & Wang, W.B. *Modulation of Swarming and Virulence by Fatty Acids through the RsbA Protein in Proteus mirabilis Infection and Immunity*. 72(12): 2019, 6836–6845. http://dx.doi.org/10.1128/IAI.72.12.6836-6845.2004

Lim, W.W. Wu, P. & Bond, H.S. *Determinants of MRSA Prevalence in the Asia Pacific Region: a Systematic Review and Meta-Analysis.* **J Global Antimicrobial Resist.** 16:2018, 17–27. doi:10.1016/j.jgar.2018.08.014

Lin, Z. Yu, J. Liu, S. & Zhu, M. *Prevalence and Antibiotic Resistance of Klebsiella pneumoniae in a Tertiary Hospital in Hangzhou, China, 2006–2020* **Journal of International Medical Research,** 50(2): 2020, 1–10

Lopeze, N. Jose, M. Quiros, P. Yaremi, Z. Vicente, T. Laura, L. Morales, S. Ana, T. Lopez, N & Francisco, J. *New Insights into the Mechanism of Aminoglycoside: An Integrative Point of View Kidney International* **American Review of Respiratory Diseases**79; 2021, 33 PMID 20861826

Lustri, B.C. Sperandio, V. & Moreira, C.G. *Bacterial Chat: Intestinal Metabolites and Signals in Host-Microbiota-Pathogen Interactions*. Infec. Immun. **Journal of University of Anbar for Pure Science** 85: 2017, (12), e00476. doi: 10.1128/IAI.00476-17

Malhotra S. & Pal. A.S. *A Review of Pharmacology of Phytochemicals from Indian Medicinal Plants.* The Internet. **Journal of Alternative Medicine**. 5(1): 2019; 23-27.

Malihar, G. & Zehra, A. *Staphylococcus aureus: A Brief Review*. **International Journal of Veterinary Science and Research.** 4(1): 2018, 020-022. DOI:

Manikandan, A. Durka, M. Selvi, M. & Anthony, S. *Sesamum Indicum Plant Extracted Microwave Combustion Synthesis and Opto-magnetic Properties of Spinel Mn* *** Co1-* Al204 nano-catalysts; Journal of Nanoscience and Nanotechnology,** 16 (1), 2018,448-456.

Martínez, M. Retamal, P. Rojas-Aedo, J. Fernández, J. Fernández, A. & Lapierre, L. *Multidrug-Resistant Outbreak-Associated Salmonella Strains in Irrigation Water from the Metropolitan Region, Chile*. **Zoonoses Public Health** 64: 2017, 299–304. doi: 10.1111/zph.12311

Martinsen, A. Chiar, B. Ismae, N. Antonio, A. Quetin-Leclercq, J. & Nicole, M. *Vascular activity of a natural diterpene isolated from Croton zambesicus and of a structurally similar synthetic trachylobane*. **Vasc Pharmacol**. 52(1/2): 2020; 63-69.

Masalha, M. Borovok, I. Schreiber, R. Aharonowitz, & Cohen, G. *Analysis of Transcription of Staphylococcus aureus Aerobic Class 1b and Anaerobic Class III Ribonucleotide Reductase Genes in Response to Oxygen.* **Journal of Bacteriology** 183: 2021, 7260-7272. Link: https://goo.gl/ChuYAJ

McKinnell, J.A. Stollenwerk, J.A. Jung, C.W. & Miller, L.G. *Nitrofurantoin Compares "Favourably to Recommended Agents as Empirical Treatment of Uncomplicated*

Urinary Tract Infections in a Decision and Cost Analysis. ***Mayo Clinic Proceedings*** 86(6): 2021, 480-8. doi:10.4065/mcp.2010.0800. PMC 3104907. PMID 21576512

Meryem, C. & Nergis, A. *Antimicrobial Resistance Profiles of Enterobacter cloacae and Klebsiella aerogenes a Tertiary Hospital in Turkey:* A five-years study. ***Medicine Science Discovery***. 8(3): 2019; 161-166

Methods of Analysis of Analytical Methods Committee of Royal Society of Chemistry . **Int. J. Antimicrob. Agents.** AMC-RSC. 2020, pp222-229

Metlay, J.P. Waterer, G.W. Long, A.C. Anzueto, A. Brozek, J. & Crothers, K. *Diagnosis and Treatment of Adults with Community-Acquired Pneumonia. An Official Clinical Practice Guideline of the American Thoracic Society and Infectious Diseases society of America.* **Am. J. Respir. Crit. Care Med.** 200, e45–e67:2019 doi: 10.1164/rccm.201908-1581ST

Miklasinska-Majdanik, M. Kepa, M. & Wojtyczka, R.D. *Phenolic Compounds Diminish Antibiotic Resistance of Staphylococcus aureus Clinical Strains.* **Int J Environ Res Public Health** 15:2018, E2321. doi:10.3390/ijerph15061188

Mims, C. Dockrell, H.M. & Goering, R.V. *Attacking the Enemy: Antimicrobial Agents and Chemotherapy: Macrolids.* **In: Medical Microbiology 3rd Edition, LondonMobsy Ltd**: 2018, p489

Monye, I. & Adelowo, A.B. *Strengthening Immunity through Healthy Lifestyle Practices: Recommendations for Lifestyle Interventions in the Management of Covid-19*. **Lifesty. Med**. 1:e7. doi: 2020, 10.1002/lim2.7

Moradigaravand, D. Reuter, S. Martin, V. Peacock, S.J. & Parkhill, J. *The Dissemination of Multidrug Resistant Enterobacter cloacae throughout the UK and Ireland*. **J Antimicrob Chemother** 2019

Nagarjuna, D. Mittal, G. Dhanda, R.S. Gaind, R. & Yadav, M. *Alarming Levels of Antimicrobial Resistance among Sepsis Patients Admitted to ICU in a Tertiary Care Hospital in India - A Case Control Retrospective Study.* **Antimicrob. Resist. Infect. Control** 7: 2018, 150. doi: 10.1186/s13756-018-0444-8

Navon-Venezia, S. Kondratyeva, V. & Carattoli, A. *Klebsiella pneumoniae: A Major Worldwide Source and Shuttle for Antibiotic Resistance*. **FEMS Microbiol Rev.** 41(3): 2017; 252–75.

O'Brien, E.C. & McLoughlin. R.M. *Considering the 'Alternatives' for Next-Generation Anti-Staphylococcus aureus Vaccine Development*. **Trends in Mol Med.** 25(3):2019, S1471–4914.

Odukoya, O.A. Sofidiya, M.O. Samuel, A.T. Ajose, I. Onalo, M. & Shuaib, B. *Documentation of Wound Healing Plants in Lagos-Nigeria: Inhibition of Lipid Peroxidation as In-vivoPrognostic Biomarkers of Activity,* **Annals of Biological Research,** 3(4): 2022, 1683-1789

Ogunbajo, A. Ogunwande, I.A. Gbadamosi, H. Giwa, R. & Flamini, G. *Chemical Composition of the Leaf Essential Oils of Croton zambesicus*; **European Journal of Medicinal Plants,** 4(12): 2019, 1524-1533.

Okokon, J. Davies, K. & Bassey, A. *Psycopharmacological study of Ethanol Leaf Extract of Solenostemon monostachyus*; **Journal of Applied Pharmaceutical Research,** 9(3): 2020, 59-63,

Okokon, J.E. & Nwafor, P.A. *Antiplasmodial Activity of Root Extract and Fractions of Croton zambesicus*. **J. Ethnopharm**. 121: 2019; 74-78.

Okokon, J.E. Dar, A. & Choudhary, M.I. *Immunomodulatory, Cytotoxic and Antileishmanial activities of Phytoconstituents of Croton zambesicus Phytopharmacol.* **J. Ethnopharm** 4(1): 2018; 31 – 40.

Okokon, J.E. Nwafor, P.A. & Noah, K. *Nephroprotective effect of Croton zambesicus root extract against gentimicin-induced kidney injury.***Asian Pac J Trop Med.** 4: 2021; 969-972.

Olajide A.A. & Omomagiowawi. I.E. *Antimicrobial Activity of Croton zambesicuson Staphylococcus aureusand Streptococcus species*.**Research Gate Journal of Science.**2018, 4511-19

Olajide, A.A. Goshen, D.M. & Blessing, C. *Antimicrobial Activity Of Chitosan, Moringa Oleifera, Croton Zambesicus And Their Synergistic Activity As Immune Booster.***Int. J. Adv. Res**. 9(10), 2021, 495-502

Olorunfemi, P.O. Onaolapo, J.A. & Ibrahim, K.E. *Prevalence and Antibiotic Susceptibility of Community acquired Methicillin Resistant Staphylococcus aureus from Healthy Students of University of Jos.* **J. Pharmacy & Bioresources** . Vol. 17 no. 2, 2019, pp. 131-141 (September 2020)

Onanuga A. & Awhowho, G.O. *"Antimicrobial Resistance of Staphylococcus aureus Strains from Patients with Urinary Tract Infections in Yenagoa, Nigeria,"***Journal of Pharmacy & Bioallied Sciences**, vol. 4, no. 3, 2021, pp. 226,

Onwusonye, J.C. Muritala, I.K. Odu, D.A. Uzomba, N.I. Igbonnaya, N. & Duruamaku, C.C. *Oral acute toxicity study of methanol leaf extracts of Croton Zambesicus in mice*. **Merit Research Journal of Pharmacy and Pharmaceutical Sciences** Vol. 2(1) 2019, pp. 001-005.

Parry, C.M. Hien, T.T. Dougan, G. White, N.J. & Farrar, J.J. *Typhoid Fever.* **N Engl J Med.** 28: 2022, 347(22):1770-82

Patridge, S.R. Collis, C.M. & Hall, R.M. *Class 1 Integron Containing a New Gene Cassette, aadA10, associated with Tn1404 from R151.* **Antimicrobe Agents Chemother** 46: 2022, 2400–2408. http://dx.doi.org/10.1128/AAC.46.8.2400- 2408.2002

Paul, A.N. & Jude, E.O. *Antimicrobial Activity of Root Extract and Crude Fractions of Croton zambesicus* : **Pakistan Journal of Pharmaceutical Sciences** 23(1): 2021b, 114-8.

Pawłowska, B. & Sobieszczańska, B.M. *Intestinal Epithelial Barrier: The target for pathogenic Escherichia coli. Adv. Clin. Exp. Med*. 26 (9), 2017, 1437–1445. doi: 10.17219/acem/64883

Phan, H. & Lehman, D. *Cerebral Abscess Complicating Proteus mirabilis Meningitis in a Newborn Infant.* **Journal of Child Neurology** 27(3): 2022, 405-407. https://doi.org/10.1177/0883073811422271 *Pharmacists.Delmar*. **J Antimicrob Chemother** 2018, p.296. ISBN 9781428361065

Pichichero, M.E. *"Cephalosporins can be prescribed safely for penicillin-allergic patients"* **The Journal of Family Practice**. 55 (2): 2021, 106-12 PMID 16451776

Prescott, M. Harley, P. & Klen, D. *General Microbiology. McGrawHill Publishing Company* **Int. J. Antimicrob. Agents**, New Delhi 7th edition, 2018, 163-850.

Qian, M. Zhao, T. & Li. R. *Targeting the R domain of coagulase by active vaccination protects mice against lethal Staphylococcus aureus infection*. **Microbes Infect.** S1286–4579. (18): 2018, 30177-1.

Radhakrishnan, A. Als, D. & Mintz. E.D. *"Introductory Article on Global Burden and Epidemiology of Typhoid Fever,"* **The American Journal of Tropical Medicine and Hygiene,** vol. 99:2018 3_Supplement, pp. 4–9.

Rakitina, D.V. Manolov, A.I. Kanygina, A.V. Garushyants, S.K. Baikova, J.P. & Alexeev, D.G. *Genome Analysis of E. coli Isolated from Crohn's Disease Patients.* **BMC Genomics,** 18 (1): 2017, 544. doi: 10.1186/s12864-017-3917-x

Ramasamy, A. Das, S. Mani, V. Sengottuvelu, S. & Prabhu, V. *Evaluation of Anti-diarrheal Potential of Hydroalcoholic Extracts of Leaves of Murraya koenigii in Experimental Animals,* **Journal of Dietary Supplements,** 13: 2016, 393–401.

Ramirez, D. & Giron, M. *Enterobacter Infections*. In: StatPearls (Internet). Treasure Island (FL): **StatPearls Publishing**; 2020.

Rasheed, M.K. Hasan, S.S. Babar, D. & Ahmed, S.I. "*Extensively Drug-Resistant Typhoid Fever in Pakistan*," **The Lancet Infectious Diseases**, vol. 19, no. 3, pp. 242-243, 2019.

Reuben, K.D. Abdulrahman, F.I. Akpan J.C. & Shyranda. B.A. *Comparison of the Chemical Constituents and Invitro Antibacterial Efficiencies of the Extracts of Croton zambesicus Stem Bark:* **Journal of Medicinal Plant Research** 2021, 4(19).

Russo, T.A .& Marr, C.M. *Hypervirulent Klebsiella pneumonia.* **Clin. Microbiol. Rev**. 32, e00001–e00119:2019 doi: 10.1128/CMR.00001-19

Russo, T.A. & Marr. C.M. *Hypervirulent Klebsiella pneumoniae.* **Clin. Microbiol. Rev.** 32, e00001–e00119. 2019, doi: 10.1128/CMR.00001-19

Sabat, A.J. Tinelli, M. & Grundmann, H. *Daptomycin Resistant Staphylococcus aureus Clinical Strain with Novel Non-Synonymous Mutations in the mprF and vraS Genes: A New Insight into Daptomycin Resistance.* **Front Microbiol.** 9:2018, 2705. doi:10.3389/fmicb.2018.02705

Saif, T.J. & Farhan, A.S. *Klebsiella Pneumonia: Epidemiology, Virulence Factors and Treatment.* **Journal of University of Anbar for Pure Science** (JUAPS). 14 (2): 2020, 5 – 10

Salatino, A. Salatino M.L.F. & Negri, G. *Traditional Uses, Chemistry and Pharmacology of Croton species (Euphorbiaceae)* **J. Braz. Chem. Soc**. 18: 2019; 11–33.

Salimiyan, R.K. Ghazvini, K. and Farsiani, H. *Clinical and Pathogenesis Overview of Enterobacter Infections*. **Rev Clin Med.** 6(4): 2020; 146-54.

Samah, A.A. Aisha Z. & NaserEldin. B.M. *Antimicrobial Activity of Croton zambesicus, Acacia ehrenbergiana, and Fagoniacretica.* **National Journal of Advanced Research**. Vol 2; Issue 3; 2019, Pg No. 16-20

Sampson, J.H. Phillipson, J.D. Bowery, N.G. O'Neill, M.J. Houston, J.G. & Lewis, J.A. *Phytotherapy Research*, **J. Chem. Soc**. 14, 2020, 24.

Santos, B.V. Melchior, K. & Moreira, C.G. *Escherichia coli as a multifaceted Pathogenic and Versatile Bacterium*. FCIMB. **J. Antimicrob. Chemother.** Vol 10, 2020

Shah, A. Marwat, S.K. Gohar, F. Khan, A. Bhatti, K.H. & Amin M. *Ethnobotanical Study of Medicinal Plants of Semi-Tribal Area of Makerwal & Gulla Khel (lying between Khyber Pakhtunkhwa and Punjab Provinces)*, **Pakistan. Am. J. Plant Sci**., 4: 2018, 98-116.

Singh, A. & Singh, P.K. *"An Ethnobotanical Study of Medicinal Plants in Chandauli District of Uttar Pradesh, India*," **Journal of Ethnopharmacolgy**, Vol. 121, No. 2, 2019, pp.324-329. doi:10.1016/j.jep.2008.10.018

Stanaway, J.D. Reiner, R.C. & Blacker, B.F. *"The Global Burden of Typhoid and Paratyphoid Fevers: A Systematic Analysis for the Global Burden Of Disease Study"* **The Lancet Infectious Diseases,** vol. 19, no. 4, 2019, pp. 369–381,

Suleyman, G.R. & Zervos, M.J. "Safety and Efficacy of Commonly used Antimicrobial Agents in the Treatment of Enterococcal Infections: A Review.".Expert Opinion on Drug Safety**. Int. J. Antimicrob. Agents** 15 (2): 2019, 153-67 doi: 10.1517/14740338.2016.1127349 PMID 26629598

Surwade, P. Ghildyal, C. & Weikel, C. *Augmented antibacterial Activity of Ampicillin with Silver Nanoparticles against Methicillin-resistant Staphylococcus aureus (MRSA).* **J Antibiotics.** 2(1):2019, 50–53. doi:10.1038/s41429-018-0111-6

Tacconelli E. & Magrini, N. *Global Priority List of Antibiotic-Resistant Bacteria to guide Research, Discovery, and Development of New Antibiotics.* **World Health Organization,** 2017.

Taylor, T.A. & Unakal, C.G. *"Staphylococcus aureus," In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*; **J**. **Adv. Urol.** 2019.

Tian, L. Sun, Z. & Zhang, Z. *Antimicrobial Resistance of Pathogens Causing Nosocomial Bloodstream Infection in Hubei Province, China, from 2014 to 2016: a Multicenter*

Retrospective Study. **BMC Public Health**. 18:2018, 1121. doi:10.1186/s12889-018-6013-5

Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. *"Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management,"* **Clinical Microbiology Reviews**, vol. 28, no. 3, 2019, pp. 603-661.

Verpoorte, R. *Pharmacognosy in the new Millennium: Leadfinding and Biotechnology.* **Journal of Pharmacy and Pharmacology,** 52, 2020, 253-262

Walter, S. *"Drug Discovery: History*" **Int. J. Antimicrob. Agents** 23 (3): 2020, 101-104 PMID 23892644

Wang, X. Biswas, S. Paudyal, N. Pan, H. Li, X. & Fang, W. *Antibiotic Resistance in Typhimurium Isolates Recovered from the Food Chain through National Antimicrobial Resistance Monitoring System between 1996 and 2016.* 2019, **Front. Microbiol.** 10:2019, 985. doi: 10.3389/fmicb.2019.00985

Wasfi, R. Samira, M.H. Mai, A.A. & Fahmy, L.I. *Proteus mirabilis Biofilm: Development and Therapeutic Strategies.***Frontier in Cellular and Infection Microbiology**. Vol 10. 2020, Art 414

Weniger, B. Lagnika, L. Djakou, L.B. VonthronSénéchaux, C. & Sanni, A. *L'ethnopharmacologie et la recherche de molécules antipaludéennes dans la biodiversité ivoirienne, béninoise et camerounaise.***Ethnopharmacologia** 41: 2020, 62- 70

Woods, E.I. & Adrienne, L.R. *The American Socirty of Health-System*
Xu, Z. Manukumar, H.M. & Rakesh, K.P. *Role of Bap-Dependent Multicellular Behavior of Clinically Important Methicillin-Resistant Staphylococcus aureus (MRSA) Biofilm adherence: A Key Virulence Study.* **Microb Pathog.** 123:2018, 275–284. doi:10.1016/j.micpath.2018.07.025

Yadav, N.S. Sharma, S. & Chaudhary, D.K. *Bacteriological Profile of Neonatal Sepsis and Antibiotic Susceptibility Pattern of Isolates Admitted at Kanti Children's Hospital, Kathmandu, Nepal.* **BMC Res Notes**. 11:2018, 301. doi:10.1186/s13104-018-3394-6

Yao, B. Xiao, X. Wang, F. Zhou, L. Zhang, X & Zhang, J. *Clinical and Molecular Characteristics of Multi-clone Carbapenem-Resistant Hypervirulent (Hypermucoviscous) Klebsiella pneumoniae Isolates in a Tertiary Hospital in Beijing, China.* **Int J Infect Dis**. 1(37): 2019; 107–12.

Zafar, U. Taj, M.K. & Nawaz, I. *Proteus mirabilis as a Pathogenic Organism.* **International Journal of Biosciences** | IJB | Vol. 14, No. 3, 2019: p. 443-450.

Websites

Available online at:https://www.ncbi.nlm.nih.gov/books/NBK442017/

European Center for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2018. 2019; https://www.ecdc.europa.eu/sites/default/files/documents/surveillance-antimicrobial-resistance-Europe2018.pdf Accessed May 11, 2020. https://doi.org/10.1177/0883073811422271

https://www.prota4u.org/database/protav8.asp?g=pe&p=Croton+lobatus+L.#:~:text=Croton%20lobatus%20occurs%20in%20the,east%20to%20Eritrea%20and% 20 Ethiopia.

https://www.prota4u.org/database/protav8.asp?g=pe&p=Croton+lobatus+L.#:~:text=Croton%20lobatus%20occurs%20in%20the,east%20to%20Eritrea%20and% 20 Ethiopia.

Books

A Textbook of Pharmacognosy. Bailler Tindall Ltd, London; 1989:53

A textbook titled Plant Alkaloids, 2008 pg 6-466

Handbook of Plant Nutrition. Edited by Allen V. Barker and Davids Pulbeam, 2007, 156-189.

Allen's Commercial Biological Analysis, 1992, Vol. See pages 167 to 171.

Analytical Methods of the Analytical Methods Committee of the Royal Society of Chemistry.:1986: AMC-RSC.pp222-239.

AOAC (1984) 14th edition. Pages 187-188. INC IIII, North Nineteenth Street, suite 210 Arlington VA 222-114.

Organic Analysis, 1979, vol. IX,

Phytotherapies in Dera Ismail Khan District, KPK, Pakistan", Pakistan Journal of Botany, vol. 43, No. 3, 2011, pages 1453-14624.

Qureshi, R. "Floristic and Ethnobotanical Survey of Nara Desert Region, Sindh", Pakistan Research Repository, Shah Abdul Latif University, Khairpur, 2004, pp. 1-454.

Royal Society of Chemistry Analytical Methods Committee Methods of Analysis 1986: AMC-RSC.pp222-229

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Appendices

Appendix 1

In Vitro **Antimicrobial Activity of the Aqueous extract of** ***Croton zambesicus*** **Leaf on** ***Escherichia coli*** **Showing Zones of Inhibition**

Appendix 2

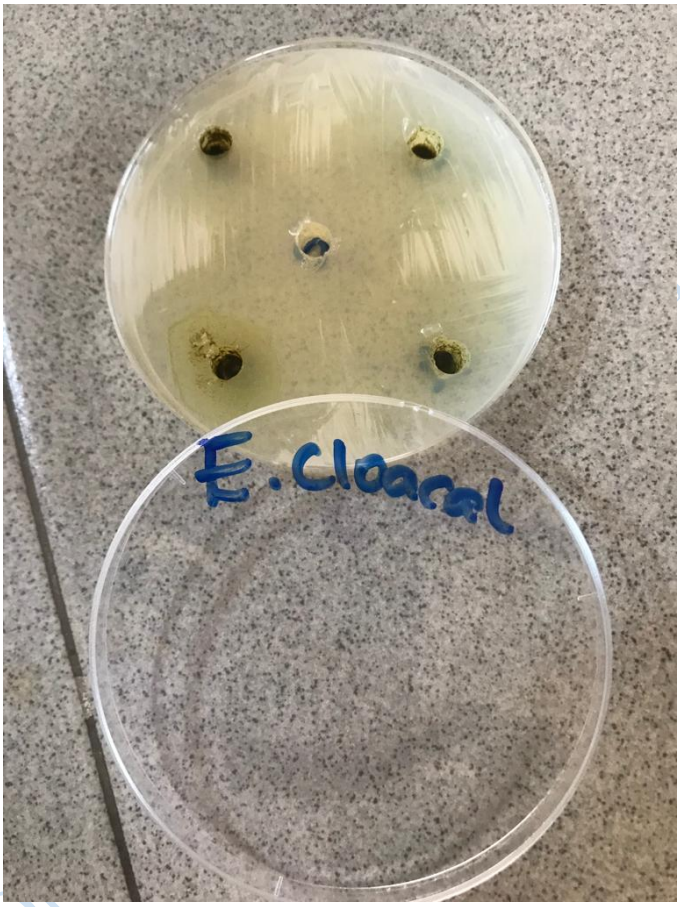
An Aqueous Extract of *Croton zambesicus* Stem Demonstrated Antibacterial Action against *Escherichia coli* in Vitro, with Zones of Inhibition

Appendix 3

In Vitro Antibacterial Activity of *Croton zambesicus* Stem Acetone Extract against *Enterobacter cloaca* Revealing Zones of Inhibition

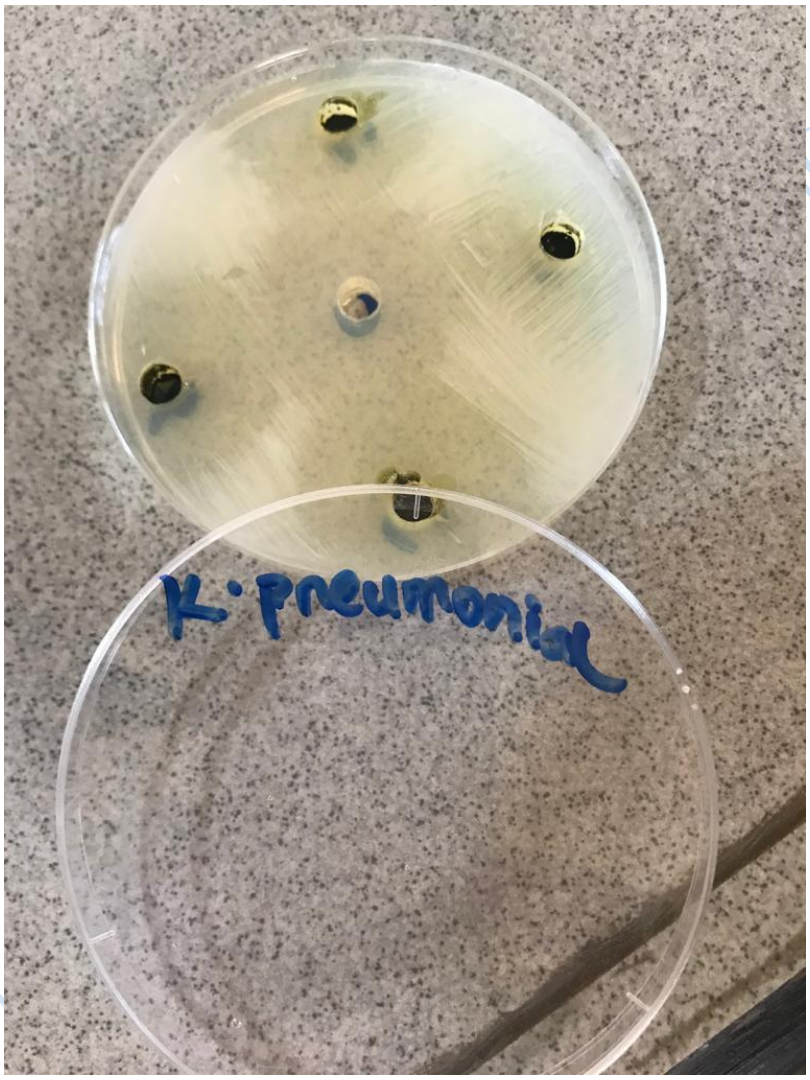
Appendix 4

***In Vitro* Antimicrobial Activity of *Croton zambesicus* Leaf Acetone Extract on *Enterobacter cloacal* showing Zones of Inhibition**



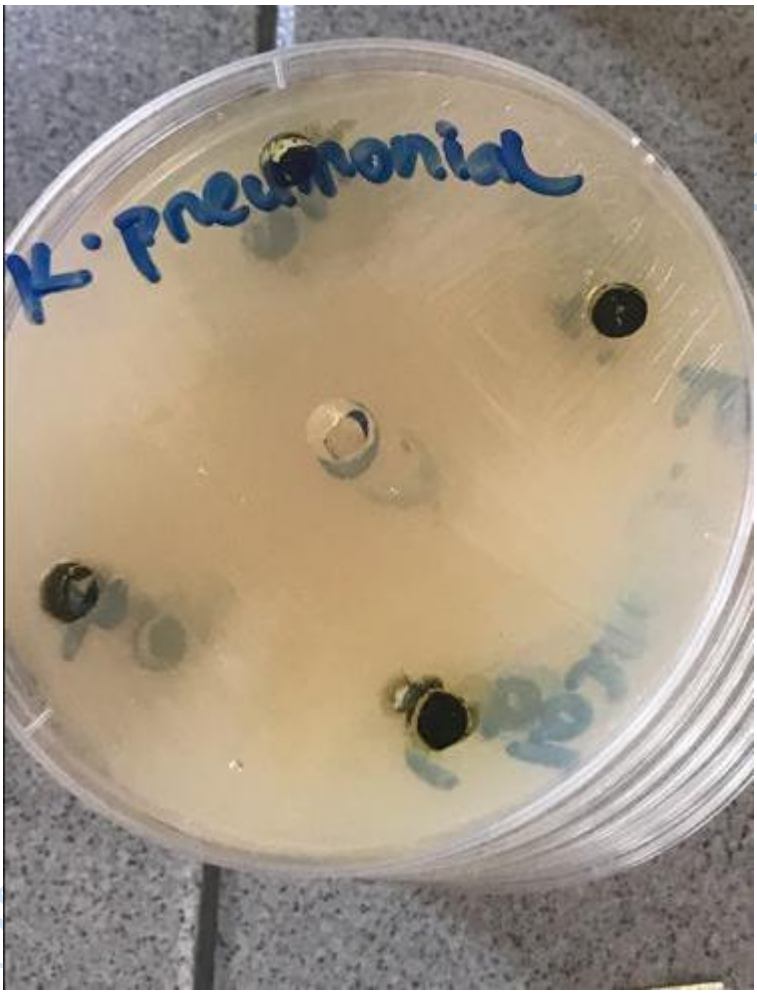
Appendix 5

***In Vitro* Antimicrobial Activity of Acetone Extract from *Croton zambesicus* Leaf on *Klebsiella pneumonia* Showing Zones of Inhibition**



Appendix 6

In Vitro Antimicrobial Activity of Acetone Extract from Croton zambesicus Leaf on Klebsiella pneumonia Showing Zones of Inhibition

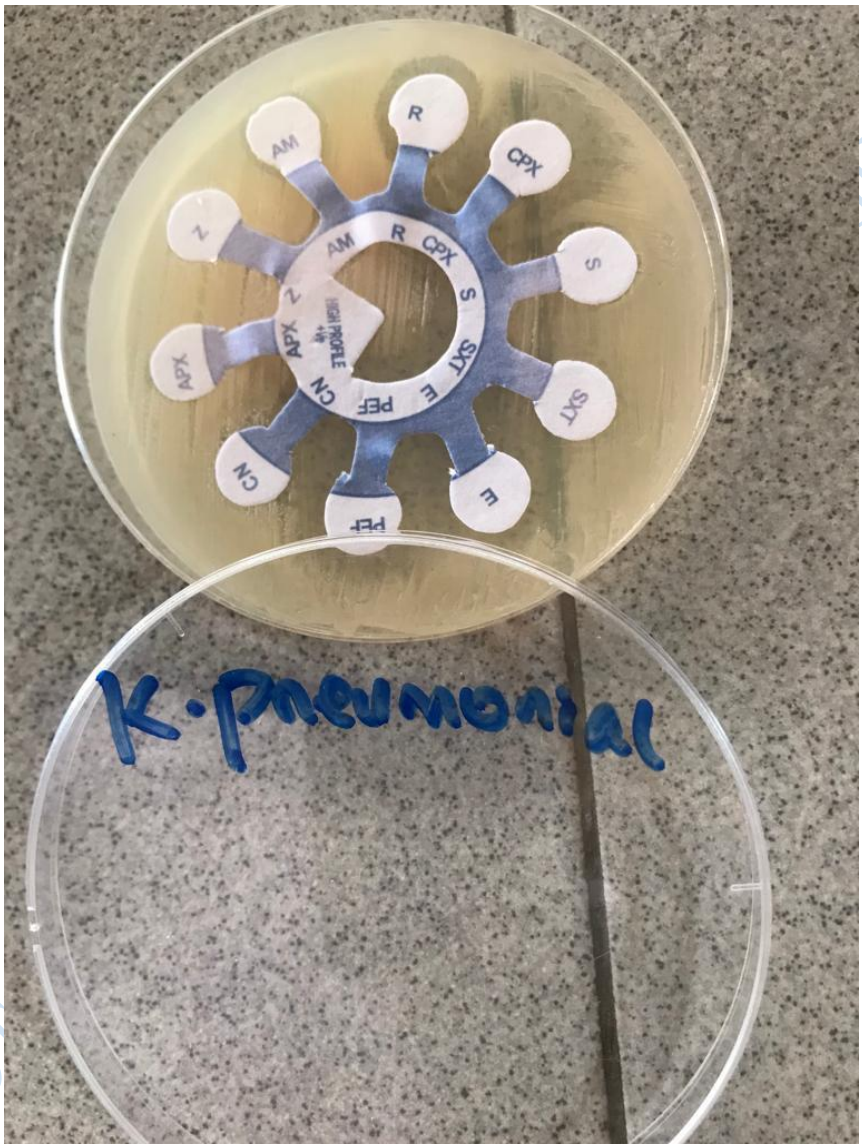


Appendix 7

In Vitro Antimicrobial Activity of Croton zambesicus Leaf Petroleum Ether Extract against Salmonella showing Zones of Inhibition

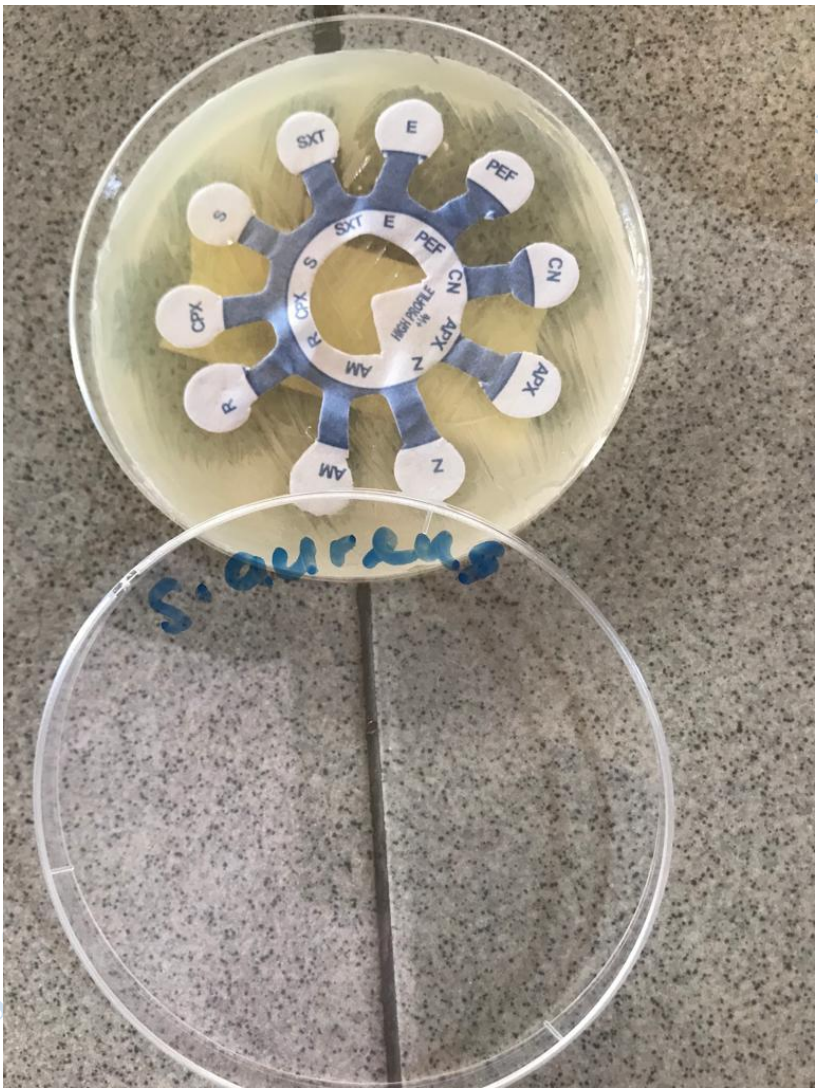
Appendix 8

Disc of in Vitro Antimicrobial Activity of Antibiotics in Klebsiella pneumonia showing Zones of Inhibition



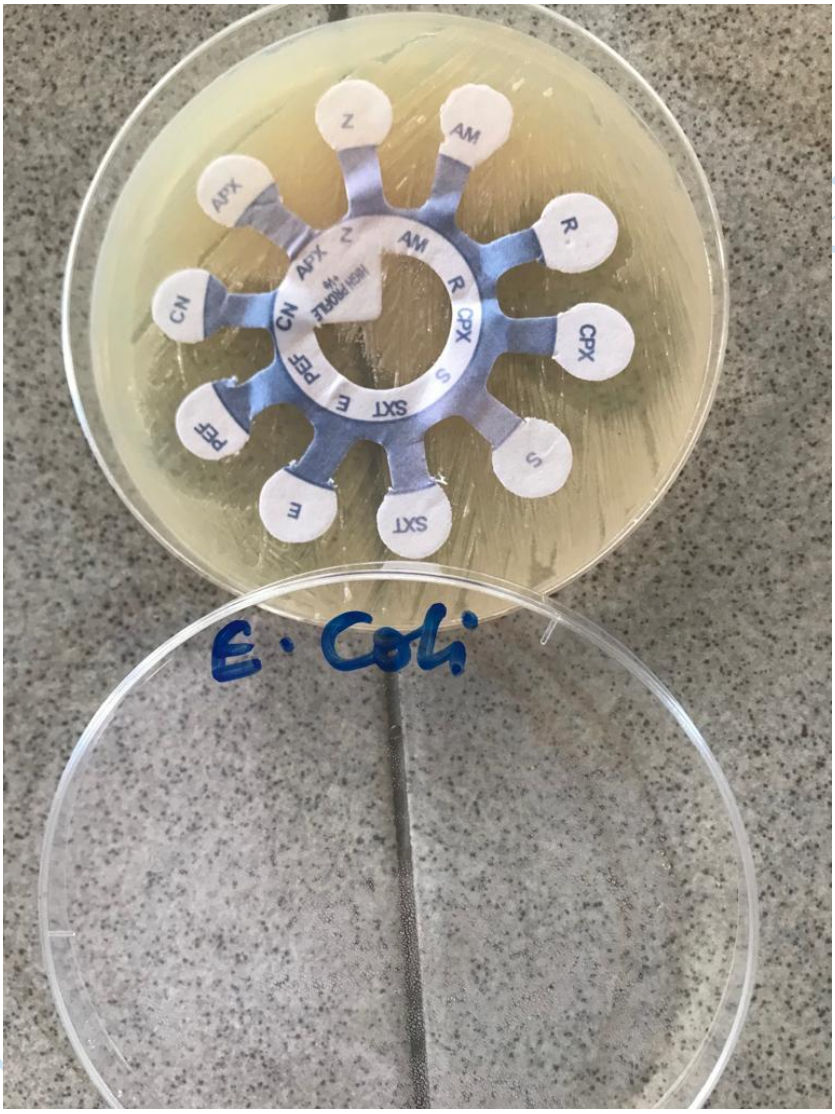
Appendix 9

Disc of in Vitro Antimicrobial Activity of Antibiotics on Staphylococcus aureus Showing Zones of Inhibition



Appendix 10

Disc of In Vitro Antimicrobial Activity of Antibiotics in Escherichia coli Showing Zones of Inhibition



Appendix 11

In Vitro Antimicrobial Activity Disk of Antibiotics in Enterobacter cloacal Showing Zones of Inhibition

Appendix 12

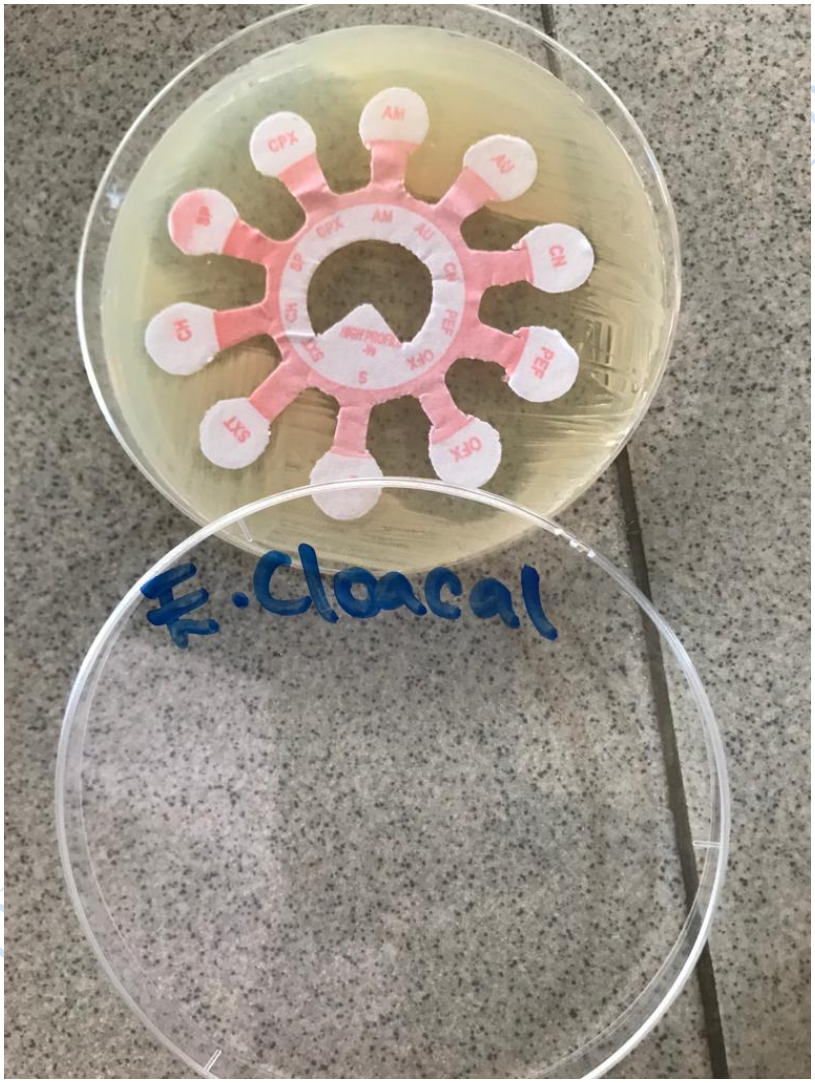
Disc of in Vitro Antimicrobial Activity of Antibiotics on Salmonella showing Zones of Inhibition

Appendix 13

In Vitro Antimicrobial Activity Disk of Antibiotics in P. miribilis Showing Zones of Inhibition

Appendix 14

***In Vitro* Antimicrobial Activity of Gram-Negative Antibiotic Disk on *Enterobacter cloaca* showing Zones of Inhibition**



Appendix 15

***In Vitro* Antimicrobial Activity of Gram-Negative Antibiotic Disk on *Salmonella* Showing Zones of Inhibition**

Biodata

A. Personal Data

1.	Full name:	Olawale Ayoola Badiru
	Address:	No 10 Agoro design Araromi Gbekuba Apata Ibadan, Oyo State
	Email address:	olawalejefbadiru@yahoo.com, Phone number: 08123524479
2.	Date of Birth:	12/06/1990, Place of birth: Plateau State
3.	Nationality:	Nigerian
4.	Name and address of next of kin:	Badiru Olabode: No 10 Agoro layout Araromi Gbekuba Apata Ibadan, Oyo State.

B. Educational Background

Educational Institutions Attended with Dates and Qualification

i.	Lasokun Araromi Community Primary School Apata Ibadan	2000
	Primary School Leaving Certificate	
ii.	Fola Model College Felele Ibadan	2009
	Senior Secondary School Certificate	
iii.	Ajayi Crowther Oyo University	2016
	B.Sc. Microbiology	
	Nigerian Youth Service Corps NYSC Certificate	2017

C. Work Experience with Dates

i. Union Bank Sagamu - Direct Sales Agent	2021-2022
ii. Guinness Box Office Ring Road Ibadan - Sales Representative	2020-2021

D. **Awards and Scholarships (if applicable)** Nil

E.	**Membership in Professional Academic Bodies**	Nil
F.	**Publication (if applicable)**	Nil
	1. Thesis/Dissertation	Nil
	2. Book/Monographs	Nil
	3. Scientific Articles	Nil
	4. Notable Academic or Professional Achievements:	Nil
G.	**Major Conferences/Workshops Attended**	Nil

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Signature Date

University Compliance Certification

This is to certify that the thesis of Badiru Olawale Ayoola with registration number LCU/PGD/001751, at the Department of Biological Sciences, Faculty of Basic Medical and Applied Sciences, Lead City University, Ibadan, Nigeria is in full compliance with the University approved form and style.

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Signature Date