PHYTOCHEMICAL COMPOUNDS AND ANTIMICROBIAL EFFICACY OF *JATROPHA CURCAS* EXTRACTS ON *ESCHERICHIA COLI* FROM DIABETIC FEMALES IN KITUI COUNTY, KENYA

CELESTINE KANINI KYEMBENI

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DECLARATION AND APPROVAL

Declaration by student

This Thesis is my original work and has never been offered for an award of a degree in any other University or any other award.

Signed: ……. Date: …….

Celestine Kanini Kyembeni,

MMLS/2018/27108

Approval by Supervisors

This thesis has been submitted for examination with our approval as University Supervisors.

Signed: ……. Date: …….

Dr. Stanley Kang'ethe, Ph.D.

Department of Medical Laboratory Sciences, Medical School

Mount Kenya University

Signed: ……. Date: …….

Dr. Peter G. Kirira, Ph.D.

School of Pure and Applied Sciences,

Mount Kenya University

DEDICATION

This thesis is dedicated to my lovely son Blair and to my parents Robert and Monica for their, prayers, support and inspiration.

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In a special way, I do acknowledge individuals whose inputs resulted in the success of this Thesis. Being in good health and energetic necessity not be assumed and thus I thank the Almighty father. I extremely appreciate my supervisors Dr. Stanley Kang'ethe and Dr. Peter Kirira for their commitment to walk with me, relentlessly and tirelessly advising, mentoring and unlocking my limits on every step of this Thesis.

ABSTRACT

Multidrug drug resistance world is a predominant drift in microbiology domain and thus desires to be addressed with innovative drugs or substitute approaches of management. Traditional medicine in which plants plays a key role remains a challenge in today's world due to lack of proper validation and documentation mechanism. Plants typically are known to contain concoctions of phytochemicals, tributary metabolites that work independently, additively, or in synergy to better the health of individuals. The study area, Kitui County- Kenya, traditional healers have been using *Jatropha Curcas* leaves and stem bark plant extracts as a multidrug extract. Chemicals ingredients, biological analysis and concoction effects of *Jatropha Curcas* crude extracts remains a research gap in the study area. The efficacies of the *Jatropha Curcas* herbal preparation have little or no validation at all neither is it documented. This experimental study was aimed at determining the phytochemicals compounds and antimicrobial efficacy of *Jatropha curcas* extracts on *Escherichia coli* clinical isolates from diabetic females identified with urinary tract infection in which establishment of the phytochemicals present in *Jatropha curcas* crude plant leaves and stem bark extracts, singly and as a concoction was done and antimicrobial efficacy of *Jatropha curcas* leaves and stem bark extracts, singly and as a concoction, on *Escherichia coli* clinical isolates of diabetic females identified with urinary tract infection. The *Escherichia coli* clinical isolates was obtained from diabetic females identified with urinary tract infection at Kitui County referrals Hospital and transported to Mount Kenya University, Sufficient amount of freshly mature crude *Jatropha curcas* leaves and stem bark were harvested from Kitui County. It is at East African Herbarium in the National Museums of Kenya where Voucher specimen's analysis on morphological characteristics took place and matched with the others documented in the East Africa Herbarium. Phytochemistry analysis took place at East African Herbarium in the National Museums of Kenya in form of ethyl acetate, acetone, aqueous and methanol solutions for the compounds Saponins, Alkaloids, Tannins, Flavonoids, Phenol, Sterol, Terpenoids, Coumarins and Glycosides in which the profiles indicated different extraction solvents do have different effects on the phytochemical composition. After, they were subjected to clinical isolates of *Escherichia coli* of diabetic females identified with urinary tract infection from kitui county referrals hospital for Antimicrobial Efficacy by evaluating their Minimum Inhibition Concentration and Minimum Bactericidal Concentration where failure to proceed to Minimum Bactericidal Concentration from Minimum Inhibition Concentration was a Static indication of *Jatropha curcas* extracts to *E.coli* organism. Incubation of Culture media known to have no inoculation to check the sterility was done, known *Escherichia coli* standard ATCC 29218 was used as a control, a well-known standard Positive and Negative control drug was used in support of control measures. Minitab version 17.0 statistical software, ONE WAY, and TWO WAY ANOVA was used to analyze the data. Ethical endorsement was sought from Kitui County Hospital Research team management, NACOSTI, and consent was sought from the study populations.

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LIST OF ABBBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
CAM	Complementary and Alternative Medicine
FBS	Fasting Blood Sugar
HIV	Human Immunodeficiency Virus
HUS	Hemolytic uremic Syndrome
IMViC	Indole, Methyl Red, Voges Proskauer, and Citrate
MBC	Minimum Bactericidal Concentration
MDR	Multi Drug Resistance
MHA	Mueller- Hinton Agar
MIC	Minimum Inhibition Concentration
MKU	Mount Kenya University
MRSHA	Mannose Resistance Haemagglutination of E.coli
MSHA	Mannose Sensitive Haemagglutination of E.coli
MSU	Mid-Stream Urine
NACOSTI	National Commission for Science, Technology and Innovation
PPE	Personal Protective Equipment's
REMA	Resazurin-Based Microplate Assay
TSI	Triple Sugar Phosphate
UTI	Urinary Tract Infections
WHO	World Health Organization

CHAPTER ONE

INRODUCTION

1.1 Background Information

Communicable diseases remains a global challenge , giving a weighty disease load with a life expectancy of 56 years per average, accounting for about 62% of deaths. E.g. malaria and HIV (Tiffany, 2013). Regardless of accomplishments to governor Communicable diseases, the status of health has deteriorated partially thus increasing Non Communicable Diseases which gives 28% deaths in the year 2010; where 2% was diabetes as it is quoted by Tiffany, 2013. Prevalence of diabetes in Kenya approximates at 3.3% and is forecasted to escalate to 4.5% by 2025 (Tiffany, 2013). Occurrence of diabetes is estimated to be 16 % in rural Kenya (Theuri *et al*., 2019) and in Kitui County, is at 29.1% .(https://www.healthdata.org/kenya-kitui, 2019, accessed January 2020.)

Diabetes is metabolic condition that is characterized by increasing blood glucose. Disease and death in patients with diabetic is normally triggered by many infections including urinary tract infection which remains to be a burden in the diabetic community globally. Patients with diabetes have malfunctioning bladder prompting urine buildup which serves as a conducive state to the bacteria to cultivate, grow and hence lead to urinary tract infection. High glucose level in urine provides a conducive environment for pathogenic microorganisms *Escherichia coli*. Urinary tract infection (UTI) is a conjoint disease in female diabetics due to their; anatomical small urethra, easy detoxification of urinary tract with fecal flora, previous history of UTI, parity, contraceptives use leading to hormonal changes, social-economic status including individual hygiene and changes in urine chemistry where elevated glucose facilitates bacterial growth.

Continued allopathic treatment with different antibiotics advances resistance to the current prescription treatment regimes. Prolonged or frequent use of medication has led to a continuous investigation of different ways of treatment of UTI in diabetic female patients and substitute therapies (Geetha *et al*., 2011). *Escherichia coli* is the utmost major cause of UTIs at approximately 80% populating the colon (Ejrnaes, 2011).

Herbal usages for UTIs in diabetes in Kitui County predates generations. Herbal therapies may relieve urinary tract infections through combating the *Escherichia coli* bacteria, diminishing irritation and curing urinary tract tissues and hence help in the prevention of future occurrences (Geetha *et al*., 2011).The beneficial outputs of these therapeutic plants can defensibly be attributed to; their phytochemical contents particularly the flavonoids, terpenoids lignans, sterols, phenolic acids, stilbenes, alkaloids, tannins and saponins (Nyamai *et al*., 2016). An approximate of 80% of the domain`s inhabitants count on on plants for their major wellness as it is stipulated by WHO (Doughari, 2006, Turker and Usta, 2008, Pawan *et al*., 2015).

Jatropha curcas, a Euphorbiaceae family, is a consequence of two Greek words jatr'os (specialist) and troh'e (food), demonstrating its valuable use in conventional medication (Komakech & Omujal, 2017). It is accepted to have begun from Mexico and northern Focal America on the grounds that in these areas, the most noteworthy hereditary variety was found (Pecina-Quintero *et al*., 2014, Elisa Senger 2018 and Rahu *et al*., 2021,).

Subjugation of *Jatropha* occurred in old times in Mexico (Dias et al., 2012; Sanou *et al*., 2015, Elisa 2018). Portuguese sailors were responsible for spreading *Jatropha* (Montes-Osorio et al., 2014,Elisa 2018) and it is to a great extent utilized as living wall, restorative plant or for clean purposes by the neighborhood occupants (Jiofack *et al*.,

2010; Yongabi *et al*., 2011,Elisa 2018,). *Jatropha* is developed for food creation just in the South of Mexico, neighborhood occupants that designed from the social gathering of Totonacas support it in their home nurseries since notable times (Valdes-Rodriguez *et al*., 2013, Elisa 2018).

The plant *Jatropha curcas* is a comical foundation of numerous natural yields, maximum of which have been broadly of value to human health in the management of numerous health conditions. The traditional healers use various portions of the plant: leaves, branches, fruits, seeds, latex, stem bark, twigs, and roots in different ways including bacterial infections treatment in traditional folk medicine (Komakech & Omujal 2017, Rahu *et al*., 2021). For antibiotic resistance befalls as part of a natural evolution course, meaningfully was reduced but not stopped, thus different antibiotics will be necessary to keep up with resistant bacteria. This experimental study is intended at assessing the phytochemicals and antimicrobial efficacy of *Jatropha curcas* leaves and stem bark extracts, singly and as a concoction, on *Escherichia coli,* a known gram-negative bacteria and a common contributing instrument of urinary tract infection (UTI) in diabetic females attending Kitui County hospital, in Kitui County, Kenya.

1.2 Statement of Problem

The Urinary tract infection is dominated in the world of diabetic patients than it is the world of non-diabetic patients where *E.coli* is the chief bacterium (Al-Asoufi *et al*., 2017). Urinary tract infections are the most frequently befallen disease with a ratio of 1:8 in males and females (Geetha *et al*., 2011).Extraordinary glucose existence in the urine provides a favorable environment of bacteria growth, and thus high glucose occurrence certificates urinary occupation with bacteria, an indication signifying high occurrence of urinary tract infection (UTI) amongst diabetic patients (Salvatore, 2011).

Escherichia coli is the greatest common causes of UTIs at approximately 80% populating the colon (Ejrnaes, 2011).

Scientific research study for UTIs in diabetic patient was significant for management and stoppage of renal complication in which mandatory urine culture was of great help for all diabetic patients, hence justifying a foundation gap in establishing treatment of UTI with *Jatropha Curcas* extracts via establishing the plants phytochemicals and their antimicrobial efficacy.

Jatropha Curcas plant is grown in the study area (Kitui County) as a medicinal plant. The area traditional healers have been using *Jatropha Curcas* leaves and stem bark plant extracts as a multidrug extract in reference to as an antidiabetic and antimicrobial. However, chemicals ingredients, biological analysis and concoction effects of *Jatropha Curcas* crude extracts remains a research gap. The efficacies of the *Jatropha Curcas* herbal preparation have little or no validation at all neither is it documented, thus remaining a research gap to be addressed in this study. It will therefore be domineering that the chemical ingredients, bioassay analysis, and concoction effects analysis are carried out. Documentation and validation of the phytochemicals will also be addressed, thus supporting the key target of this study, "To determine the Phytochemical compounds, and antimicrobial efficacy of *Jatropha Curcas* crude extracts on *Escherichia coli* clinical isolates from diabetic females identified with urinary tract infection''.

1.3 Study Justification

Jatropha Curcas leaves and stem bark extract use has been in the rise without documentation and scientific study in Kitui County, Mwingi Sub County in Mwingi central constituency. This transpires due to stout believes, myths, practices, unreachable

and scanty health care facilities (Omwenga et al., 2015). Antimicrobial efficacy and phytochemical compounds of *Jatropha Curcus* has not been scientifically studied against clinical isolates *E.coli* from identified diabetic females with UTI, attending Kitui Level IV Hospital, however the diabetic females have been using it to manage U.T.I. It will be of great significance to study, spent time and resources in this experimental laboratory procedure for it will establish bioactive compounds with their desirable therapeutic potential, and antimicrobial efficacy of *Jatropha Curcas* leaves and stem bark crude extracts, singly and as concoction, which will be of benefit to Kitui County female diabetic patients and globally by; relieving UTIs through destroying the *E.Coli* bacteria, healing of urinary tract tissues, decreasing urinary tract irritation, providing effective alternatives to prescription medication currently in use, reducing health care expenses by preventing future occurrences, and strengthening plus toning the immune system bark.

1.4 Research Questions

1. Which are the phytochemical composites of Jatropha Curcas crude leaves and stem bark extracts?

2. How is the antimicrobial efficacy of Jatropha Curcas crude leaves and stem bark extracts singly on Escherichia coli clinical isolates from diabetic females identified with U.T.I.?

3. How is the antimicrobial efficacy of Jatropha Curcas crude leaves and stem bark extracts as a concoction on Escherichia coli clinical isolates from diabetic females identified with U.T.I.?

1.5 The study Objective

General objective drives to determine the Phytochemical compounds and antimicrobial efficacy of *Jatropha curcas* crude extracts on *Escherichia coli* clinical isolates from diabetic females identified with urinary tract infection.

1.5.1 The study specific objectives

1. To establish the phytochemicals present in *Jatropha curcas* crude plant leaves and stem bark extracts.
2. To determine in vitro antimicrobial efficacy of *Jatropha Curcas* leaves and stem bark extracts singly, on *Escherichia coli* clinical isolates of diabetic females identified with urinary tract infection.
3. To determine in vitro antimicrobial efficacy of *Jatropha Curcas* leaves and stem bark extracts as a concoction, on *Escherichia coli* clinical isolates of diabetic females identified with urinary tract infection

1.6. Hypothesis

Null hypothesis (H0)

Jatropha Curcas crude leaves and stem bark extracts singly do not have an efficacy against *Escherichia coli* clinical isolates from diabetic females identified with U.T.I.

Jatropha Curcas crude leaves and stem bark extracts as a concoction do not have an efficacy against on *Escherichia coli* clinical isolates from diabetic females identified with U.T.I.

1.7 Scope of the study

It is in Kitui County, Kitui Level IV Hospital, where the study took place covering a population of 211 diabetic females with urinary tract infection of ages 21 to 85 years

contacted using consented questionnaires for a period of six months. Primary isolation of *Escherichia coli* were done at Kitui Level IV Hospital in Kitui County and transferred to Mount Kenya University Microbiology laboratory department for Presumptive analysis, Purification and Susceptibility test with the *Jatropha Curcas* leaves and stem bark extracts singly and as a concoction. The *Jatropha Curcas* plants parts were harvested from Kitui County, in Mwingi Sub-County, Mwingi central constituency, Boru location which is around 171 kilometers from Nairobi City, off the Eastern part of Kenya, and fetched to National Museum of Kenya, Nairobi for processing.

1.8 Study limitations

The study was restricted to the isolated clinical isolates which may not be universal and standard thus contributing to results variability based on the nature of clinical isolate despite the quality control measures applied. Oblige of related literature due to lack of documentation, validation and scientifically studied on the phytochemical compounds and antimicrobial efficacy of the leaves, stem bark and the concoction of both extracts of *Jatropha Curcas* on *Escherichia coli* clinical isolates of diabetic females identified with urinary tract infection may be another limitation.

1.9 Study delimitation

The study choice was delimitated by study objectives; the phytochemical compounds of *Jatropha Curcas* crude leaves and stem bark extracts, and their antimicrobial efficacy on *E.Coli* clinical isolates from diabetic female patients identified with UTI in Kitui County.

1.10 Study Assumption

It was anticipated that the study subjects based on *Jatropha Curcas* leaves, stem bark and the concoction of both extracts on *Escherichia coli,* a laboratory experimental study that all questionnaires were answered truthfully, the chosen sample represent the study population, the used instruments were validated, calibrated, and reliable based on the engaged quality control measures applied during the study period and the study population consented to the study. However, intervening variables like non-consenting were expected due to patient's attitudes, fear of acceptance and lack of knowledge, while Government policies and Management of UTI indicated by drug resistance was assumed as Moderating variables.

1.11 Conceptual frame work;

Conceptual framework covered:

- ✓ Dependent variables
- ✓ Independent variables
- ✓ Intervening variables
- ✓ Moderating variables

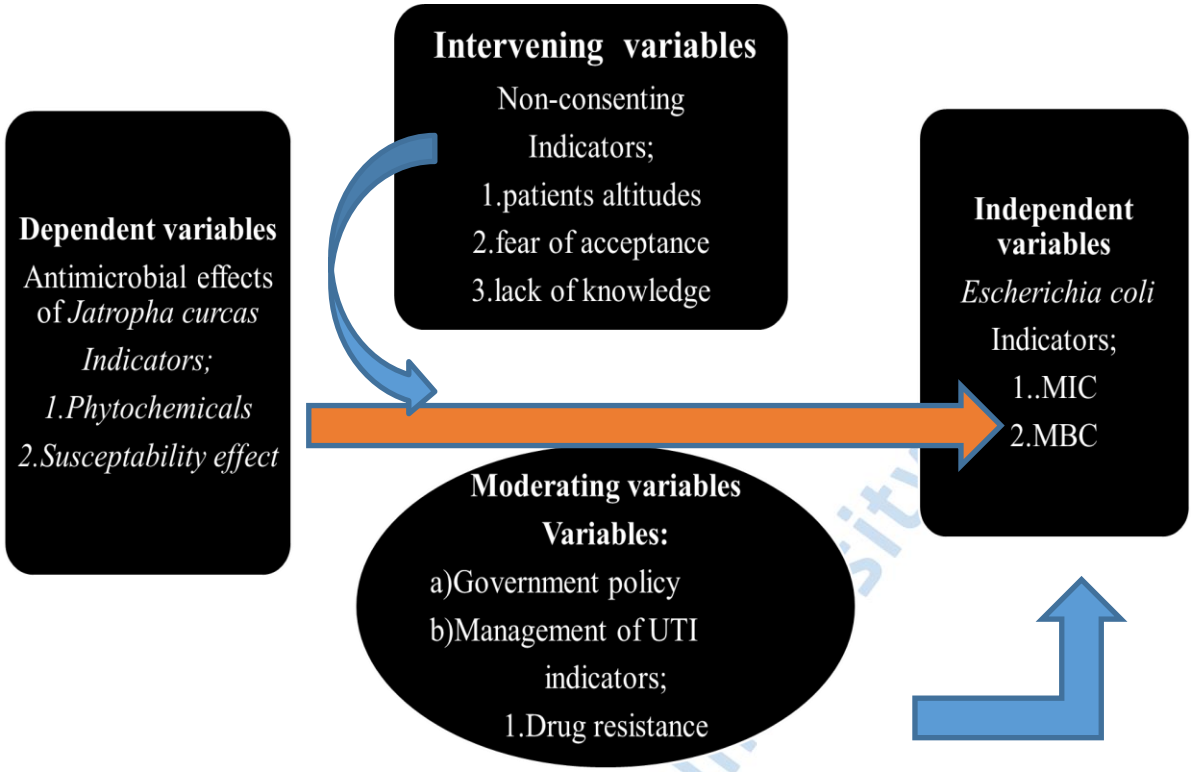


Figure 1: Conceptual Framework

1.12Study significance;

1. The phytochemicals - will help in gauging on the antimicrobial ingredients of the *Jatropha curcas* extracts
2. Antimicrobial effects –will help in prescription choices of antimicrobial medication
3. Minimum Inhibitory Concentration – will help in prescription and dosages
4. Minimum Bactericidal Concentration – will guide on prescription and dosages

1.13 Operational definition of key terms

Antimicrobial efficacy- power of a product to guard against microbes by damaging them at the cellular level, which harms their ability to reproduce in the environment.

Clinical isolates- This denotes the separation of a strain from natural, mixed inhabitants of living microbes

Herbal medicine- Herbal medicine is the habit of plants to delicacy disease and boost general health and prosperity.

Medicinal plants-It is any plant with therapeutic properties or exercise beneficial pharmacological influence on the human or animal body.

Minimum Bactericidal Concentration - MBC is the most reduced centralization of antibacterial specialist expected to obliterate a specific bacterium (Wiegand et al., 2008).

Minimum Inhibition Concentration- MIC is the least centralization of antimicrobial or medication that will block the clear development of microbes after short-term hatching (Levison, 2004),

Phytochemical screening- states of taking out, screening and credentials determination of the medicinally active constituents found in plants.

Qualitative analysis- The testing of an ingredient to define the features of its chemical ingredients

Susceptibility testing- Susceptibility testing is a term used to determine which antimicrobials will hinder the growth of the bacteria

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

This chapter focuses of the review of the previous literature which are correlated to the study. It begins by highlighting the plants historical background. It gives the historical relationships of plants, animals and humans, how medicinal plants are gaining ground as alternative cure of diseases. It goes ahead to give an account of UTI as the most common disease occurring among male and female a ratio of 1:8, and affects people of all ages during the course of their lifespan. It concludes by looking at *Escherichia coli* infections in revealing that it happens globally and have been testified on all continent apart from Antarctica.

2.1 Plant 1Historical background

Nature has always been a golden sign of a projecting sign of coexistence despite the evolutions of life. The relationship of plants, animals and humans started long time ago, where complex system barks of life were provided with all basic need from plants. Plants naturally contain a combinations of diverse phytochemicals, that act independently, additively, or in synergy thus boosting the immune system bark of an individual. Secondary metabolites of the plant's heritages rises the strength of the vigorous complex or phytochemicals, reducing the side effects, leading to an additive and a hostile effect (Fawzi, 2013).

Medicinal plants are currently in demand and their acknowledgement is increasingly progressing with evolution. Among these are the presentation of herbs and extracts for their medicinal capabilities which can be outlined to origins of traditions, myths, and practices, believes and words used to identify such plants and manage diseases to excellence (Mamedov, 2012).

Traditional Medicine is described as one of the unquestionable alternative ways to attain full health care attention of the world's population by the WHO, where it is an important part in the survives of many who are unable to get western medicine in most of the African citizens (Samuel *et al.,* 2014). Traditional medicine in some zones is one of the initial sets of answer appliances for medical disasters whilst in others the whole health care system is hinged on medicines rooted in native practice, myth and belief. Though the application and standards of traditional medicine are commencing to increase recognition, African traditional medicine is still facing contests which accentuate its analysis (Samuel *et al.,* 2014).

Communicable diseases remains a global challenge , giving a weighty disease load with a life expectancy of 56 years per average, accounting for about 62% of deaths. E.g. malaria and HIV (Tiffany, 2013). Regardless of accomplishments to governor Communicable diseases, the status of health has deteriorated partially thus increasing Non Communicable Diseases which gives 28% deaths in the year 2010; where 2% was diabetes as it is quoted by Tiffany, 2013. Prevalence of diabetes in Kenya approximates at 3.3% and is forecasted to escalate to 4.5% by 2025 (WHO, Tiffany, 2013). Occurrence of diabetes is estimated to be 16 % in rural Kenya (Theuri *et al*., 2019)

Traditional medicine, though being extensively used globally, has been observed with a lot of skepticism by conventional health practitioners and the exercise faces many challenges. The herbs are natural products and their phytochemical ingredients differ on some factors, e.g. the plant species, part used as well as the packing, used chemotypes, ground type, sun, harvest time, humidity, and the geographic zone which are very key. These variabilities can result in substantial modifications in a pharmacological activity

connecting pharmacodynamic with pharmacokinetic subjects and is worth reliable to achieve high pharmaceutical quality for traditional medicine. Scientific research that proves efficacy was of greater help to overcome most of the challenges facing traditional medicine (Samuel *et al.,* 2014 and Rahu *et al*., 2021).

Secondary metabolites of the plant's heritages rises the strength of the vigorous complex or phytochemicals, reducing the side effects, leading to an additive and a hostile effect. (Prasad *et al.,* 2012 Fawzi, 2013, and Rahu *et al*., 2021).

2.1.2 Jatropha curcas

The *Jatropha* genus fits in tribe Joannesieae of the Euphorbiaceae family comprising of nearly 170 acknowledged species, being a drought-resistant shrub, it is sketchily spread in the wild areas of South and Central America, India, Africa and South-East Asia. *Jatropha* a name of the genus, is derived from jatr´os meaning doctor and troph´e meaning food, implying drug usage. It has a height of around 3-5m, but under auspicious surroundings, can manage a height of eight to ten Meters (Kumar and Tewari 2015).

In a life period of above Fifty (50) years, it is known to have branches which are thick, straight trunk and grey - reddish bark, camouflaged by large white patches, and green leaves measuring around 6 by 15 cm with 5 to 7 shallow lobes. Dormancy of *Jatropha Curcas* shrub is encouraged by fluctuation of heat, rainwater and sunny weather. The branches have whitish latex. Five roots are known to be made from seeds, one tap root and four horizontal roots. THE Fertilization is through bugs. The seeds are dark with 18 mm long by 10 mm wide. (Kumar and Tewari 2015). Cuttings plants foster just horizontal roots. Inflorescences are made terminally on branches. The plant is monoecism and blossoms are unisexual. Fertilization happens through bugs. After

fertilization, a trilocular ellipsoidal natural product is made. The exocarp stays plump until the seeds are adult. The seeds are dark and in the normal 18 mm long (11 - 30) and 10 mm wide ready Jatropha organic products (7 - 11).

Jatropha Curcas begins from Focal America. From the Caribbean, *Jatropha Curcas* was scattered by Portuguese sailors through the Cape Verde Islands and previous Portuguese Guinea (presently Guinea Bissau) to different nations in Africa and Asia. Jatropha sustains anyplace barring waterlogged grounds. It develops under a broad precipitation systems from 250 to more than 1200 mm for each annum. Currently it is sustained in all tropical and subtropical nations.It isn't self-spreading and must be planted. It develops very well in excess of 600 mm of precipitation each year, and it endures long dry spell periods.

J. Curcas is a multipurpose plant with several authentic and latent uses particularly in medicinal uses. This plant characteristically contains combinations of different chemical compounds that may act independently, additively or in synergy to advance the health of an individual. (Prasad et al., 2012) Many biologically active constituents have been sequestered and branded from all parts of the *Jatropha* plant. Their methodology of action have been deliberate in associate to a great number of applications of *J. Curcas* in traditional medicines. However, its full potential is a long journey both technically and economically for several reasons. (Prasad *et al*., 2012).

The starring role of *J. Curcas* in medicinal uses needs more consideration for it is indicative of valid potentials in the pharmaceutical field. The Commercializing on the medicinal produce resulting from *J. Curcas* may be of more value than using it as a fuel switch. (Prasad *et al*., 2012 and Rahu *et al*., 2021)

Quantitative and qualitative investigations of bioactive mixtures from plant materials depends on the assortment of appropriate extraction technique .Customary medication and restorative plants use in a large portion of arising nations has been extensively noticed and around 80% of the total populace depends on natural meds than present current medication . (Mohammed, 2018) Plants contain endless dynamic mixtures, for example, alkaloids, steroids, tannins, glycosides, unpredictable oils, fixed oils, gums, phenols and flavonoids which are saved in their exact parts like leaves, blossoms, bark, seeds, natural products, and root. (Mohammed, 2018).

Jatropha Curcas Health Benefits

Jatropha Curcas has been described to consume various health benefits due to its large number of therapeutic utilizations (Bialey and Doys, 2001). The name *Jatropha Curcas,* significance Specialist's supplement, was connected with its various restorative purposes. The restorative purposes of this species healthfully and benefits goes from outside, inner and, surprisingly, dental (Agbogidi and Ekeke, 2011). Various pieces of the plant including the leaves, organic products, plastic and bark contains glycosides, tannins, phytosterol, flavonoids and steroidal sapogenins that shows extensive variety of restorative properties (Duke, 1994; Edeoga *et al*., 2005; Agbogidi and Eruotor, 2012). Flavonoids are phenolic intensifies that are engaged with plant collaboration (allelopathy, restraint of germination and development) while glycosides are blended for amino acids. (Agbogidi, Akparobi, and Eruotor, 2013)

Leaves of *Jatropha Curcas*

Leaves are seen as antiparasitic, applied to scabies, rubefacient for loss of motion, ailment and furthermore applied to hard growth (Duke et al., 2002; Aliyu, 2006). Leaves sap is additionally utilized on honey bee or wasp sting. When beat, are applied

on the eyes of a pony to dread flies in India. Leaves contain apigenin, vitexin and ansovitexin which when pooled with different variables empower them to be utilized against strong agonies (El-Ekanali, 2010 and Agbogidi and Ekeke, 2011) and abundant infections as they contain potential antimicrobial achievement thus advancing the immune system. Little of the bioactive particles are not scientifically analyzed, validated, documented and concluded in the market as fresh material for several herbal industries (Renisheya *et al*., 2011). The potential action activity mechanisms of antimicrobial drugs against microorganisms include; meddling with synthesis of the cell wall, the cell membrane disruption, nucleic acid and protein synthesis inhibition, enzymatic activity, metabolic pathways and folate fatty acid inhibition (Tenover, 2006). (Agbogidi, Akparobi, and Eruotor, 2013), Making a bandage from the leaves and placing it on the sore tooth will help relieve pains (Rejore and Batra, 2003).Leaves sap can also be scrubbed on babies gums aiding with teething. The leaves tea help with fever lessening, fever, jaundice and gonorrhea. (Agbogidi, Akparobi, and Eruotor, 2013)

Jatropha Curcas **Stem bark**

Young leaves stem barks have been in use to treat urinary infections with success. The tender twig is used as tooth brush (Gill, 1992; Hasfort, 2000). The barks tea is given to people with leprosy and rheumatism. (Agbogidi, Akparobi, and Eruotor, 2013)

Antimicrobial potential of therapeutic *Jatropha Curcas*

An antimicrobial specialist is characterized as a feeder metabolite delivered by microscopic organisms that has inhibitory person against microorganisms which incorporate anti-infection agents and engineered compounds yet with negligible consequences for mammalian cells (Elliott, 2007). A broad scope of therapeutic plant

removes is utilized to oversee different outside restorative purposes. (Krishnananda *et al*., 2017) Therapeutic plants are plentiful wellspring of antimicrobial particles. A large number of therapeutic plants separates are utilized to regard various contaminations as they have possible antimicrobial action. A portion of these bioactive particles are screened and exchanged market as unrefined substance for incalculable home grown enterprises (Renisheya *et al*., 2011). Experts turned their consideration back close acquiring benefits from restorative plants subsequent to seeing more symptoms of manufactured drugs contrasted with their advantages (Bushra *et al*., 2012). It is assessed that around 35,000 to 70,000 plants species are utilized as therapeutic plants out of 422,127 detailed overall plant species (Bibi *et al.,* 2011). In Pakistan 80% of the populace having a place with the provincial regions relies upon the conventional prescriptions (Munir *et al.,* 2013).

The utilization of plant and its items has a long history that began with people medication and throughout the long term has been acclimatized into conventional and allopathic medication. Since vestige, many plants species vouched for have pharmacological properties as they are known to claim different feeder metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, tirpenes which is hence, ought to be taken advantage of to battle the sickness causing microbes with the development in Science and Innovation, striking headway has been made in the field of medication with the disclosures of numerous regular and manufactured drugs. Anti-microbials are evidently one of the main remedial disclosures of the twentieth century that had effectiveness against serious bacterial contaminations. Be that as it may, just a single third of the irresistible illnesses known have been treated from these manufactured items. This is a result of the rise of safe microorganisms that is certain the

outcome of long stretches of boundless unpredictable use, unremitting and abuse of anti-toxins.

Anti-infection resistance has expanded considerably in the new years and is representing a consistently expanding helpful issue. One of the techniques to lessen the resistance to anti-toxins is by utilizing anti-infection obstruction inhibitors from plants.

Restorative plants have been utilized as customary medicines for various human illnesses for millennia and in many regions of the planet. Subsequently, scientists certainly stand out to more secure phytomedicines and naturally dynamic mixtures disconnected from plant species utilized in home grown meds with OK remedial record for the improvement of novel medications. *J. Curcas* differently known as physic nut, cleansing nut or pig nut (Uche and Aprioku, 2008; Igbinosa *et al*., 2009) is utilized in old stories solutions for treatment of different afflictions, for example, skin 2009. They additionally recommended that the antibacterial movement of the methanolic concentrate of the leaves of *J. Curcas* against 13 bacterial species including *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The concentrate introduced obvious inhibitory action against the individual organic entities (Akinpelu *et al*., 2009).

Jatropha Curcas is a wellspring of feeder metabolites of therapeutic significance. The leaf, organic products, plastic and bark contain glycosides, tannins, phytosterols, flavonoids and steroidal sapogenins that display extensive variety of restorative properties. The plant item display antimicrobial movement. (Krishnananda *et al*., 2017)

India is undeniably the country with the biggest potential for *Jatropha,* the most exceptional conversation, significant level choices, greatest number and kinds of establishments included and the broadest assortment of encounters in the field of *Jatropha*, including most related issues and questions.

Figure 2. Leaves of *Jatropha Curcas*

Figure 3. Stem bark s of *Jatropha Curcas*

Photo courtesy of Kitui County medicinal plants by the researcher

Pharmacological potential of *Jatropha Curcas*

(Egharevba, Omoregie, & Folashade, 2013, Aladodo 2013 & Aina, 2016)

A.Antioxidant activity

Hydro-alcoholic concentrate of the leaves, stem bark and base of *Jatropha Curcas* had demonstrated groundbreaking cancer prevention agent movement by in vitro cell reinforcement models like DPPH extremist rummaging action, nitric oxide revolutionary searching action, hydroxyl revolutionary searching action, lessening power strategy and hydrogen peroxide revolutionary rummaging action.

B. Hepatoprotective activity

Methanolic part of Jatropha Curcas demonstrated hepatoprotective movement on aflatoxin b1 actuated hepatic carcinoma in creatures.

C. Wound healing activity

Home grown salve containing the leaf and bark concentrate of Jatropha Curcas in wistar pale skinned person rodents rushes the mending system by accumulating the skin breaking strength, granulation tissue breaking strength, wound withdrawal, dry granulation tissue weight and hydroxyproline levels.

D. Antimetastatic and Antiproliferative activity

Methanolic part of Jatropha Curcas was perused up for its adversary of metastatic activity including B16F10 melanoma cells in C57BL/6 mice. It was focused on using MTT (3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide; Thiazolyl blue) look at and the IC50 was seen as 24.8 µg/mLE. Antimicrobial action

Jatropha Curcas Methanolic, ethanolic and water concentrates of stem bark has the invitro antimicrobial activity against S.aureus, P.aeruginosa, Escherichia coli, S.faecalis and different organisms

F. Antidiabetic activity

Jatropha Curcas leaves indicated an Antihyperglycemic effect of 50% ethanolic extract, a study done in normal and alloxan induced diabetic rats.

G. Anti-inflammatory activity

The methanol remove showed foundational and critical mitigating action in intense carrageenan-actuated rodent paw edema and an action against formalin prompted rodent paw edema, notwithstanding, turpentine-actuated exudative changes and cotton pellet-prompted granular tissue development in mice and rodents.

H. Pregnancy terminating effect

Fetal resorption was characteristic with methanol, oil ether and dichloromethane removes showing the abortifacient properties of the natural product in rodents. It proposed that the interference of pregnancy happened at a beginning phase after implantation.

I. Antiulcer activity

Methanolic concentrate of Jatropha Curcas showed the antiulcer action involving anti-inflamatory medicine prompted gastric sores in Wister rodents.

J. Anthelmintic activity

Aqueous concentrate of leaves have anthelmintic action against Pheretima Poshtuma.

K. Antifungal Activities

Ethanolic extract of *Jatropha Curcas seed* cake indicated antifungal activities against important fungal phytopathogens: Fusariumoxysporum, Pythiumaphanidermatum, Lasiodiplodiatheobromae, Curvularialunata, Fusariumsemitectum, Colletotrichumcapsici and Colletotrichumgloeosporioides. The extract contained phorbol esters mainly responsible for antifungal activities. (Egharevba et al., 2013, Aladodo 2013, Aina, 2016)

Table 1; *Jatropha Curcas* different parts uses

Therapeutic indications of plant part	Remedy Plant form	References
LEAVES		
• Vaginal bleeding treatment	Infusion preparation from the leaves	Singh et al. (1984)
• Wound healing	Leaves applied to wounds	Staubmann et al. (1999)
• Fever	Decoction is used internally and externally	Staubmann et al. (1999
• Rheumatism	Leaf decoction is applied externally	Staubmann et al. (1999
• Jaundice	Application of the leaves	Staubmann et al. (1999 Thomas 1989
• Lymphocytic leukemia	Ethanolic extract defatted leaves and twigs	Hufford and Oguntimein (1978)
• Anti-parasitic activity	Sap and crushed leaves	Henning (1997)
• Malaria	Leaves application	Irvine (1961), Oliver (1986)
• Mouth infections, guinea worm sores	Leaves application	
• Promote lactation	Crushed leaves applied to the breast	Parveen et al. (2007)
• Dysentery and colic	The juice of the leaves is used	Parveen et al. (2007)
STEM BARK S, BRANCES AND TWIGS		
• Gumboils and strengthen the gums	Twigs are used as toothbrush	Parveen et al. (2007)
• Inhibits HIV induces cytopathic effects with low cytotoxicity	Water extract of the branches	Matsuse et al. (1999)
• Strong antimicrobial agents	Extract from the branches	Igbinosa et al. (2009)
• Aid antimicrobial activities	Secondary metabolites extract from the stem bark exert antimicrobial activities through different mechanisms	Igbinosa et al. (2009)
• Gumboils and strengthen the gums	Twigs are used as toothbrush	Parveen et al. (2007)
• Inhibits HIV induces cytopathic effects with low cytotoxicity	Water extract of the branches	Matsuse et al. (1999)

(Source, Prasad and Khan 2012)

Leaves and stem barks Pharmacological potential

A few extractions techniques really do exist to extricate compound from plants. These strategies can be call regular (conventional utilizing from quite a while in the past) and new (current and grew all the more as of late). Regular techniques are known to utilize natural solvents or water and at environmental strain while new strategies really do utilize pressure and/or raised temperatures (Mohammed, 2018) .The Various Strategies utilized for taking out are fundamental for the variety of dynamic parts of plant tissues from the started parts through utilization of appropriated solvents. During extraction process, solvents move into the strong plant materials and solubilize the mixtures with comparable extremity, and subsequently taking note of dissolvable decision is of fundamental significance alongside utilization of a viable extraction strategy. Solvents choice standard 'like breaks down like' is vital. Consequently polar solvents will extricate out polar substances and nonpolar substances by non-polar solvents. (Mohammed, 2018)

Phytochemical screening of ethanolic extract of *Jatropha Curcas*

The segregation of phytochemicals segregation with their usage as a solo compound entity as good as a substitute has led to replacement of plant extracts' usage, an alert that may be there are benefits of the crude medical use or its consistent extracts as opposed to secluded solo complexes, where it is attaining great momentum in the scientific civic as it is stipulated by Fawzi. (2013***)***

Table 2: *Jatropha curcas* phytochemicals and therapeutic indicators

Plant part	Chemical compositions	Therapy Indicators	The plant part plus form of remedy for the therapeutic indicator
The leaves	Flavonoids, sterol alkaloids,	vaginal bleeding treatment	leaves infusion is used
		Healing of Wound	application of leaves to wounds
		Fevers	internally and externally decoction is used
		Rheumatism	External application of Leaf decoction
		Jaundice	Application of the leaves
		Lymphocytic leukemia	leaves and twigs defatted ethanolic extract
		Action against parasitic activity	crushed leaves and sap is used
		Malaria	leaves Application is done
		Mouth infections (sores)	leaves Application is done
		Promote lactation	leaves Application is done to the breast
		Dysentery and colic	Leaves juice is used
The stem bark s	The steroids, glycosides, saponins, , Tannins, alkaloids and flavonoids	Known to facilitate antimicrobial actions	The secondary metabolites extract are used antimicrobial doings via diverse machineries

(Source Maksudur *et al.*, 2012).

***Jatropha Curcas* leaves and stem bark phytochemicals, therapeutic indicators and remedies.**

Table 3: Phytochemical results

Saponins	+
Tannins	+
Alkaloids	+
Anthraquinones	+
Terpenoids	+
Flavonoids	+

Key: + = phytochemicals in the extracts.

(Source, Ifayefunmi ., 2018)

Jatropha Curcas exhibited to be compelling over the utilization of anti-toxins by stopping the movement of *Pseudomonas aeruginosa* which ended up being safe when tried with standard anti-infection agents. The antibacterial movement of the concentrate could be improved in the event that the parts are filtered, and in this manner the plant *Jatropha Curcas* holds a commitment as an expected wellspring of new medication for treating contaminations brought about by clinical microbes. (Ifayefunmi *et al*., 2018)

Phytochemical constituents of *J. Curcas* extracts.

Table 4; phytochemical constituents of *J. Curcas* extracts

Extracts	Alkaloid	Coumarins	Flavonoids	Steroids	Tannins	Phenol
Bark ME	-	+	+	+	+	-
Bark EA	+	+	+	-	+	+
Roots ME	+	-	+	+	-	-
Roots EA	+	+	-	+	+	+
Immature leaves ME	+	-	+	-	+	+
Immature leaves EA	+	+	+	+	-	+
Mature leaves ME	+	-	+	+	+	+
Mature leaves EA	+	+	-	-	-	-
Fully mature leaves ME	+	+	-	+	+	+
Fully mature leaves EA	+	+	+	-	+	+
Immature seed oil ME	+	-	+	+	-	+
Immature seed oil EA	-	-	+	+	-	+
Mature seed oil ME	+	+	+	-	+	+
Mature seed oil EA	+	+	+	-	+	+
Fully mature seed oil ME	+	+	+	-	-	+
Fully mature seed oil EA	+	+	-	+	+	+

Key;

ME Methanol, EA Ethyl Acetate,

+: Presence, -: Absence

(Source, Rajesh *et al.*, 2016)

2.2 Urinary Tract Infections

UTI is the most common disease occurring among female and male in a ratio of 8:1, and affects people of all ages during the course of their lifespan. UTIs are known to be caused by pathogenic bacteria such *as Escherichia coli, Staphylococcus saprophyticus, Klebsiella pneumoniae, Proteus mirabilis* and fungi *Candida albicans*. (Sowjanya *et al.*, 2017). A higher prevalence of UTI is perceived in diabetic patients than it is with the non-diabetic, with a higher severity leading to complications, ranging from dysuria to organ damage and even demise due to pyelonephritis, if not managed in time (May *et al*., 2016)

Herbal usages for UTIs in diabetes have been used for centuries in Kitui County. Herbal therapies may relieve urinary tract infections through combating the *Escherichia coli* bacteria, diminishing irritation and curing urinary tract tissues and hence help in the prevention of future occurrences (Geetha *et al.*, 2011).The beneficial outputs of these therapeutic plants can defensibly be attributed to; their phytochemical contents particularly the flavonoids, terpenoids lignans, sterols, phenolic acids, stilbenes, alkaloids, tannins and saponins (Nyamai *et al.*, 2016).

Intricate UTI is a contamination in a urinary parcel with useful or primary irregularities (eg. in staying catheters and renal calculi). The inclining variables of host, for example, age, catheterization, diabetes mellitus and spinal line injury causing convoluted UTIs. During many-sided UTI cystitis of long term or hemorrhagic cystitis happens. (Sowjanya et al., 2017).

Most *Escherichia coli* associated UTIs are in the bladder and urethra, and extreme cases can affect the kidneys. (Geetha *et al.*, 2011) Urinary system is normally a sterile anatomic site in health individuals and hence sterile urine. Infection frequently happens

when bacteria access the system through urethra followed by attachment and multiplication. (Geetha *et al.,* 2011) The progression of blood filtration is found to take place in the kidneys with urine as an end product. Urine is passed to urinary bladder via the ureters and expelled out of the body via the urethra. UTIs can resort from endogenous or exogenous, descending and ascending infections respectively. Either way the ureter, urethra or bladder will be affected. Females are prone to UTIs from poverty throughout reproductive period and post menopause. (Aadhan & Anand, 2017)

Risk factors of UTI

A risk factors is a something that raises the chances of getting a health problem which can be age and gender, genetics, sexual activity and underlying health problems. (Geetha *et al*., 2011).Females are mostly prone UTIs in comparison to males due to their short urethra whose access for pathogens is succeeding the anus and vagina, thus setting a foundations for disease causing organism. Other risk factors for UTI include; the sexual activities, birth control mode, menopause staging, diabetes condition and catheter use (Geetha *et al.,* 2011).

Herbal therapy in Urinary tract infection

Herbal medicine has been widely practiced throughout in different health conditions among different communities globally (Geetha *et al*., 2011). Herbal therapies may boot out urinary tract infections by combating the bacteria, reducing irritation and curing urinary tract infection. Some Herbs also help in inhibiting forthcoming occurrences (Geetha *et al.,* 2011). Urinary tract infection is normally arrested with prescription antibiotics. Nevertheless, it is progressively acknowledged that using antibiotics often may subsidize to periodic UTIs and increased habit use on antibiotic use may

additionally deteriorate the immune system. Natural therapies can offer an operational substitute to authorized medicines in use. (Geetha *et al*., 2011).

2.3 Escherichia coli

Escherichia coli infections happens globally and have been testified on all continent apart from Antarctica. (*Food security and public health Center, 2012).*

Escherichia coli dominates the gut of all warm-blooded animals making the most aerobic flora. Morphologically it is a Gram-negative, non-sporulating and facultative anaerobic with characteristically rod-shaped cells, around 2.0 micrometers (μm) long by 0.25-1.0 μm in diameter, and a cell volume of 0.6–0.7 μm. *Escherichia coli*, a facultative anaerobe, has defined Optimum growth temperature of 37 °C, though growth and development can occur at up to 45 °C.(Fecal *Escherichia coli). Escherichia coli* growth and development can be achieved in a diversity of laboratory condition nutritionally and atmospheric respectively (facultative anaerobe)

Pathogenicity

Escherichia coli is the most dominant causative agent for UTI due to diverse virulence factors that contribute to elicitation of infection. Among the virulence factors are adherence organelles that upsurge the chances of attachment of *E.coli* to uroepithelial tissue which is an essential stage of UTI, other virulence influences include; aerobactin, cytotoxic necrotizing factor and haemolysin that vary in the act leading to UTI (Al-Asoufi *et al*., 2017). Extraordinary glucose presence in the urine offers a conducive environment of bacteria growth, in which, they reproduce and create basis for infection; moreover, high glucose presence permits urinary occupation with bacteria, an evidence signifying high occurrence of urinary tract infection (UTI) amongst diabetic patients (Salvatore, 2011). Shiga toxin causes primary damage of the red blood cells, blocking

the body's filtering system in the kidneys, leading to hemolytic-uremic syndrome (HUS). Female's hygiene is important and significant observation to avoid or minimize auto infection owing to the anatomical proximity of vaginal and anal opening. During voiding it is advisable to wipe rear to anterior. Anal sex is a major contributing factor to infection with gut flora, while vaginal sex unhygienic practices should be avoided as a way of prevention and control of urinary tract infections.

The Correlation of E. *coli* and Urinary Tract Infections

UTI are global bacterial infection with around 25% of all women infections mostly occurring in the lower urinary tract (bladder and urethra) and is complicated when it ascends to the kidneys and *E.coli* is a major contributing etiological agent.

E. coli infections can be sporadic infections originating from the host intestinal tract. (Bergeron *et al*., 2012).

Symptoms of a UTI

Symptom of UTI varies with individual and gender, among other parameters. UTI is uncomfortable and downright painful. Some of the common symptoms are:

- ✓ Stout, tenacious need to urinate
- ✓ Sore and scorching urination
- ✓ Slightly minimal amounts of urine is passed
- ✓ Strong-smelling, cloudy urine
- ✓ Bloody urine
- ✓ Pain in the upper back and sides
- ✓ Pelvic pressure

CHAPTER THREE

RESEARCH MATERIALS AND METHODS

3.1: Introduction

This is an Experimental and cross sectional method of research, in which the *Escherichia coli* clinical isolates were obtained from diabetic females identified with urinary tract infection at Kitui County Referrals Hospital via questioners (Appendix 2) and transported to Mount Kenya University. Sufficient amount of freshly young crude *Jatropha Curcas* leaves and stem barks were harvested from Kitui County and attached to herbarium sheets, compelled hard in order to level and dry, then branded. It is at East African Herbarium in the National Museums of Kenya where Voucher specimen's analysis on morphological characteristics took place and matched with the others documented in the East Africa Herbarium. Phytochemistry analysis was done at East African Herbarium in the National Museums of Kenya in form of ethyl acetate, acetone, aqueous and methanol solutions, then after was subjected to clinical isolates of *Escherichia coli* for antimicrobial efficacy by evaluating their minimum inhibition zones, minimum inhibition concentration and minimum bactericidal concentration at mount Kenya university-Microbiology laboratory.

3.2 Study design

The *Jatropha Curcas* leaves, stem bark and a concoction of both extracts were subjected to the solid-liquid extraction method in which ethyl acetate, acetone, methanol and aqueous were used as solvents based on their polarity index Ethyl acetate 4.4,Acetone 5.1,Methanol 5.1 and Aqueous 10.2. (Doughari, 2012). The stem bark s and leaves extracts were analyzed for phytochemicals tests. The *Escherichia coli* colony isolates from known screened diabetic females with UTI via consent and questionnaire tools (Appendix 2) in sampling of participants of Kitui County level IV Hospital during

the period of study was subjected to the extracts of fresh *Jatropha Curcas* leaves, stem bark and a concoction of both in triplicates for an overnight incubation at 37^0C with controls to certify that all the measurements attained in the study are accurate, precise and reproducible.

3.3 Site of study

The jatropha Curcas are grown in the study area (Kitui County) as medicinal plants having been introduced by colonialist as a natural fence and adopted by the locals. They were harvested from Kitui County, Mwingi Sub-County, Mwingi central constituency (Appendix X), Boru location, which is around 180 kilometers from Nairobi City off the Eastern part of Kenya, with an estimated area of 30,496.4 KM^2, and fetched to National Museum of Kenya, Nairobi for processing. *Escherichia coli* were isolated at Kitui County level IV Hospital in Kitui County and transferred to Mount Kenya University Microbiology laboratory for biochemical test.

3.4 Study population

This study population involved all the consented non pregnant diabetic females patients identified with urinary tract infection at Kitui County Level IV Hospital, during the period of study.

3.5 Sampling method and size

To calculate the study sample size, a prevalence rate of 50% estimated by Fishers and Van Belle (2004) was used, as no previous similar study had been done at Kitui Level IV Hospital.

Formula; $n = \frac{t^2 \text{ x } p(1-P)}{m^2}$

In which:

n = the anticipated sample scope (for objective population of above 10,000)

t^2 = the standard normal deviation, which agrees with 95% confidence level (1.96)

P = proportion in the objective population is projected to 50% (prevalence of non-established prior data of *E.coli*)

m = precision, degree of error, set at 0.05

Therefore, n = $\frac{1.96^2 \times 0.5(1-0.5)}{0.05^2}$

= 384

With a target population below 10,000, the sample was modified using the following method.

nf = n/[1+ (n/N)]

Where;

nf – Anticipated sample scope (when the population is less than 10,000).

n – Sample scope (when population is more than 10,000) deliberated above as 384.

N – Number of estimated female Diabetes patients, In this case three hundred and eighty four (384).

=192, plus 10% of the non-respondents which is totaling to **211**

3.6 The Selection criteria

3.6.1 The Inclusion criteria

1. Diabetic females identified to have Urinary tract infection attending Kitui County level IV Hospital with consent

3.6.2 Exclusion criteria

1. Non-Diabetic females identified with Urinary tract infection attending Kitui county level IV Hospital

2. Non-consenting diabetic females identified with Urinary tract infection attending Kitui County Level IV Hospital

3.7 Study material

3.7.1Plant material collection

Fresh fully mature crude *Jatropha Curcas* leaves and stem barks were harvested from Kitui County, Mwingi Sub-County with satisfactory bio-conservation procedures (WHO, 2003).

Reaping was done in a dry weather sunrise (Prajapati, Purohit, Sharma, & Tarun, 2010). The two crude extracts were wrapped with paper foil and ferried to East African Herbarium in the National Museums of Kenya for extraction and phytochemical analysis. Voucher specimens was stored.

Figure 4: Raw material harvesting

Fresh fully mature raw materials harvesting, photo courtesy of the researcher

3.8. Experimental and laboratory procedures

3.8.1Processing of plant materials

Preparing the medicinal plants for experiments reasons is an initial step and extremely key in accomplishing quality examination result. It includes extraction and assurance of value and amount of bioactive constituents prior to continuing with the planned organic testing. The significant stages remembered for gaining quality bioactive atom are the

determination of a proper dissolvable, extraction strategies, phytochemical screening methodology, and

Identification procedures. Solvents usually utilized in extraction of restorative plants are polar dissolvable e.g., water, alcohols, middle polar e.g., CH3)2CO, dichloromethane, and nonpolar e.g., n-hexane, ether, chloroform. (Abdullahi. Abubakar, Mainul, 2020)

Sufficient amounts of harvested freshly mature crude *Jatropha Curcas* leaves and stem barks from Kitui County were processed within 72hours of collection. They were put on herbarium sheets, compelled to level, to dry and branded. At East African Herbarium in the National Museums of Kenya the voucher specimens were authenticated on the foundation of morphological features and equated with other voucher specimens documented in the East Africa Herbarium. The harvested plant supplies were splashed systematically with running tap water, then air-dried under the shadow at room temperature for 3-4 weeks. Air-dried materials were pre-crushed and later grounded into a fine powder using an electric blender, then soaked in respective solvents for a day in the ratio of 1:2 by weight volume (w/v) correspondingly. Methanol, ethyl acetate, acetone and aqueous were used separately to extract the leaves and stem bark s via Soxhlet extraction method (Gopalasatheeskumar, 2018, Abdullahi. Abubakar, Mainul, 2020,) (Protocol 1).

The methanol and ethyl acetate extracts were shaken well two times a day and then sieved. The deposits were then vaporized under reduced pressure to get a gummy deposit with the aid of a rotary evaporator. The extracts were evaluated in terms of kilograms and put in storage in a sterile glass bottle at 4°C awaiting scientific screening. The aqueous extracts, on the other hand, was prepared by soaking the dry powder in 1:10 ratio weight by volume, in distilled water for 2hrs with alternating mixing, after

which the content was sieved through filter papers (0.45 μm) and the aqueous extracts exposed to lyophilization to eradicate the solvent used for extraction and later the dried extracts were refrigerated prior to the experiment (Muhuha, 2018).

Figure 5: Soxhlet extraction method

Soxhlet Leaves extraction method taking place (Photo courtesy of the researcher)

Post extraction process

Soxhlet extraction process extracted materials are then endorsed to processes like concentrating the extract, evaporating the solvent and storage of the extract at normal air was used as a process of drying to the concentrate extract. The collected extract was stored in a tightly closed container, stored in a refrigerator after covering it with an aluminum foil. The extract was then diluted with the solvent and subjected to a further isolation process by use of chromatography technique (Gopalasatheeskumar, 2018)

Table 5: Plant yield extract percentages

Plant part	Type of solvent	Yield extract Percentages By Soxhlet extraction method
LEAVES	Acetone	11.9 %
	Aqueous	12.1%
	Ethylacetate	7.7%
	Methanol	12.8%
STEM BARKS	Acetone	11.1%
	Aqueous	13.0%
	Ethylacetate	6.3%
	Methanol	14.3%

Amid all the yield percentage obtained, Methanolic extract was instituted the highest amount compared to other solvents. (Krishnananda *et al*., 2017)

3.8.2 Phytochemical screening

Phytoconstituents as a natural occurring bioactive composites established in plants, working with nutrients and fibers to procedure a cohesive measure of the defense scheme towards numerous diseases defines the medicinal worth of plants established in parts of the plants where they provide a certain physiological accomplishment on the human immune system bark. Screening of *Jatropha curcas* leaves and stem bark phytochemicals such as alkaloids, tannins, Phenol, coumarins, steroids, glycosides,

flavonoids, sterols, terpenoids and saponins were carried out as described in Protocol I. (Silva *et al*., 2014 and Abdullahi, Mainul , 2020)

A. Test for saponins

About 1g of *Jatropha Curcas* leaves and stem bark s were positioned in an isolated test tube and water introduced, then shaked and left to stand for two minutes. Tenacious frothing was an indication of saponins (Ahmad, 2007)

B. Test for alkaloids

Approximately 1g of *Jatropha Curcas* leaves and stem bark s were added to 5ml of 10% sulphiric acid separately, placed in water bath for 2 minutes to warm, and then sieved. Two drops of Meyer's reagent were introduced followed by addition of chloform in each, then the mixture was vaporized to leave a solid deposit which was liquefied in sulphiric acid. Meyer's reagent addition resulted to development of whitish to buffy precipitate indicating presence of alkaloids in each extract (Oranga, 2020).

C. Test for glycosides

Keller-Kilian test; Around 50 grams of *Jatropha Curcas* leaves and stem barks were extracted with 10ml of 10% alcohol, through boiling for around 2 minutes in a water bath separately, the extracts were left to cool and then sieved, 2mls and 1ml of 5% glacial acetic and 5% ferric chloride solutions were added respectively in each extract. The subjects were boiled and chilled separately, then shifted to an experimental tube with 2ml concentrated sulphiric acid. Development of reddish brown edge of the dual liquids signified existence of deoxy sugar characteristic of cardenolides, a top level which gradually progresses a bluish green color (steroidal nucleus) indicates existence of glycosides (Kiran & Prasad 2015).

D. Test for phenols

Approximately 1g of *Jatropha Curcas* leaves and stem bark s each separately were mixed with 70% ethyacetate, exposed for boiling in a water bath for 5min, then the extracts was sieved then left to cool.5% ferric chloride was added, a green precipitate development gave an indication of phenol presence. (Ahmad, 2007)

E. Test for tannins

Ferric Chloride Test: Around 1g of *Jatropha Curcas* leaves and stem bark s each respectively was mixed with 10ml water, boiled in a water bath and sifted. 3 drops of 5% concentrated Ferric chloride solution was introduced to each 2ml filtrate separately, establishment of a green precipitate indicated tannins presence. (Kiran and Prasad 2015)

F. Test for coumarins

Few drops of ammonia were added onto different filter papers, then a drop of *Jatropha Curcas* leaves and stem bark extracts was introduced independently. Fluorescence indication on the filter paper confirmed coumarins existence (Sangeetha *et al.,* 2014)

G. Test for Flavonoids

A 2mls of *Jatropha Curcas* leaves and stem bark extract each separately we treated with 5 drops 5% concentrated ferric chloride solution. Black-red color development indicated existence of flavonoids (Kiran and Prasad 2015)

H. Test for Sterols

A 2mls extracts of *Jatropha Curcas* leaves and stem bark in different tubes were liquefied in 2mls acetic anhydride and 2ml chloroform and a 2ml concentrated sulphiric acid introduced in each. Observation of a translucent green color was an indication of sterols presence (Sabri *et al*., 2012)

I. Test for terpenoids

5mls of *Jatropha Curcas* leaves and stem bark extracts each separately were mixed with 2mls chloroform and the 3mls concentrated sulphiric acid introduced in each .A reddish brown color formation at the interface was an indication of terpenoids (Ablude, 2001)

Figure 6. Phytochemical screening

Saponins test of both stem bark and root barks aqueous extraction (Photo courtesy of the researcher)

3.8.3 Clinical sample collection

Members who met inclusion criteria were well versed for the study, filled the questionnaire (Appendix 2), and signed the informed consent form. Mid-stream urine (M.S.U) was collected with the participant's identification number, Code number, age, time, and date of collection (Protocol 5, 6 and 7).

3.8.3.1 Micro-organism

Escherichia coli isolates was obtained via mid -stream urine culture of various female diabetic patients identified of urinary tract infection at Kitui county hospital in Kitui County, Kenya (Protocol 3).

3.8.3.2 Controls

The culture media was nurtured inoculation less of any isolates thus testing its sterility. After preparation was inoculated with acknowledged *Escherichia coli* bacteria isolate to confirm their growth support. Positive control known acknowledged drug and Negative control known acknowledged drug was used in support of control measures

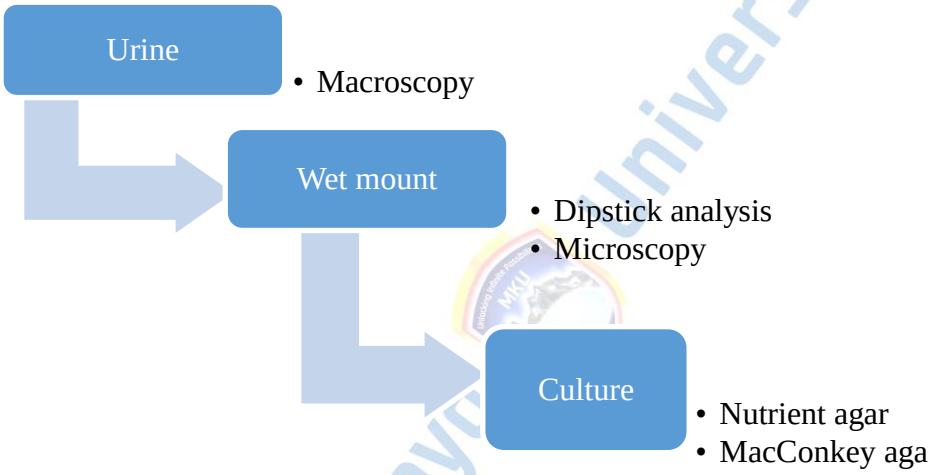
3.8.3.3 Fasting blood sugar test

Fasting glucose testing (FBS) is a tool for people with diabetes. It will help assess how well diabetes is being managed. By prick of a finger, a small drop of blood was drawn, and a Fasting blood test run. (Protocol 4)

3.8.3.4 Collection of urine sample

Mid - stream urine (MSU) was used for culture to investigate the presence of *Escherichia coli* in the urinary tract. A MSU was collected into a sterile bottle. Patients was informed to pass a slight amount of urine into the toilet or latrine to certify that bacteria, cells or parasites that have entered the lower urethra from the vagina or perennial area are flushed away, and then collect about 20ml of urine into the sterile bottle, the remaining urine in the bladder is passed (Protocol 5). Macroscopy and Microcopy diagnosis was performed, then by a dipstick deliberated to identify urine nitrite and to indirectly estimate the number of segmented neutrophils (Protocol 6,7 and 8.)

3.8.3.5 Culturing urine sample



Urine sample was mixed to re-suspend existing microorganism.

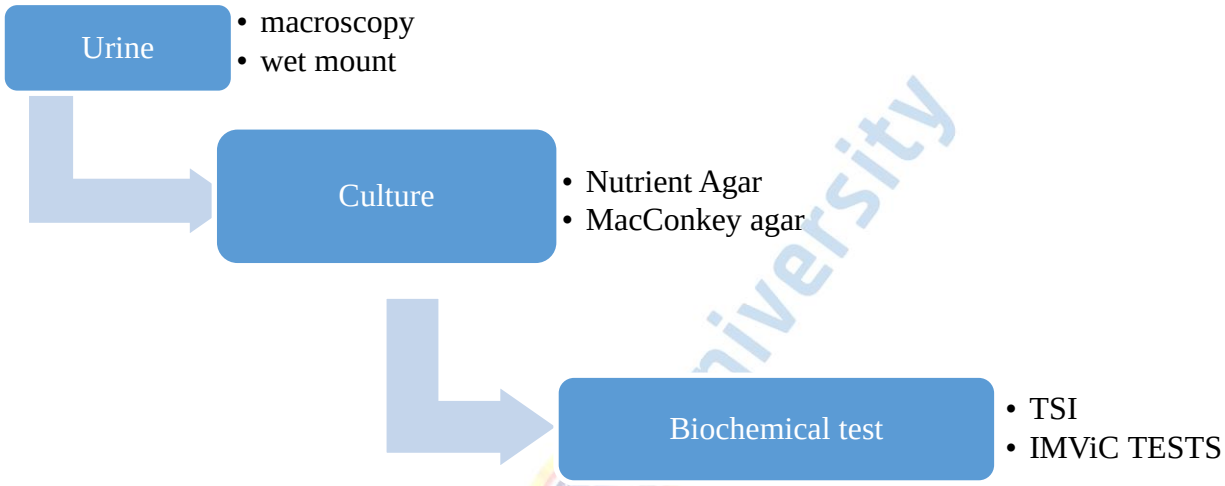
A 10 µl standardized loop was dished in upright position in the urine and removed, the collected fluid was inoculated in Nutrient and MacConkey agars respectively.

The respective inoculated Medias was incubated for an overnight at an optimum growth temperature of 37°C (Protocol 9).

3.8.3.6 Biochemical Identification of *Escherichia coli*

Triple sugar iron (TSI) and Indole (I), Methyl Red (MR), Voges Proskauer (VP) and Citrate (C) utilization (IMVC) are the five biochemical tests commonly utilized for

entero-bacteriaceae classification. *Escherichia coli* produces acid and gas under Triple sugar iron, *it* is Indole and MR positive VP and citrate negative (IMVC++ - -), H2S is not formed and urea is not hydrolyzed (Protocol 9).



3.8.4 Anti-bacterial assay

Escherichia coli clinical isolates was suspended in glycerol. The Mueller Hinton agar was set then dispersed in petrish dishes in which the clinical isolates was inoculated and incubated overnight at 37°C to enable recovery of micro-organism

3.8.4.1 Preparation of media

A. Preparation Mueller Hinton agar

Mueller-Hinton agar (MHA), was the paramount medium to use for routine susceptibility testing using the Kirby Bauer disc diffusion method for non-fastidious bacteria. It is also the most acknowledged medium used for most broth dilution testing as the condition contents are well preserved.

B. Preparation McFarland turbidity standard

The adjustment of the turbidity and concentration of the inoculums will use McFarland turbidity standard preparation when performing antimicrobial susceptibility test.

3.8.4.2 The diffusion method of Kirby Bauer disc

A modified diffusion technique of Kirby-Bauer disc for Antimicrobial Susceptibility Testing by measuring zones of inhibition was applied. Gray shading indicated a confluent lawn of bacterial growth while the white circle indicated absence of growth of the test organism.

3.8.4.3 Measurement of Inhibition Zones

300 mg of every unrefined plant separate was broken down in 1000 µl (1ml) of DMSO. A stock miniature weakening technique was followed to decide the base inhibitory Focus for the dynamic rough concentrates against E.coli consulting with the Clinical Standard Establishment (CLSI) (Korir et al., 2012b). The tests were acted in 96 well miniature titer plates. Sequential multiplying weakening's were acted in which the succeeding fixation all around was half of the focus in the past well. The MIC was resolved just where the plant remove areas of strength for introduced action (≥ 9 mm) by the circle dispersion technique (Mariita et al., 2010a). The wells were loaded up with 50 µl of the Muller Hinton stock for bacterial strains. Then, 50 µl of the plant remove (made by dissolving 300 mg of each concentrate in 1000 µl (1ml) of DMSO for complete disintegration) were administered into the principal a long time before sequential weakening's. The sequential weakening's were achieved by moving 50 µl of Muller Hinton stock including the concentrate from the initial well through the second, third and fourth wells. Then 50 µl of the test disconnects were dispersed into each well. One line of wells was utilized as regrettable control of the development of the

microorganisms in the medium, while 50 µl of the anti-microbial ciprofloxacin were utilized as a positive control. Miniature titre plates were covered. Microbes were brooded at 37 ºC for 24 hrs (Kitonde et al., 2013) Least inhibitory Fixations not set in stone by recording the most reduced centralization of the dynamic concentrates that restrained development of miniature organic entities when contrasted with the control stock turbidity (Wagete *et al*., 2010; Kitonde *et al*., 2013).

Inhibition zones measurements and interpretation after incubation time was to determine the antimicrobial potential of *Jatropha Curcas* crude plant extracts leaves and stem bark s against *E.coli*, designated by clear zones of growth inhibition around each disc diameter and was documented in millimeters by use of a ruler on the undersurface of the plate minus opening the lid. Inhibition diameter zones was dignified by the boundaries of the dense growth. The inhibition zones was compared with the zone-size interpretation of acknowledged growth inhibition zones and documented as susceptible, intermediate or resistant to each crude drug (CLSI, 2015).

3.8.5 Minimum Inhibition Concentration and Minimum Bactericidal Concentration

The determination of the MIC value using the REMA was based on the color change indicator as a result of growing bacteria interaction with the indicator, known to change from blue to pink purple in presence of bacteria growth. (Maya *et al*., 2019)

Culture methods

The test organic entities was put on Supplement Agar (NA) slants, ready from Supplement Stock base (Oxoid CM1) at 2·5 °C, All miniature creatures utilized in MIC measures were two times passed on 16-18 h societies filled in Supplement Stock (NB) (Oxoid CM1)

Assay media

Through the resazurin MIC strategy (REMA), the inocula were weakened to the suitable cell densities in NB containing 0·15% (w/v) agar for all test societies for which BHIB with 0·15% (w/v) agar was utilized. Before vaccination, test media were liquefied by steaming and tempered to 37 °C, at which temperature they stayed fluid. (Maya *et al*., 2019)

Inoculum densities

The cell fixation expected to cause decrease of resazurin inside 2 not entirely set in stone for every one of the test creatures. Sequential 10-crease weakening's of each culture were ready. NB. Aliquots (1·7 ml) were administered into tubes containing 0·2 ml 'messy' (0·15%, w/v) agar, and 0·1 ml resazurin arrangement. The cylinders were brooded for 2 h at 37 °C, at which point aliquots from adjoining blue (oxidized) mauve and pink (diminished) weakening cylinders were tried by the plate count strategy. Sequential twofold weakening's (0·005-1·25%, v/v) of the concentrates were ready by vortexing in room-temperature.

The resazurin examine medium was then vaccinated with the test organic entity to yield a last cell thickness expected to lessen resazurin. The inoculum thickness was affirmed by plate count. A clean 96-well microtitre plate with top was set up with every one of the test microorganisms. (n_8) as follows:

- Column 1–9, 170 ml inoculum plus 20 ml of a plant extract;
- Column 10, 170 ml inoculum plus 20 ml plant diluent (**positive control**);
- Column 11 and 12, 170 ml sterile resazurin assay medium plus 20 ml plant diluent (**Negative control and Blank, respectively**).

Well items were totally blended utilizing the micropipette Two plate were ready for every organic entity and brooded at 37 °C for either 3·5 h or 18 h. After brooding, 10 ml of resazurin arrangement was added to all aside from segment 12, to which 10 ml of refined water was added.Following a second brooding of 2 h at 37 °C, wells were evaluated outwardly to decide the MIC values. (Maya et al., 2019).Values of MIC obtained determined the proceeding of MBC which was not done.

3.9 Validity and Reliability of the data

Validity will ensure precise presentation and elucidation of the results, this was ensured by use of acknowledged *Escherichia coli ATCC 29218* from Microbiology section, National public health reference laboratory, Nairobi as a control bacteria.

Reliability of this study was the constancy of a degree that guarantees uniformity of this study. The consistency was confirmed by usage of the positive and negative control thus measuring both Minimum inhibition concentration and minimum bactericidal concentration.

3.10 Data analysis

Questionnaires were crisscrossed for comprehensiveness at the end of assortment period. Data was kept in an individual laptop where excel spread sheet software was used protected by a password. A flash disk backup was used and protected with password. Later the stored raw data was subjected to Minitab application for statics analysis, then it was exposed to descriptive statics then articulated as mean, standard error of mean. The statistical effects of leaves and stem bark extracts ingredients on *Escherichia coli* was analyzed by ONE WAY ANOVA followed by Turkey's post hoc test for pair wise comparison. A TWO WAY ANOVA was used to conclude on the effect of different leaves and stem bark ingredients. Graph pad prism application was

used to calculate standard error of the mean and the output, then after be presented as mean + or – standard error of the mean. (Misonge et al., 2015)The *p* values of less or equal to 0.05 was reflected important and the scrutinized data presented in tables and graphs

3.11 Ethical consideration

The right of voluntary consent, Principal of anonymity, Implication of confidentiality, and the necessity of data collection as per ethical consideration by Mwinzi, 2012, was achieved through principled authorization pursued from Mount Kenya University Ethical Review Commission. Authorization was pursued from Kitui County Hospital Research team management. Permit to carry out the research was obtained from NACOSTI and informed consent from the study populations (Appendix III, IV, V, VI, VII, VIII, and IX)

CHAPTER FOUR

RESEARCH RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Phytochemical Compounds

Table 6: Qualitative phytochemical compounds profiling

	Phytochemical test	Name of test	Theoretical test results		Actual test results in respective extracts solvents							
			Positive	Negative	Leaves				Stem bark			
					M	E. Ac	Aq		M	E. Ac	Aq	
1	Saponins	Froth test	Tenacious frothing	No Tenacious frothing	+	-	+	+	+	-	+	+
2	Alkaloids	Dragendoff's reagent	whitish to buffy precipitate	No whitish to buffy precipitate	+	+	+	+	+	+	+	-
3	Tannins	Ferric chloride test	green precipitate	No green precipitate	+	-	+	+	+	+	+	+
4	Flavonoids	Ferric chloride test	Black-red color	No Black-red color	-	+	+	+	-	+	+	+
5	Phenol	Ethyl acetate test	green precipitate	No green precipitate	+	-	+	+	+	+	+	+
7	Sterols	Liebermann burchard's test	translucent green color	No translucent green color	+	+	+	-	+	+	+	-
8	Terpenoids	Liebermann burchard's test	A reddish brown ring	A reddish brown ring	+	+	+	+	+	+	+	+
9	Coumarins	Ammonia test	Fluorescence indicatio	No Fluorescence	-	-	-	-	+	-	+	-

			n on the filter paper	indication on the filter paper									
10	Glycosides	Keller killian's test	Reddish brown ring slowly becoming bluish green colour	Reddish brown ring slowly becoming bluish green colour	-	-	-	-	+	+	-	-	

Key; M-Methanol, E.Ac-Ethyl Acetate, A-Acetone, Aq- Aqueous

4.1.2 Antimicrobial Efficacy

4.1.2.1Susceptibility testing of Clinical isolates and Control bacteria

A total of 211 female Diabetic patients were engaged for the research as a sample size via testing their Fasting blood sugars. Urinalysis via Macroscopy, Dipstick and Microscopy was done to rule out Urinary tract infection followed by Culture in Cled media which confirmed 101 female diabetic patients to have UTIs of suspected *E.coli* growth via their morphological characteristics. Out of the 101 suspected *E.coli* clinical isolates, 70 confirmed to be true *E.coli* via the biochemical test used.as illustrated in Table 8 and as per Protocol 1, 4,5,6,7,8 and 9.

Chart 1: Susceptibility testing flow chart illustration

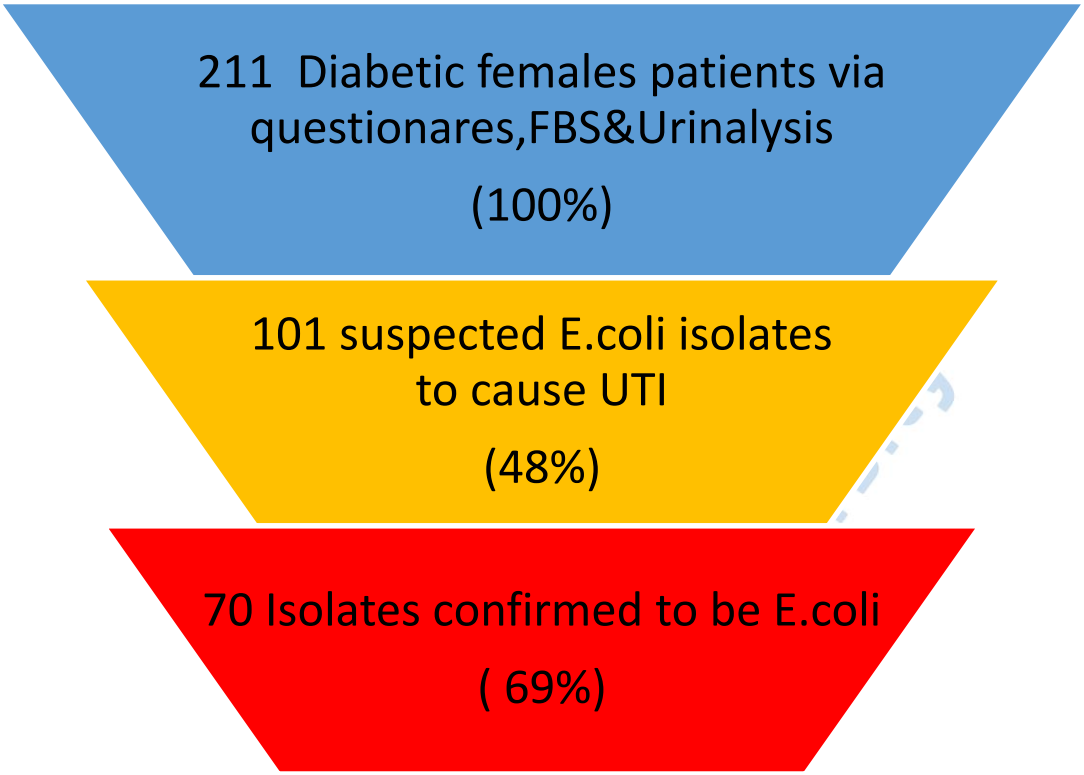


Table 7: Fasting Blood Sugar and Urinalysis Findings

Sugars &Urinalysis parameters	Ages(Yrs.) 21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	76-80	81-85
FBS	2	5	8	11	16	14	11	10	9	7	5	2	1
Glucose	2	5	8	11	16	14	11	10	9	7	5	2	1
Bilirubin	0	0	0	0	0	0	0	0	0	0	0	0	1
Ketones	0	0	1	2	3	1	1	0	0	0	0	1	1
SG	2	5	8	11	16	14	11	10	9	7	5	2	1
PH	2	5	8	11	16	14	11	10	9	7	5	2	1
Blood	0	0	0	1	1	0	0	0	0	0	0	0	0
Protein	0	0	1	0	0	0	0	0	0	0	0	0	1
Urobilinogen	0	0	0	0	0	0	0	0	0	0	0	0	1
Nitrates	1	0	1	2	0	0	1	0	0	0	0	0	0
leucocytes	2	5	8	11	16	14	11	10	9	7	5	2	1

Fasting blood sugar (FBS) and Urinalysis findings for known diabetic females against different age groups.

KEY; FBS= Values above 8mml/l for a known diabetic patient

Numbers indicates positivity patients' rate despite the intensity of the findings

Table 8: Presumptive and Purification of *E.coli* Primary isolates and Control bacteria

S/N	Motility	Indole	Lysine decarboxylation	Lysine deamination	Simmons Citrate	Eosin methylene blue	REMARKS on suspected E.Coli results
1	+	-	+	-	-	+	**False**
2	+	+	+	-	-	-	**False**
3	+	+	+	-	-	+	**True**
4	+	-	+	-	-	+	**False**
5	+	-	+	-	-	+	**False**
6	+	+	+	-	-	+	**True**
7	+	+	+	-	-	+	**True**
8	+	-	+	-	-	+	**False**
9	+	-	+	-	-	-	**False**
10	+	-	+	-	-	-	**False**
11	+	+	+	-	-	+	**True**
12	+	-	+	-	-	+	**False**
13	+	+	+	-	-	+	**True**
14	+	+	+	-	-	+	**True**
15	+	+	+	-	-	+	**True**
16	+	+	+	-	-	+	**True**
17	+	+	+	-	-	-	**False**
18	+	+	+	-	-	-	**False**
19	+	+	+	-	-	+	**True**

20	+	+	+	-	-	+	**True**
21	+	+	+	-	-	+	**True**
22	+	+	+	-	-	+	**True**
23	+	-	+	-	-	+	**False**
24	+	+	+	-	-	+	**True**
25	+	+	+	-	-	+	**True**
26	+	+	+	-	-	+	**True**
27	+	+	+	-	-	+	**True**
28	+	+	+	-	-	+	**True**
29	+	+	+	-	-	+	**True**
30	+	-	+	-	-	-	**False**
31	+	+	+	-	-	+	**True**
32	+	+	+	-	-	+	**True**
33	+	-	+	-	-	-	**False**
34	+	+	+	-	-	+	**True**
35	+	+	+	-	-	+	**True**
36	+	+	+	-	-	+	**True**
37	+	+	+	-	-	+	**True**
38	+	+	+	-	-	+	**True**
39	+	+	+	-	-	+	**True**
40	+	+	+	-	-	+	**True**
41	+	+	+	-	-	+	**True**
42	+	+	+	-	-	-	**False**
43	+	-	+	-	-	-	**False**
44	+	+	+	-	-	+	**True**
45	+	+	+	-	-	+	**True**

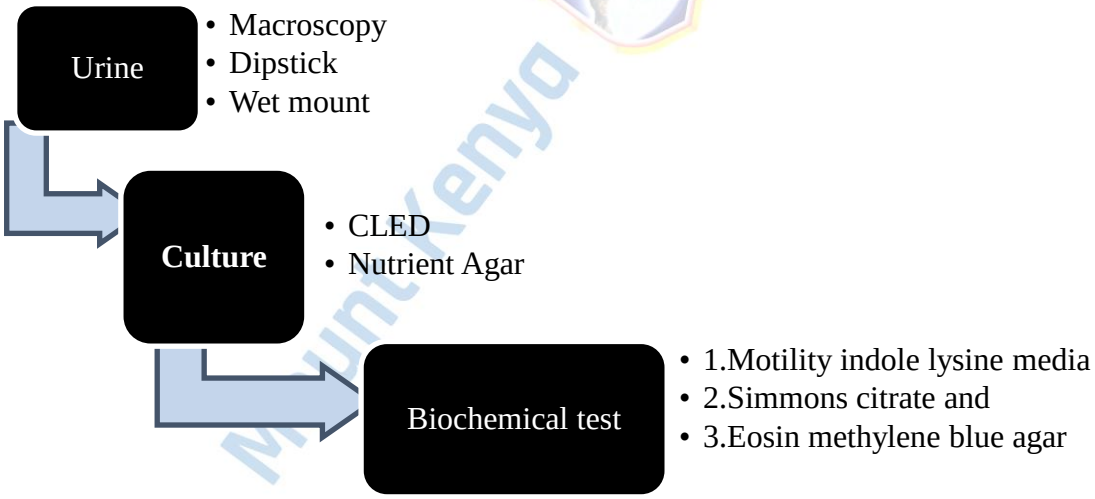
46	**+**	**-**	**+**	**-**	**-**	**-**	**False**
47	**+**	**-**	**+**	**-**	**-**	**-**	**False**
48	**+**	**+**	**+**	**-**	**-**	**-**	**False**
49	**+**	**+**	**+**	**-**	**-**	**+**	**True**
50	**+**	**+**	**+**	**-**	**-**	**+**	**True**
51	**+**	**+**	**+**	**-**	**-**	**+**	**True**
52	**+**	**+**	**+**	**-**	**-**	**+**	**True**
53	**+**	**+**	**+**	**-**	**-**	**+**	**True**
54	**+**	**+**	**+**	**-**	**-**	**+**	**True**
55	**+**	**+**	**+**	**-**	**-**	**-**	**False**
56	**+**	**+**	**+**	**-**	**-**	**+**	**True**
57	**+**	**+**	**+**	**-**	**-**	**+**	**True**
58	**+**	**-**	**+**	**-**	**-**	**-**	**False**
59	**+**	**-**	**+**	**-**	**-**	**-**	**False**
60	**+**	**+**	**+**	**-**	**-**	**+**	**True**
61	**+**	**+**	**+**	**-**	**-**	**+**	**True**
62	**+**	**-**	**+**	**-**	**-**	**-**	**False**
63	**+**	**+**	**+**	**-**	**-**	**+**	**True**
64	**+**	**+**	**+**	**-**	**-**	**-**	**False**
65	**+**	**+**	**+**	**-**	**-**	**+**	**True**
66	**+**	**+**	**+**	**-**	**-**	**+**	**True**
67	**+**	**+**	**+**	**-**	**-**	**+**	**True**
68	**+**	**+**	**+**	**-**	**-**	**+**	**True**
69	**+**	**+**	**+**	**-**	**-**	**-**	**False**
70	**+**	**+**	**+**	**-**	**-**	**+**	**True**
71	**+**	**+**	**+**	**-**	**-**	**+**	**True**

72	+	+	+	-	-	+	**True**
73	+	+	+	-	-	+	**True**
74	+	+	+	-	-	+	**True**
75	+	+	+	-	-	+	**True**
76	+	+	+	-	-	+	**True**
77	+	-	+	-	-	-	**False**
78	+	+	+	-	-	+	**True**
79	+	+	+	-	-	+	**True**
80	+	+	+	-	-	+	**True**
81	+	+	+	-	-	+	**True**
82	+	+	+	-	-	+	**True**
83	+	-	+	-	-	-	**False**
84	+	-	+	-	-	-	**False**
85	+	+	+	-	-	+	**True**
86	+	+	+	-	-	+	**True**
87	+	+	+	-	-	+	**True**
88	+	+	+	-	-	+	**True**
89	+	+	+	-	-	+	**True**
90	+	+	+	-	-	+	**True**
91	+	+	+	-	-	+	**True**
92	+	+	+	-	-	+	**True**
93	+	-	+	-	-	-	**False**
94	+	+	+	-	-	+	**True**
95	+	+	+	-	-	+	**True**
96	+	-	+	-	-	-	**False**
97	+	+	+	-	-	+	**True**

98	+	+	+	-	-	-	**False**
99	+	+	+	-	-	+	**True**
100	+	+	+	-	-	+	**True**
101	+	+	+	-	-	+	**True**
Control E.Coli ATC29218	+	+	+	-	-	+	**True**

4.1.2.2 Presumptive and Purification of the primary clinical isolates

Presumptive and Purification of the 101 primary isolates of *E.Coli* through biochemical tests gave a total of 70 pure *E.Coli* clinical isolates which were further pooled together for efficacy test with Jatropha curcas extracts with controls.



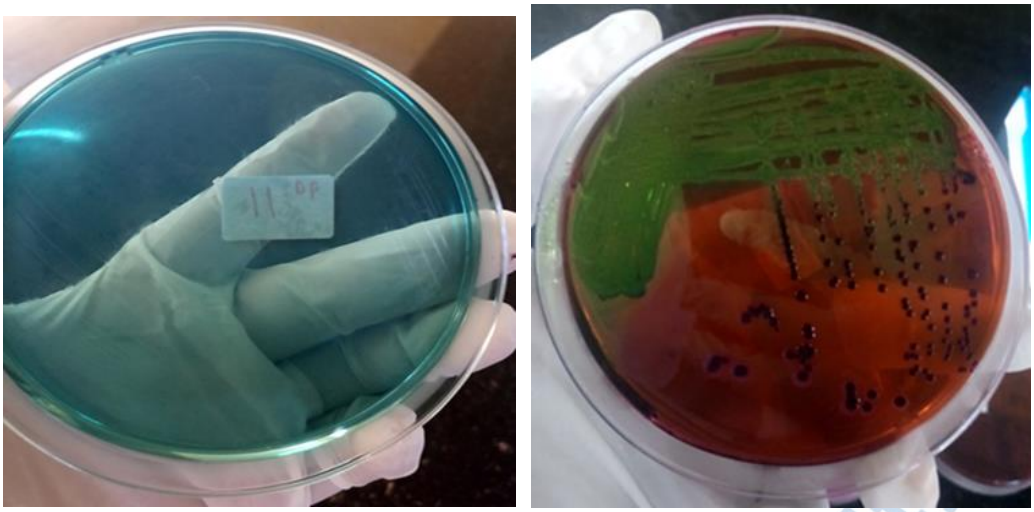


Figure 7: S/No. 11 Simmons Citrate Negative and S/No 22 Eosin Methylene Blue agar (Green sheen, LFs and acid produce)

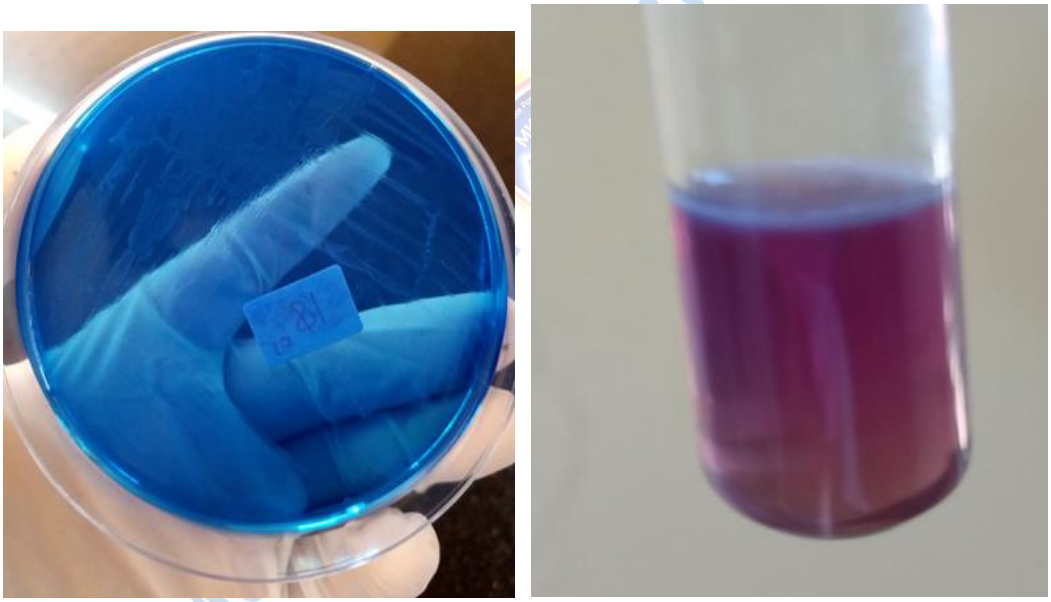


Figure 8: S/No 18 = Simmons Citrate positive and M-Positive, LDC-Positive, LDA-Negative

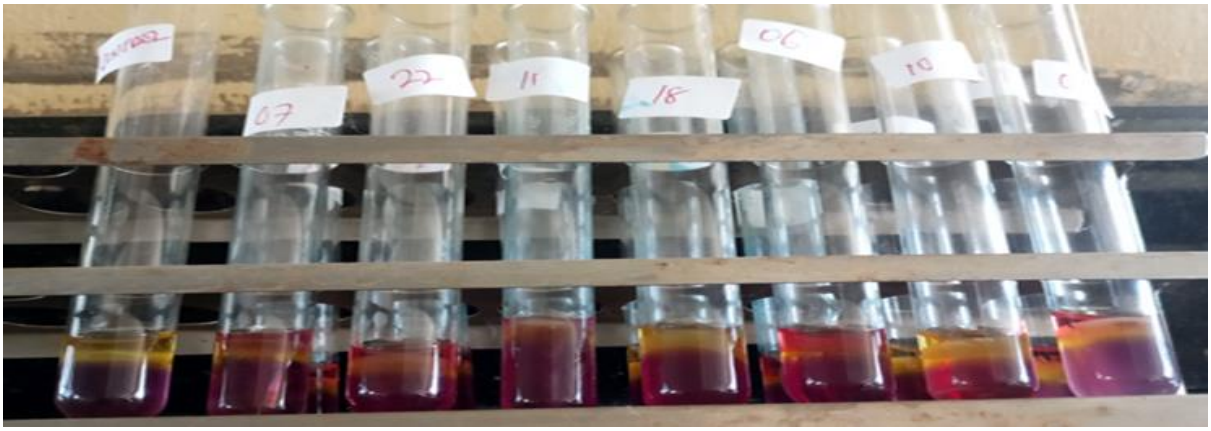


Figure 9: Indole Test

Positive-red color change at the top, on addition of 4 drops of Kovac's reagent (07, 22, 11, 06 and 09**)**

Negative - No color change at the top, on addition of 4 drops of Kovac's reagent (18 and 10) (Presumptive and Purification raw data, Appendix VII)

4.1.2.3 Inhibition Zones

Antimicrobial activity of methanol, acetone, aqueous and ethyl acetate solvents against Clinical isolate *E.Coli* and *E.Coli* Control bacteria ATCC 29218

A piloting J.C leaves extracts inoculation on *E.coli* Inoculation in triplicate was done based on key solvents**.**

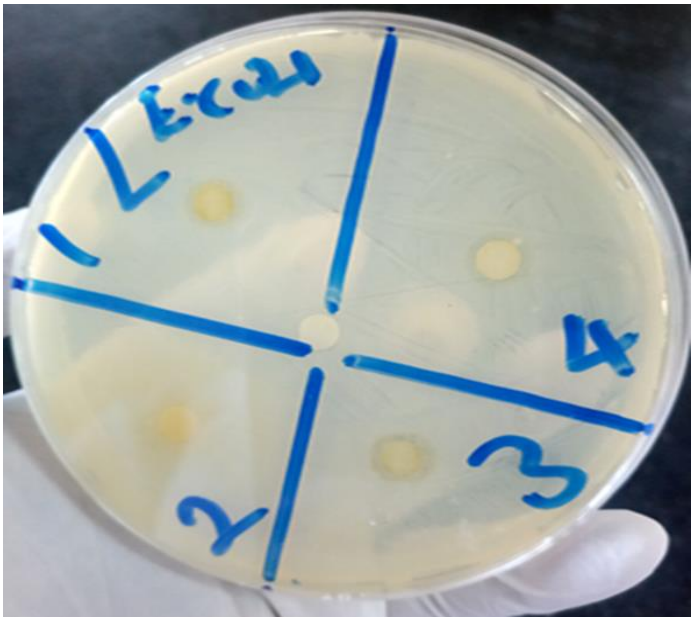


Figure 10: A piloting J.C leaves extracts inoculation on *E.coli*

Key1.Acetone, 2.Aqueous, 3.Ethyl acetate, 4.Methanol and at the Center- Negative control Isolates piloting analysis concluded to have a minimum diffusion efficacy of 100 mg/ml.

Further dilutions of 800 mg/ml, 400 mg/ml, 200 mg/ml and 100 mg/ml were prepared, inoculations and incubation done in triplicate.

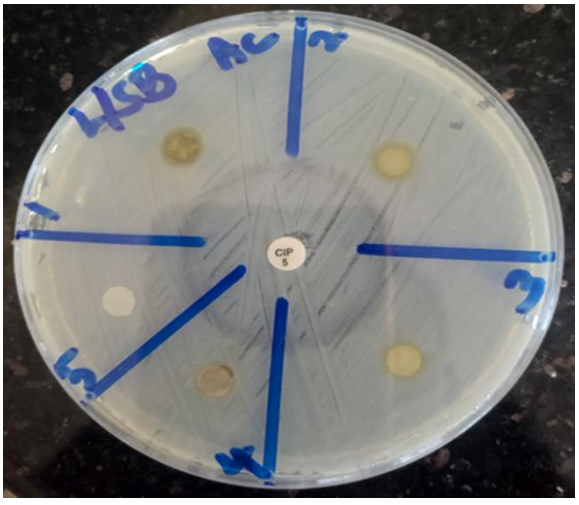


Figure 11: J.Curcas Concoction piloting inoculation on *E.coli*

Dilutions of 800 mg/ml, 400 mg/ml, 200 mg/ml and 100 mg/ml inhibition zones (mm) of Leaves and Stem bark concoction extracts

4.1.2.3.1 Leaves extracts Inhibition Zones (mm)

Table 9: Leaves extracts Inhibition Zones (mm)

SOLVENT	Concentration(mg/ml)	T1(mm)	T2(mm)	T3(mm)	MEAN(mm)
AQUEOUS	800	9	9	9	9
	400	8	8.5	8	8.1
	200	8	8	8	8
	100	7	7	7	7
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan .,2011)	34	34	34	34
ACETONE	800	8.5	8	8	8.1
	400	8	7.5	7	7.5
	200	7.5	7	7	7.1
	100	7	6.5	6	6.5
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan .,2011)	34	34	34	34
ETHYL ACETATE	800	9	9	9	9
	400	8	8	8	8
	200	7	7	7	7
	100	7	7	7	7
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan .,2011)	34	34	34	34
METHANOL	800	8	9	8	8.3
	400	7	8.5	7	7.5
	200	7	8	7	7.3
	100	7	7.5	6	6.8
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan .,2011)	34	34	34	34

4.1.2.3.2. Stem barks inhibition zones (mm)

Table 10: Stem barks inhibition zones

SOLVENT	Concentration(mg/ml)	T1(mm)	T2(mm)	T3(mm)	MEAN(mm)
	800	8	9	9	8.7
	400	7	8	8	7.7
	200	6	8	8	7.3
AQUEOUS	100	5(no activity)	5	5	5
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan.,2011)	34	34	34	34
	800	8	8	8	8
	400	7	7	7	7
	200	7	7	7	7
ACETONE	100	5	5	5	5
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan.,2011)	34	34	34	34
	800	5	5	5	5
	400	5	5	5	5
ETHYL	200	5	5	5	5
ACETATE	100	5	5	5	5
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan.,2011)	34	34	34	34
	800	8	5	5	6
	400	8	5	5	6
	200	5	5	5	5
	100	5	5	5	5
METHANOL	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan.,2011)	34	34	34	34

4.1.2.3.3 Concoction of leaves and stem barks extracts inhibition zones (mm)

Table 11: Concoction of leaves and stem barks extracts inhibition zones (mm)

SOLVENT	Concentration(mg/ml)	T1(mm)	T2(mm)	T3(mm)	MEAN(mm)
AQUEOUS	800	8	8	8	8
	400	7	7	7	7
	200	6	6	6	6
	100	5	5	5	5
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan .,2011)	34	34	34	34
ACETONE	800	10	9	10	9.7
	400	8	8	8	8
	200	7	7	7	7
	100	6	6	6	6
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan .,2011)	34	34	34	34
ETHYL ACETATE	800	8	8	8	8
	400	7	8	7	7.3
	200	7	7	7	7
	100	7	7	7	7
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan .,2011)	34	34	34	34
METHANOL	800	9	8	8	8.3
	400	8	7.5	7.5	7.7
	200	8	7.5	7.5	7.7
	100	7	7	7	7
	NEG CONT	-	-	-	-
	POS.CONT, (Ciprofloxacin 25mg/mm(Daniyan .,2011)	34	34	34	34

Leaves, stem bark s and their concoction analysis Mean inhibition zones comparison Findings in different Extraction solvents in different concentration (800,400,200 and 100.)

4.1.2.4 Minimum Inhibition Concentration

Double serial dilution of the extracts was done and the lowest concentration that inhibited growth was recoded in milligrams per milliliter. Minimum inhibitory concentration that was able to prevent growth of micro-organism. MIC 50 is the concentration that is able to inhibit growth of 50% of the micro-organism while MIC90 is the Minimum concentration that inhibits growth of 90% of the bacteria.

The minimum inhibitory concentration of the plant extracts was determined by the broth micro dilution method in 96 well microtitre plates for MIC.

The determination of the MIC value was done using the Resazurin-based microplate assay (REMA) method, (Maya Dian Rakhmawatie at al., 2019)based on the change of color resulting from interaction between indicators with the growing test bacteria, changing from blue to pink purple in presence of bacterial growth.

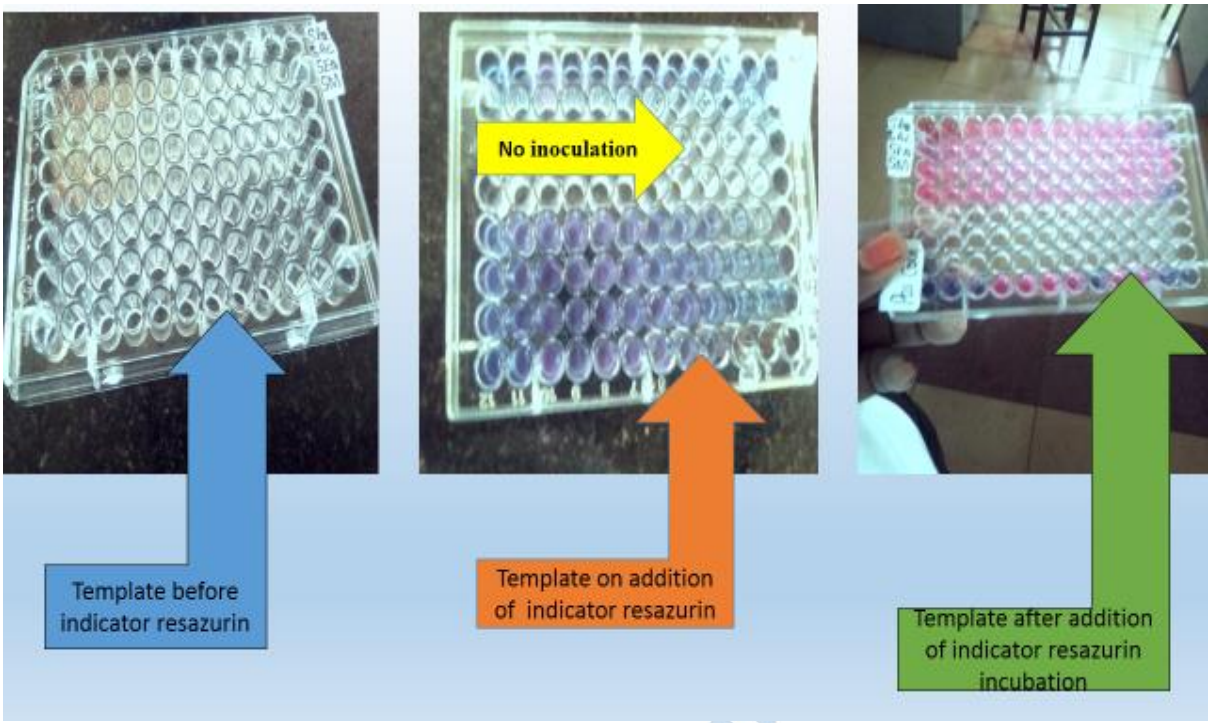


Figure 12: Resazurin-Based Microplate Assay Plates

4.1.2.4.1 Leaves extracts MIC

Table 12: Leaves extracts MIC 50

		SERIAL DILUTIONS Mg/ml	Mg/ml	NEGATIVE CONTROL (Nitrate broth +*E.coli* 29218)	POSITIVE CONTROL (Nitrate broth	SOLVENTS AQUEOUS	ACETONE	ETHYL ACETATE	METHANOL
LEAVES EXTRACTS	A. MIC 50	400		Growth	No Growth	Negative	Negative	Negative	Negative
		200		Growth	No Growth	Negative	Negative	Negative	Negative
		100		Growth	No Growth	Negative	Negative	Negative	Negative
		50		Growth	No Growth	Negative	Negative	Negative	Negative
		25		Growth	No Growth	Negative	Negative	Negative	Negative
		12.5		Growth	No Growth	Negative	Negative	Negative	Negative
		6.255		Growth	No Growth	Negative	Negative	Negative	Negative
		3.125		Growth	No Growth	Negative	Negative	Negative	Negative

Leaves extracts MIC 90

		SERIAL DILUTIONS Mg/ml	NEGATIVE CONTROL (Nitrate broth +*E.coli* 29218)	POSITIVE CONTROL (Nitrate broth +*E.coli* 29218+ CIP 25Mg/ml)	SOLVENTS AQUEOUS	ACETONE	ETHYL ACETATE	METHANOL
LEAVES EXTRACTS	B. MIC 90	400	Growth	No Growth	Negative	Negative	Negative	Negative
		200	Growth	No Growth	Negative	Negative	Negative	Negative
		100	Growth	No Growth	Negative	Negative	Negative	Negative
		50	Growth	No Growth	Negative	Negative	Negative	Negative
		25	Growth	No Growth	Negative	Negative	Negative	Negative
		12.5	Growth	No Growth	Negative	Negative	Negative	Negative
		6.255	Growth	No Growth	Negative	Negative	Negative	Negative
		3.125	Growth	No Growth	Negative	Negative	Negative	Negative

4.1.2.4.2. Stem barks extracts MIC

Table 13: Stem bark s extracts MIC 50

		SERIAL DILUTIONS Mg/ml	Mg/ml NEGATIVE CONTROL (Nitrate broth +*E.coli* 29218)	POSITIVE CONTROL (Nitrate broth +*E.coli* 29218+ CIP 25Mg/ml)	SOLVENTS AQUEOUS	ACETONE	ETHYL ACETATE	METHANOL
STEM BARK EXTRACTS	A. MIC 50	400	Growth	No Growth	Negative	Negative	Negative	Negative
		200	Growth	No Growth	Negative	Negative	Negative	Negative
		100	Growth	No Growth	Negative	Negative	Negative	Negative
		50	Growth	No Growth	Negative	Negative	Negative	Negative
		25	Growth	No Growth	Negative	Negative	Negative	Negative
		12.5	Growth	No Growth	Negative	Negative	Negative	Negative
		6.255	Growth	No Growth	Negative	Negative	Negative	Negative
		3.125	Growth	No Growth	Negative	Negative	Negative	Negative

Stem bark s extracts MIC 90

		SERIAL DILUTIONS Mg/ml	Mg/ml NEGATIVE CONTROL (Nitrate broth +*E.coli* 29218)	POSITIVE CONTROL ((Nitrate broth +*E.coli* 29218+ CIP 25Mg/ml)	SOLVENTS AQUEOUS	ACETONE	ETHYL ACETATE	METHANOL
STEM BARK EXTRACTS	B. MIC 90	400	Growth	No Growth	Negative	Negative	Negative	Negative
		200	Growth	No Growth	Negative	Negative	Negative	Negative
		100	Growth	No Growth	Negative	Negative	Negative	Negative
		50	Growth	No Growth	Negative	Negative	Negative	Negative
		25	Growth	No Growth	Negative	Negative	Negative	Negative
		12.5	Growth	No Growth	Negative	Negative	Negative	Negative
		6.255	Growth	No Growth	Negative	Negative	Negative	Negative
		3.125	Growth	No Growth	Negative	Negative	Negative	Negative

4.1.2.4.3 Concoction of leaves and stem bark extracts MIC

Table 14: concoction of leaves and stem bark extracts MIC 50

CONCOCTION OF LEAVES AND STEM BARK EXTRACTS A. MIC 50	SERIAL DILUTIONS Mg/ml	NEGATIVE CONTROL (Nitrate broth +*E.coli* 29218)	POSITIVE CONTROL ((Nitrate broth +*E.coli* 29218+ CIP 25Mg/ml)	SOLVENTS AQUEOUS	ACETONE	ETHYL ACETATE	METHANOL
	Mg/ml						
	400	Growth	No Growth	Negative	Negative	Negative	Negative
	200	Growth	No Growth	Negative	Negative	Negative	Negative
	100	Growth	No Growth	Negative	Negative	Negative	Negative
	50	Growth	No Growth	Negative	Negative	Negative	Negative
	25	Growth	No Growth	Negative	Negative	Negative	Negative
	12.5	Growth	No Growth	Negative	Negative	Negative	Negative
	6.255	Growth	No Growth	Negative	Negative	Negative	Negative
	3.125	Growth	No Growth	Negative	Negative	Negative	Negative

Concoction of leaves and stem bark extracts MIC 90

CONCOCTION OF LEAVES AND STEM BARK EXTRACTS

B. MIC 90

SERIAL DILUTIONS Mg/ml	NEGATIVE CONTROL (Nitrate broth +*E.coli* 29218)	POSITIVE CONTROL ((Nitrate broth +*E.coli* 29218+ CIP 25Mg/ml)	SOLVENTS AQUEOUS	ACETONE	ETHYL ACETATE	METHANOL
400	Growth	No Growth	Negative	Negative	Negative	Negative
200	Growth	No Growth	Negative	Negative	Negative	Negative
100	Growth	No Growth	Negative	Negative	Negative	Negative
50	Growth	No Growth	Negative	Negative	Negative	Negative
25	Growth	No Growth	Negative	Negative	Negative	Negative
12.5	Growth	No Growth	Negative	Negative	Negative	Negative
6.255	Growth	No Growth	Negative	Negative	Negative	Negative
3.125	Growth	No Growth	Negative	Negative	Negative	Negative

4.2 DISCUSSION

4.2.1. Phytochemical Compounds

4.2.1.1 Leaves

Phytochemical compounds Alkaloids, Tannins, Phenol, and Sterol were extracted by Methanol solvents which concurs with Rajesh et al., 2016. Presence of Flavonoids, Sterol and Alkaloids therapeutic indicators despite the solvent used coincides with Maksudur *et al*., 2012 and Prasad et al., 2012 findings. Presence of Flavonoids in Methanol extracts of immature and mature leaves as per Rajesh *et al*., 2016 contrasted with this study findings, however its deficiency in fully mature leaves agreed with this study. Presence of Coumarins under solvents Ethyl Acetate and Methanol in fully mature leaves in reference to Rajesh *et al*., 2016 differed with this study. Despite the age of the leaves, Tannins presence agreed with Rajesh *et al.*, 2016 findings under Methanol and Ethyl Acetate solvents. Phenol compound findings of this study concurred with Rajesh *et al*., 2016. Findings.

4.2.1.2 Stem barks

Phytochemical compounds Alkaloids, Tannins, and Glycosides presence of this study agreed with Prasad *et al*., 2012 however absence of flavonoids varies with the Prasad *et al*, 2012 findings. Alkaloids and flavonoids compound in Ethyl Acetate solvent presence and their absence in Methanol solvent of this study agreed with Rajesh *et al*., 2016 findings. Coumarins presence in Methanol extract concurred with Rajesh *et al*., 2016 however his presence in Ethyl Acetate differed with this study.

4.2.1.3 Common compounds for the leaves and stem barks

This study common Phytochemical compounds Saponins, Tannins, Alkaloids, Terpenoids and Flavonoids therapeutic indicators presence corresponded with Ifayefunmi., 2018 discoveries. Alkaloids, Tannins and Flavonoids common compounds

agreed with Rajesh *et al.*, 2016 and Ifayefunmi., 2018. Coumarins seemed to be a common compound as per Rajesh *et al.*, 2016 however this study results indicated to be found only in stem barks.

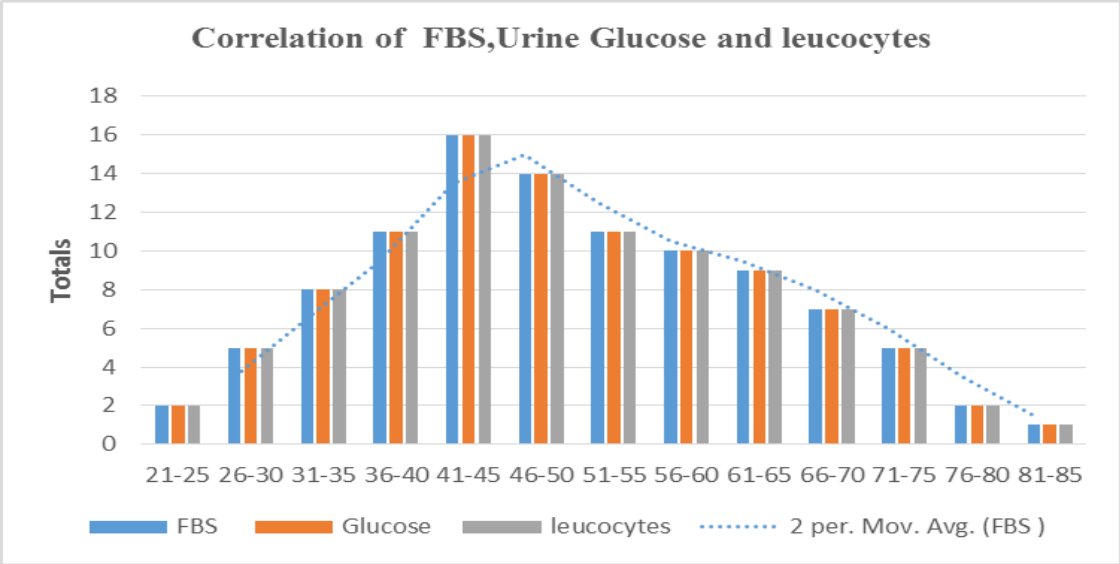
4.2.2 Antimicrobial Efficacy

4.2.2.1 Correlation of FBS and Urinalysis

Table 15: Correlation of FBS and Urinalysis

Sugars &Urinalysis parameters totals	Ages(Yrs.)												
	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	76-80	81-85
FBS	2	5	8	11	16	14	11	10	9	7	5	2	1
Glucose	2	5	8	11	16	14	11	10	9	7	5	2	1
leucocytes	2	5	8	11	16	14	11	10	9	7	5	2	1

Chart 2: Correlation of Fasting Blood Sugar with Urine glucose and leucocytes

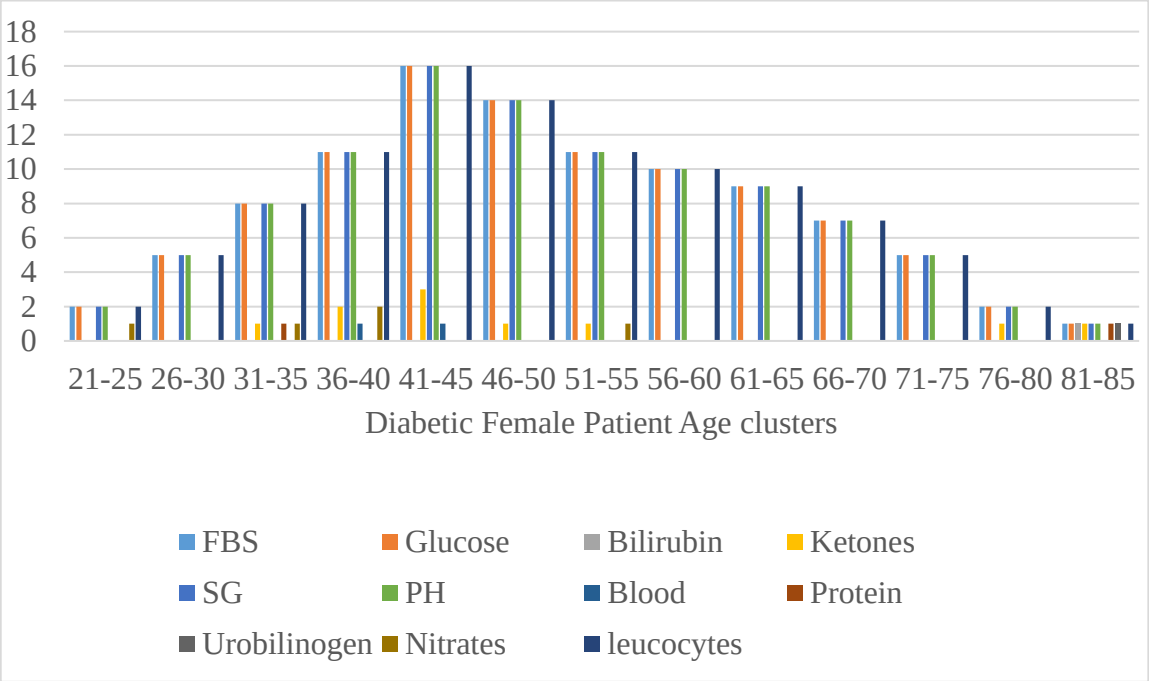


KEY; FBS= Values above 8mml/l for a known diabetic patient

Total Numbers indicates positivity patients' rate despite the intensity of the findings

The findings indicated a Fasting blood sugar at age's 41-45years to be correlating with high leucocytes frequency at the same age intervals and thus fasting blood sugar levels are directly proportional to leucocytes values.

Chart 3: Correlation of FBS and Urinalysis



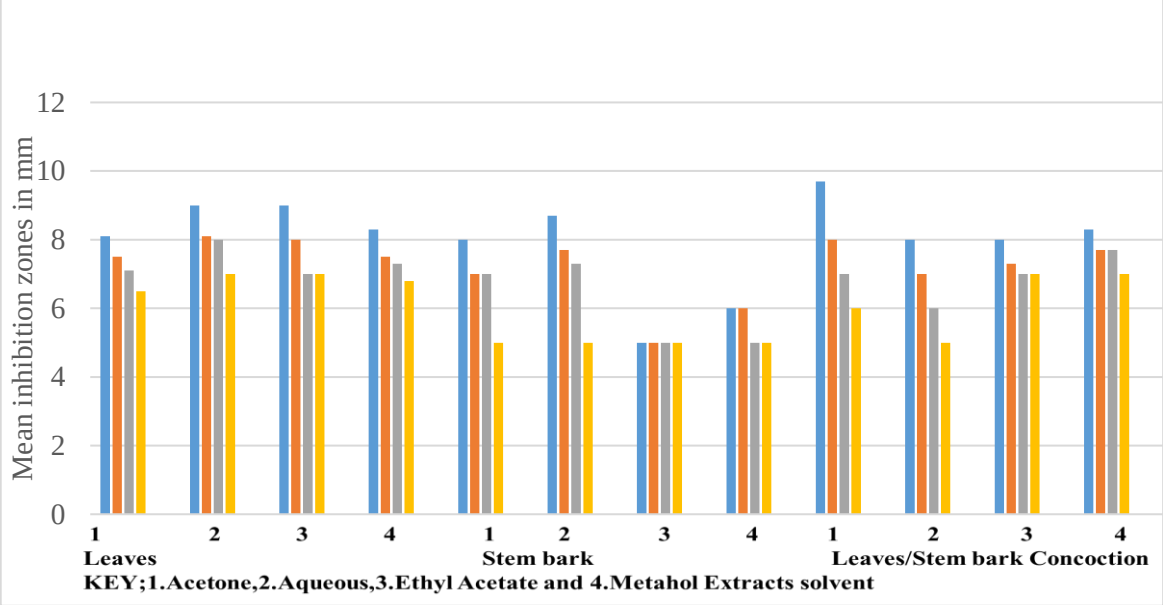
KEY; FBS= Values above 8mml/l for a known diabetic patient

Total Numbers indicates positivity patients' rate despite the intensity of the findings

The findings indicated a Fasting blood sugar at age's 41-45years to be correlating with high leucocytes frequency at the same age intervals and thus fasting blood sugar levels are directly proportional to leucocytes values.

4.2.2.2. Inhibition zones comparison

Chart 4: Comparison of Mean inhibition zones in mm of leaves, stem bark and a leave/stem bark concoction



KEY; Color codes define the different concentration dilutions. Blue= 800mg/ml, orange =400mg/ml, grey 200mg/ml and yellow 100mg/ml.

Leaves Aqueous and Ethyl acetate solvents were the best giving a mean inhibition zone of 9.0mm at 800mg/ml respectively whereas acetone was the poorest with a mean inhibition zone of 6.5 mm at 100mg/ml.

Stem bark aqueous solvent proofed to be the best with a mean inhibition zone of 8.7 mm at 800mg/ml unlike the ethyl acetate which gave the lowest mean inhibition zone of 5.0 in all the concentrations (800,400,200 and 100 mg/ml).

The mean inhibition zone of 9.7mm at 800mg/ml in acetone solvent was the most excellent for the concoction of leaves and stem bark s and also for the whole procedure at large while aqueous proofed to be the worst giving a mean inhibition zone of 5.0 mm 100mg/ml for the concoction.

4.2.2.3 Minimum Bactericidal Concentration

Minimum Bactericidal concentrations of MBC 50 AND MBC 90 was not indicative due to negative test results of all the Minimum Inhibition Concentration (MIC) dilutions of all the plant extracts. Failure to proceed to MBC from MIC was a static indication of the plant extracts to *E.coli* organism.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 SUMMARY

5.1.1. Phytochemical screening

A).Leaves

Phytochemical compounds Saponins, Phenol, and Tannins was extracted by the three common solvents; Methanol, Acetone and Aqueous. Saponins and tannins presence finding agreed with previous studies done in Nigeria on Phytochemical Screening and Antimicrobial Activity of Leaf extract of *Jatropha Curcas by Oyamal et al.,* 2016 though phenol was a new discovery and in South Africa on Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha Curcas* (Linn) by Igbinosa1 *et al*., 2009.

Ethyl acetate solvent extraction of sterols and alkaloids agrees with a discovery done Reddy, Amira and Maksudur, 2012 at Malaysia and by Sillma Rampadarath1, Daneshwar Puchooa1, and Rajesh Jeewon 2016 in Mauritius. Terpenoids was extracted in all the solvents whose presence in the larger jatropha curcas concurs with a study done by Anibijuwon, Gbala , Adedokun and Ifayefunmi 2018.

No extraction was achieved for Coumarins compounds. This agreed with a previous study done in Nigeria on Phytochemical Screening and Antimicrobial Activity of Leaf Extract of *Jatropha Curcas* by Oyamal *et al.,* 2016. Presence of flavonoids, sterol and alkaloids concurs with a research done by Reddy, Amira and Maksudur, 2012). Glycosides were not able to be detected in the different four solvent an indication result not concurring with a study done by Sillma Rampadarath1, Daneshwar Puchooa1, Rajesh Jeewon 2016 of Mauritius and Nigeria study on Phytochemical Screening and Antimicrobial Activity of Leaf Extract of *Jatropha Curcas* by Oyamal *et al*., 2016.

B) Stem bark s

Phytochemical compounds Tannins and Phenol were able to be extracted by the four solvents Methanol, Acetone, Aqueous and Ethyl Acetate solvents which agrees with a previous study done on *Jatropha curcas*: Plant of medical benefits by Reddy *et al*., 2012 at Malaysia and Sillma Rampadarath1, Daneshwar Puchooa1, Rajesh Jeewon 2016 in Mauritius.

Methanol and acetone solvent managed to extract six common compounds: alkaloids, tannins, phenol, sterols, coumarins and glycosides. Aqueous only extracted five compounds: saponis, tannins, flavonoids, terpenoids and phenol. The findings of saponins, alkaloids, tannins and sterols agrees with a study done previously in Malaysia on *Jatropha Curcas*: Plant of medical benefits (Reddy *et al*., 2012) Coumarins presences agreed with study done by Sillma Rampadarath1, Daneshwar Puchooa1, Rajesh Jeewon 2016 in Malaysia where methanol solvent was used, however they differ in some solvents where Malaysia's ethyl acetate managed to get the extract and in Kenya it was managed with acetone.

New findings on components of Phenol disagreed with previous study done in Malaysia on *Jatropha Curcas*: Plant of medical benefits (Reddy *et al.*, 2012)

C) Concoction of Leaves and Stem barks

Terpenoids was the best compound extracted in all the plant parts with the selected solvents an indicator in agreement with Anibijuwon, Gbala , Adedokun and Ifayefunmi , 2018.

Methanol and acetone was the best solvent for both leaves and stem bark s phytochemical compounds extraction. Aqueous was the poorest extraction solvent

5.1.2 Antimicrobial efficacy

5.1.2.1 Correlation of Fasting Blood Sugar and urinalysis

Fasting blood sugar was indicated to be high at age's 31-46years a suggestion of high diabetic prevalence rate as it is quoted to be at pick during child bearing ages, active sexual ages and menopause stage and a key role in UTI by Aadhan and Anand, 2017. A 211population total was the primary diabetic females selected via questioners, FBS test and urinalysis test for the study.101samples out of the selected 211 (48%) proved to have suspected E.Coli clinical isolates to cause UTI, 70 which is the 69% isolates confirmed to be true clinical E.coli giving a prevalence rate of 69%% of the causative agent of UTIs in diabetic females for this study in Kitui county, Kenya, which is not distant from the quote of Ejrnaes, 2011 stating " *Escherichia coli* is the greatest common causes of UTIs at approximately 80% populating the colon.

5.1.2.2 Inhibition Zones

Leaves Aqueous and Ethyl acetate solvents were the best giving a mean inhibition zone of 9.0mm at 800mg/ml respectively whereas acetone was the poorest with a mean inhibition zone of 6.5 mm at 100mg/ml.

Stem bark aqueous solvent proofed to be the best with a mean inhibition zone of 8.7 mm at 800mg/ml unlike the ethyl acetate which gave the lowest mean inhibition zone of 5.0 in all the concentrations (800,400,200 and 100 mg/ml).

Concoction: The mean inhibition zone of 9.7mm at 800mg/ml in acetone solvent was the most excellent for the concoction of leaves and stem bark s and also for the whole procedure while aqueous proofed to be the worst giving a mean inhibition zone of 5.0 mm 100mg/ml for the concoction

5.1.2.3 Minimum Inhibition Concentration

Leaves: Minimum inhibition zone of all the solvents in concentrations of 400, 200, 100, 50, 25, 10.5, 5.145 and 2.57 mgs/ml were Negative (Growth obtained)

Stem barks: Minimum inhibition zone of all the solvents in concentrations of 400, 200, 100, 50, 25, 10.5, 5.145 and 2.57 mgs/ml were Negative (Growth obtained)

Concoction: Minimum inhibition zone of all the solvents in concentrations of 400, 200, 100, 50, 25, 10.5, 5.145 and 2.57 mgs/ml were Negative (Growth obtained)

5.1.2.4 Minimum Bactericidal Concentration

Leaves: Minimum Bactericidal Concentration procedure was not done due to negative MIC results (growth obtained) in all the plant parts extracts

Stem barks: Minimum Bactericidal Concentration procedure was not done due to negative MIC results (growth obtained) in all the plant parts extracts

.Concoction: Minimum Bactericidal Concentration procedure was not done due to negative MIC results (growth obtained) in all the plant parts extracts

5.2 CONCLUSIONS

1. Phytochemical profiles indicates different extracting solvent has a different effect on the phytochemical composition.
2. Leaves Aqueous and Ethyl acetate solvents were the best giving a mean inhibition zone of 9.0mm at 800mg/ml respectively.
3. Stem bark aqueous solvent proofed to be the best with a mean inhibition zone of 8.7 mm at 800mg/ml.
4. The antimicrobial efficacy Mean inhibition zone of 9.7mm at 800mg/ml in acetone solvent was the most excellent for the concoction an indicator of

synergistic mode of the Plant extracts which might be contributed by coumarins chemical compound present in stem barks acetone extract solvent .

5. *Jatropha curcas* is bacterial static against *E. coli* in Urinary Tract Infections of diabetic females located at Kitui County, Kenya.
6. This study indicates a prevalence rate of 69% of *Escherichia coli* isolates as the causative agent of UTIs in diabetic females of Kitui County, Kenya

5.3 RECOMMENDATIONS

1. There is need on further plant extracts analysis in different solvents
2. Studies on concoctions of different plant extracts actions is required.
3. There is need of further immunological analysis of *Jatropha curcas* crude extract of diabetic female with UTI for a bacterial static indicator may be a sign of immunological booster to the diabetic females with UTI.
4. Follow up of this study using clinical isolates from different body parts is needed.
5. Evaluation of adverse reactions is paramount

5.4 LIMITATIONS

1. Determination of the medicinal plant extracts breakpoints
2. High-performance liquid chromatography studies for bioactive compounds

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APPENDICES

Appendices I: Protocols

Protocol I: Soxhlet extraction method.

Soxhlet extraction likewise perceived as the hot persistent extraction process with the principal benefit of complete extraction in least measure of dissolvable The device Soxhlet extractor is comprised of glass comprising of a round base flagon, extraction chamber, siphon cylinder, and condenser at the top. A dried, crushed, and ¬newly powdered plant material is put inside permeable sack (thimble) comprised of a perfect fabric or solid ¬l paper and firmly tightened. (Abdullahi¬ R. Abubakar1, Mainul Haque, 2020)

The Benefits of this method are;

1. Huge amount of drug is extracted with minimum solvent.
2. Can be used on heat stable plant materials
3. No filtration process is required.

The Drawbacks are:

Consistent shaking is not promising, and the method is not appropriate for thermolabile materials. (Abdullahi and Mainul , 2020)

Soxhlet extractor

A Soxhlet extractor is defined as a piece of laboratory apparatus designed in 1879 by Franz von Soxhlet with three components in the system bark:

(1) The top part which is a solvent vapor reflux condenser, In middle are a thimble holder with a siphon device and a side tube, and the thimble holder connects to a round bottom flask at the bottom.

Because the sample is extracted with cooled, condensed solvents, Soxhlet is slow and can take 6 to 48h.

This is a method in which powdered sample is separated a permeable sack or "thimble" produced using serious areas of strength for a paper or cellulose, which is set in thimble office of the Soxhlet device. Extraction dissolvable is held in the round base cup and warmed utilizing warming source like warming mantle. The warming temperature is based on the dissolvable utilized to extraction. Because of intensity the dissolvable in the base carafe disintegrates into the condenser and afterward dribble back to the example thimble. At the point when fluid substance arrives at the siphon arm, the fluid items is discharged into the base flagon once more and the cycle end is shown by an unmistakable arrangement in the siphon tube.(.Gopalasatheeskumar, 2018, Abdullahi and Mainul , 2020,)

Selection of solvent for Soxhlet extraction

The choice of the dissolvable for Soxhlet extraction depends on the phyto constituent disengagement process. The dissolvable ought to be not difficult to eliminate and latent. By and large the dissolvable decision depends on the rising extremity request e.g., the request for CH3)2CO, oil ether, ethyl acetic acid derivation, chloroform, methanol, ethanol and water.

Methanol is the semi polar dissolvable which can remove a large number of the Phytoconstituents and water is the polar dissolvable which is cheap dissolvable and

nontoxic. Quantities of polar constituents are disengaged by water and which is likewise appropriate for the creature studies and human examinations. (Gopalasatheeskumar, 2018)

Table 7: Selection of Solvent for Active Constituent Extraction for Soxhlet Extraction

Non polar solvent	Semi polar solvent	Polar solvent
EG;Petroleum ether, chloroform, Diethyl Ether	EG; Ethanol, Acetone	EG;Water
Phyto constituents; Alkaloids Terpenoids Coumarins Fatty acids Flavonoids	Phyto constituents; Tannins Polyphenols Polyacetylenes Flavonols Terpenoids Sterols Alkaloids	Phyto constituents; Anthocyanins Starches Tannins Saponins Terpenoids Polypeptides Lectins

, (Gopalasatheeskumar, 2018)

Soxhlet extraction was completed with General Extraction Framework bark (Buchi). Ten grams dried powder (seed, seed coat, root, leaves and bark) was taken in glass thimble and removed with solvents like methanol, liquid methanol, CH3)2CO, ethyl acidic corrosive deduction and hexane. The framework was finished for 10 cycles for each concentrate and the temperature was changed just under the edge of bubbling over of the singular solvents. An enormous piece of the dissolvable from each concentrate was evaporated by using a comparable instrument. Extra drying of the mass was finished at room temperature. The greatness of each concentrate was also noted as depicted past (Harborne, 1973) among all that the yield level of Methanolic remove was seen as high diverged from various solvents and thus conveyed further for antibacterial actions. (Krishnananda et al., 2017)

Protocol 2. Fasting blood sugar test

Procedure: Permitted procedures and patients consents will command the requirement for doing blood glucose test using a gluco-meter. Results was documented in the patients chart.

Obtaining Blood Glucose:

Manufacturer's instruction for blood glucose monitors may vary and was followed.

1. A clean, dry work surface was chosen, a glucose meter, unused sterile pricker and test strip was used.

2. Through pressing the power button of the glucometer, the code number will appear, matching the code number to the bottle of test strips.

3. A new sterile pricker was inserted into pricker holder.

4. The finger was pricked to obtain a drop of blood.

5. A drop of blood was put on the test strip as directed by the manufacturers

6. The test results was displayed in ≤ 30 seconds.

7. The meter was pressed to turn off

8. Disposal of sharps and bio-hazard materials was.

10. Documentation of results was done

15. Abnormal results /panic values was reported to the investigator

Protocol 3. Harvesting Mid-Stream Urine (MSU)

1. Procedure was explained to the patient, in which they was taught to get midstream specimen in sterilized bottle after empting a slight first volume of urine to the toilet, then the last urine into the toilet

2. Labelling of specimen appropriately with patient's details was done

Protocol 4. Urine Macroscopy

The appearance of the specimen (Monica, 2006)

Report wa4 done on:

– The Color of specimen

– The turbidity

Protocol 5. Urine microscopy

Investigation of a wet preparation

1 10 ml of well mixed urine was transferred to a labelled conical tube.

2 It was centrifuged at 500–1000 g for around 5 minutes. The top layer urine was dispensed by totally inverting the tube into a second container. This was analyzed biochemically evade contaminating the original urine.

3 Through tapping the bottom of the tube the sediment was mixed. A drop of the well-mixed sediment was transferred to a slide and microscopically examined via the 10X and 40X objective

4. Documentation of the findings was done.

(Monica, 2006).

Protocol 6. Urine Dipstick Analysis Method

Protein, Nitrite and Leukocyte are the Biochemical Dipstick key tests which are helpful in investigating UTI.

Nitrite and leukocyte strip tests

A nitrite reagent strip known also check leucocyte esterases is the *Combur 2 Test LN*, was used. (Monica, 2009).

Protein reagent strip tests

Urine protein strip tests senses mostly albumin. A fresh urine specimen is a must. (Monica, 2009).

Protocol 7.Cultural Characteristics of Escherichia coli

Nutrient agar and MacConkey agar (a differential media)

Categorization of Escherichia coli was achieved via isolating in pure forms through Nutrient agar and MacConkey agar. Gram staining was done to differentiate gram negatives from gram positives and categorize their shapes. Colony morphology of achieved via observing their cultural behavior.

Protocol 8: Biochemical identification of Escherichia coli

A.) TSI

The Triple Sugar-Iron agar test is intended to distinguish amongst the dissimilar groups of the Enterobacteriaceae. Reflection of carbohydrate consumption designs is facilitated by a TSI Agar have three fermentative sugars, lactose and sucrose in 1% fixations and glucose in 0.1% focus. Carbohydrate fermentation was specified by gas production and an alteration in the color of pH indicator from red to yellow.

B.IMVC TESTS

This is a sequence of four assessments namely:

Indole which breaks down the amino acid Tryptophan, the Methyl Red known for glucose oxidation, Voges-Proskauer which produces neutral end products and Citrate for Citrate fermentation

Appendix II: Questionnaire

Participants Identification Number…….Date……

Participants specimen Code Number……..…..Date……

My name is Celestine K. Kyembeni, a Post graduate student doing Masters in Medical Laboratory sciences, Microbiology option at Mount Kenya University, Thika. Admission Number; MMLS/2018/27108.This questionnaire is intended to gather your details. Kindly help in giving the right information. The shared information was private. The study output was used to improve health care services in the management of urinary tract infection.

Instructions;

Kindly give the required information correctly by ticking the correct option. In case of difficulties, a consultant is on standby.

A).Social demographic characteristics

1. What is your oldness?

a) 15-24
b) 25-34
c) 35-44
d) 45-54
e) 55-64

2. What is your Occupation position?

a) Employed
b) Unemployed
c) Self employed

3. What is your education level?

a) College

b) Secondary

c) Primary

d) None of the above

4. What is your marital position?

a) Married

b) Divorced

c) Single

d) Complicated

5. How do you clean your vaginal area?

a) Front to back

b) Back to front

6. What do you use to clean your vaginal area?

a) Plain water

b) Water with soap

B).Risk features of Urinary tract infections in identified diabetic patients

1. What is your todays Radom blood sugar (FBS) Values

a) 0-3Mmols/l

b) 4-8Mmols/l

c) 9-13Mmols/l

d) 14-18Mmols/l

e) 19Mmols and above

2. Have you ever had a urinary tract infection?

a) No

b) Yes

3. If No.2 is Yes, How many times in the last six months

a) Once

b) Twice

c) Thrice

d) All through

4. Are you in contraceptives as a method of family planning?

a) Yes

b) No

5. What is your parity equivalence?

a) Para 0

b) Para 1-3

c) Para 4-6

d) Para 7-10

Appendix III: Patient Informed Consent Form

Participants Identification Number…….Date……

Participants specimen Code Number……..…..Date……

Celestine Kyembeni is a Post graduate student doing Masters in Medical Laboratory sciences, Microbiology option at Mount Kenya University, Thika. Admission Number; MMLS/2018/27108.The aim of this form is to obtain consent on the Data collection information about the subject study on "To determine the Phytochemical compounds, toxicity and antimicrobial efficacy of *Jatropha curcas* crude extracts on *Escherichia coli* clinical isolates from diabetic females identified with urinary tract infection, Kitui Referral Hospital, Kitui County,". This is an experimental laboratory study aimed at collecting and analyzing urine mid-stream morning samples for isolation of *Escherichia coli* of diabetic females' identified with UTI. A study in whose specific objectives was;

To establish the phytochemicals present in *Jatropha curcas* crude plant leaves and stem bark extracts, singly and as a concoction, to evaluate toxicity intensities of the crude plant leaves and stem bark extracts using brine shrimp and to determine in vitro antimicrobial efficacy of *Jatropha curcas* leaves and stem bark extracts, singly and as a concoction, on *Escherichia coli* clinical isolates of diabetic females identified with urinary tract infection. Expected benefits of the study to subject's population was;

- To provide effective alternatives to prescription medication currently on use
- To strengthen and tone immune system bark by herbal use
- To reduce health care expenses by preventing future occurrences, and
- To relieve UTI by combating Escherichia coli

I…… (Insert full name in Capital letters)…….. (Date and signature]

Give my consent for this information.

Appendix IV: Fomu ya Kibali cha Mgonjwa

Participants Identification Number…….Date……

Participants specimen Code Number……..…..Date……

Celestine Kyembeni ni post kuhitimu mwanafunzi kufanya Masters katika Medical Laboratory sayansi,Microbiology chaguo katika chuo kikuu MlimaKenya,Thika.Admission Number; MMLS/2018/27108.Umuhimu wa fomu hii ni kupata kibali chomgonjwa cha kukusanya habari na kufahamisha kuhusu mada yaathari,mathari an kemikali za majani na magamba ya matawi ya mti *Jatropha curcas* kwenye wanawake walio na ugonjwa wa kisukari na maambukizi kwenye njia ya mkojo (UTI), katilka hosipitali ya rufaaya Kitui kauntiya Kitui nchini Kenya. Huu ni utafiti wa maabara unao lenga kuchunguzwa na kutathmni mkojo wa katikati ya kukojoa, kipimo cha kuchunguza vijidudu vya Escherichia coli kwa wanawake waliopatikana na maambukizi kwenye njia yamkojo (UTI)

Uchunguzi huu unalenga;Kutambua kemikali muhimu katika majimajiya *Jatropha curcas* matawi na stem bark kila moja peke yake na tena mchanganyiko.Kuonyesha Mathari ya majimaji kutoka Jatropha curcas matawi na stem barkbark, na Kuonyesha athari ya majimaji kutoka *Jatropha curcas* matawi na stem barkbark,kilamoja pekee na tena mchanganyiko kwa vijidudu vya Escherica coli kwa wanawake wenye ugonjwa wa kisukari waliopatika na maambukizi katika njia ya mkonjo.Faida zinazo tarajiwa kutokana na utafiti huu kwa jummani;1. Kupata matibabu tofauti na yale ya kisasa

2. Kuimarisha kinga ya mwili kwanjia ya dawa za mitishamba

3. Kupunguza gharama za afya kwakuzuia kuto tokeatena

4. Kutibu maambukizi kwanjia ya mkojo kwakuangamiza Escherichia coli.

Mimi ……. (Majina kwa herufi kubwa) …… (tarehe)……..(sahihi) nakubali kipimo yangu kutumika kwahii utaviti.

Appendix V: Vomu ya Kwitikila Kwa Muwau

Participants Identification Number…….Date……

Participants specimen Code Number……..…..Date……

Celestine K. Kyembeni nimumanyiwa wa kiwango kya Post graduate va Masters thini wa Medical Laboratory sciences, usakui wa Microbiology sukulu munene wa Mount Kenya University, Thika. Namba ya sukulu; MMLS/2018/27108. Kyelelo kya vomu ino ni kukwata witikilo wamuwau kana niwetikila ualanyo wa uvoo wakwosanya Mauvoo yulu wa Uuiti wa Matu na Makavo ma mti wa *Jatropha curcas* kwa Aka ala mena thina wa Sukali na methiawa na mathina ma dudu sya nthia ya maumao.(UTI), Ula ukwikwa vaa sivitalini munenewa kitui kaunti,musyini witu Kenya. Uu niumanyi na mtalatala ula unyuvitwe kukunikila maumao ma kwakya maithimwa dudu yitawa *Escherechia Coli* kwa aka alamethiawa na sukali, nathina wa nthia ya maumoo. (UTI)

Ukunikili uu wenda:

Kumanya kemikoo sya matu na makavo ma *Jatropha curcas* kila imwe weka naindi mbulanio,Kumantha uthuku wa matu na makavo ma *Jatropha curcus* kwisila kutumia brine shrimp na Kumantha vata wa matu na makavo ma *Jatropha curcus* kila imwe weka na indimbulanio, kwadudu ya *Escherechia coli* kwa aka ala methiawa na sukali, na thina wa nthia ya maumoo (UTI).

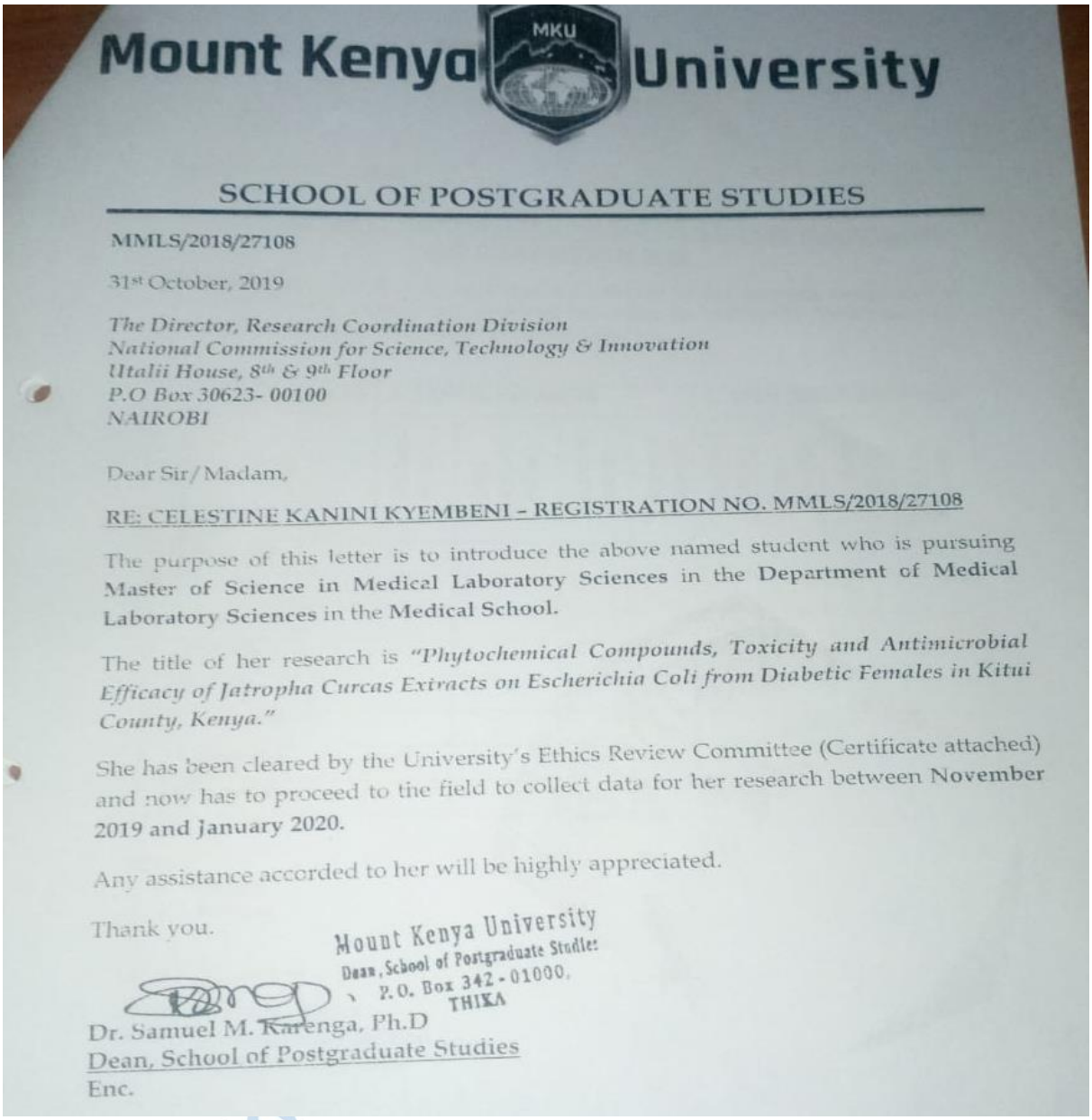
Mauseo ma mtalalata uu wa ukunikilini;

a) Kukwata uuiti mwailu na wakisasa,Kutethesya uima wamwii na dawa syamiti,
b) Kwolanganga galama ya uuiti kwisila kusuia UTI kusyoka ingi itina wa uuti
c) Kumina mauwa ma nthia ya maumao kwa kumina vyu Escherechia coli

Nye ……..

(masyitwanandetonene) ……(matuku) ……. (saii/kitole) nigwitikilauvoouunanine tikilakithimokyakwakukunikilwa

Appendix VI: Research Permit introduction letter to NACOSTI



Mount Kenya University

SCHOOL OF POSTGRADUATE STUDIES

MMLS/2018/27108

31st October, 2019

The Director, Research Coordination Division
National Commission for Science, Technology & Innovation
Utalii House, 8th & 9th Floor
P.O Box 30623- 00100
NAIROBI

Dear Sir/Madam,

RE: CELESTINE KANINI KYEMBENI – REGISTRATION NO. MMLS/2018/27108

The purpose of this letter is to introduce the above named student who is pursuing Master of Science in Medical Laboratory Sciences in the Department of Medical Laboratory Sciences in the Medical School.

The title of her research is *"Phytochemical Compounds, Toxicity and Antimicrobial Efficacy of Jatropha Curcas Extracts on Escherichia Coli from Diabetic Females in Kitui County, Kenya."*

She has been cleared by the University's Ethics Review Committee (Certificate attached) and now has to proceed to the field to collect data for her research between November 2019 and January 2020.

Any assistance accorded to her will be highly appreciated.

Thank you.

Mount Kenya University
Dean, School of Postgraduate Studies
P.O. Box 342 - 01000,
THIKA

Dr. Samuel M. Karenga, Ph.D
Dean, School of Postgraduate Studies
Enc.

Appendix VII: Research Permit from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 385648

Date of Issue: 18/December/2019

RESEARCH LICENSE

This is to Certify that Ms.. CELESTINE KYEMBENI of Mount Kenya University, has been licensed to conduct research in Kitui on the topic: PHYTOCHEMICAL COMPOUNDS, TOXICITY AND ANTIMICROBIAL EFFICACY OF JATROPHA CURCAS EXTRACTS ON ESCHERICHIA COLI FROM DIABETIC FEMALES IN KITUI COUNTY, KENYA for the period ending : 18/December/2020.

License No: NACOSTI/P/19/2713

385648

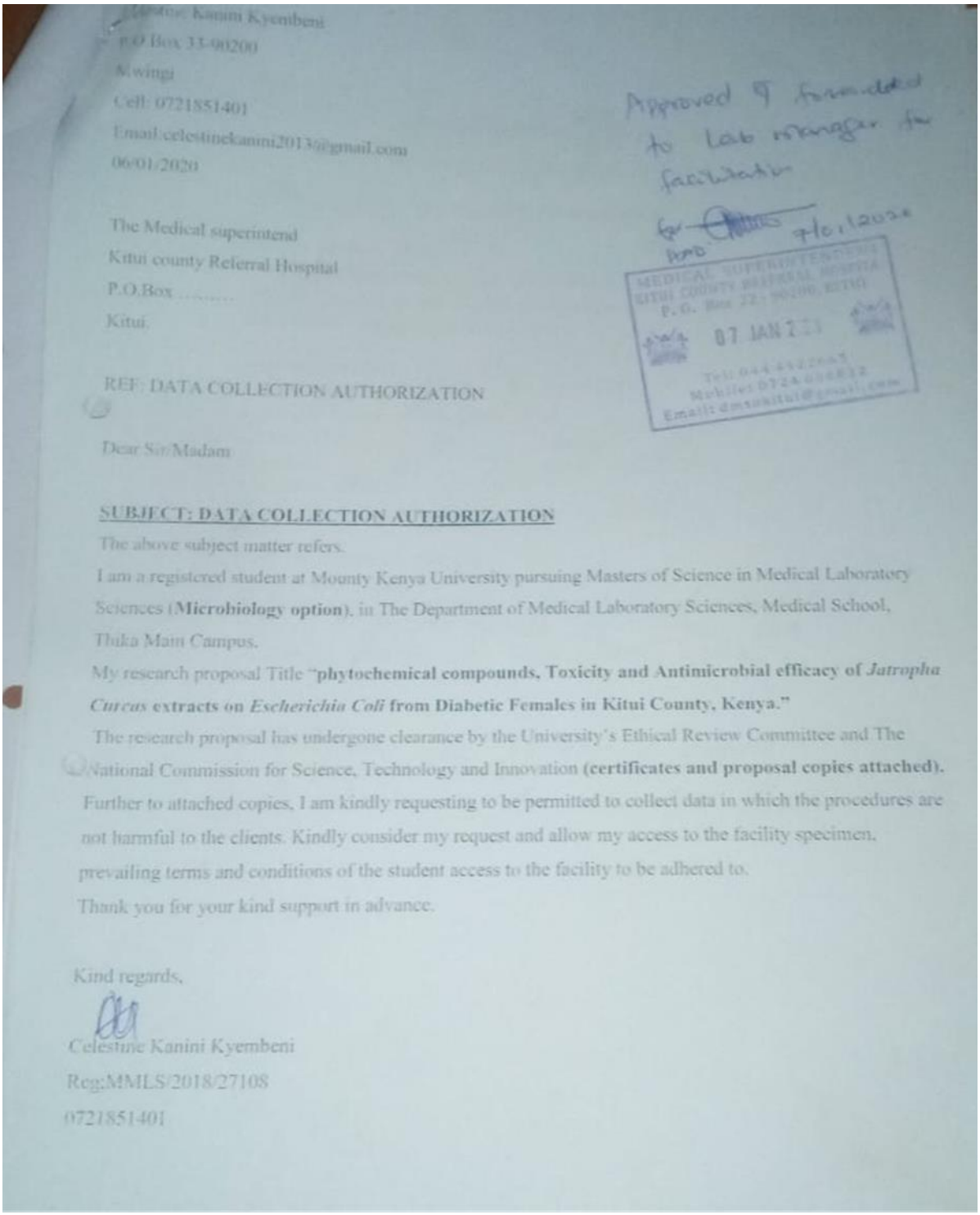
Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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

Appendix VIII Kitui Level IV Hospital Introduction letter



Celestine Kanini Kyembeni
P.O Box 33-90200
Mwingi
Cell: 0721851401
Email:celestinekanini2013@gmail.com
06/01/2020

Approved & forwarded to Lab manager for facilitation
for [illegible] 7/01/2020

The Medical superintend
Kitui county Referral Hospital
P.O.Box .....
Kitui.

MEDICAL SUPERINTENDENT
KITUI COUNTY REFERRAL HOSPITAL
P. O. Box 22 - 90200, KITUI
07 JAN 2021
Tel: 044 4422047
Mobile: 0724 606812
Email: dmskitui@gmail.com

REF: DATA COLLECTION AUTHORIZATION

Dear Sir/Madam

SUBJECT: DATA COLLECTION AUTHORIZATION

The above subject matter refers.

I am a registered student at Mounty Kenya University pursuing Masters of Science in Medical Laboratory Sciences (**Microbiology option**), in The Department of Medical Laboratory Sciences, Medical School, Thika Main Campus.

My research proposal Title "**phytochemical compounds, Toxicity and Antimicrobial efficacy of *Jatropha Curcas* extracts on *Escherichia Coli* from Diabetic Females in Kitui County, Kenya.**"

The research proposal has undergone clearance by the University's Ethical Review Committee and The National Commission for Science, Technology and Innovation **(certificates and proposal copies attached).**

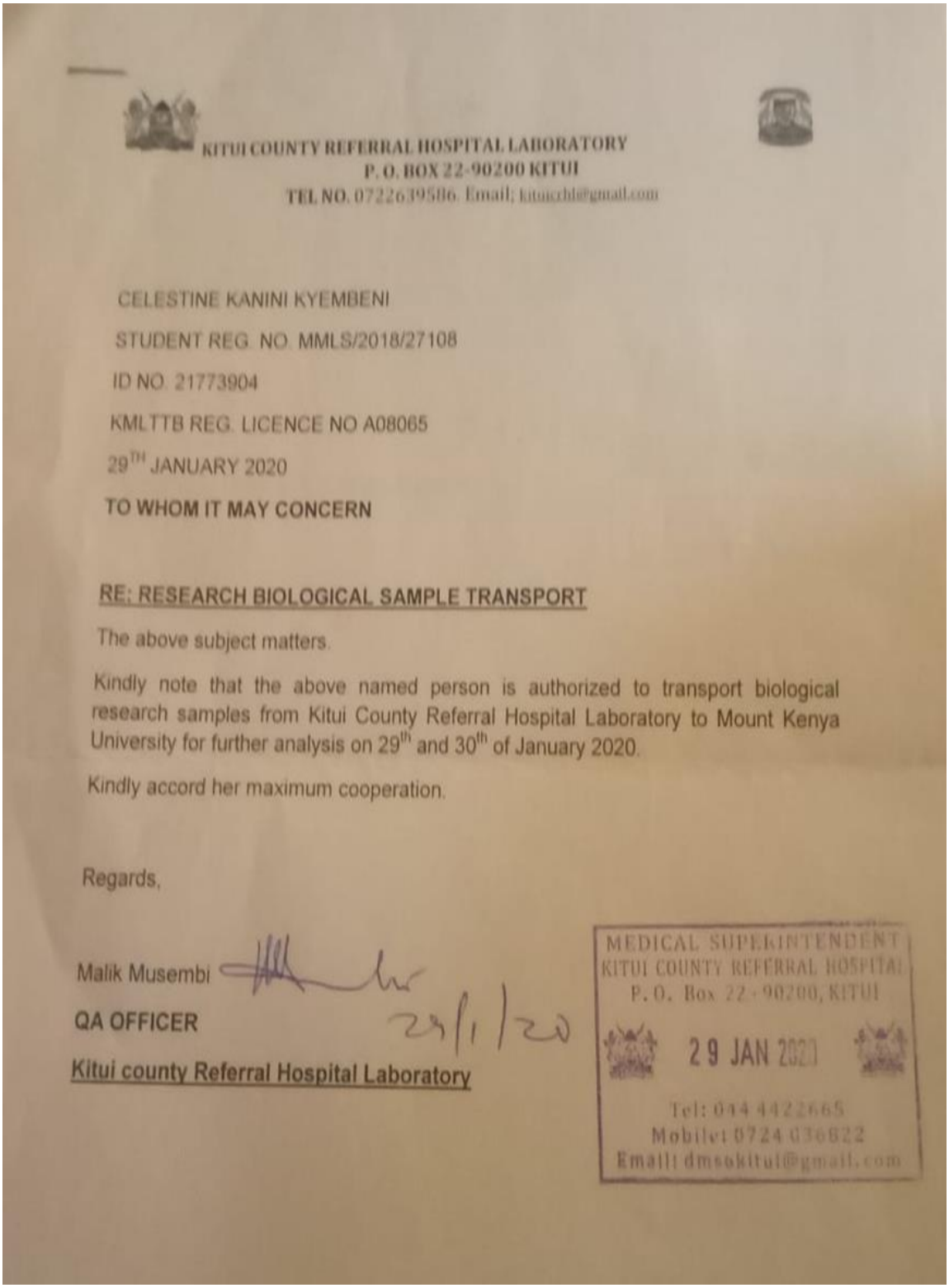
Further to attached copies, I am kindly requesting to be permitted to collect data in which the procedures are not harmful to the clients. Kindly consider my request and allow my access to the facility specimen, prevailing terms and conditions of the student access to the facility to be adhered to.

Thank you for your kind support in advance.

Kind regards,

Celestine Kanini Kyembeni
Reg:MMLS/2018/27108
0721851401

Appendix IX Biological material transport authorization form



KITUI COUNTY REFERRAL HOSPITAL LABORATORY
P. O. BOX 22-90200 KITUI
TEL NO. 0722639586. Email; kituicrhl@gmail.com

CELESTINE KANINI KYEMBENI

STUDENT REG. NO. MMLS/2018/27108

ID NO. 21773904

KMLTTB REG. LICENCE NO A08065

29TH JANUARY 2020

TO WHOM IT MAY CONCERN

RE: RESEARCH BIOLOGICAL SAMPLE TRANSPORT

The above subject matters.

Kindly note that the above named person is authorized to transport biological research samples from Kitui County Referral Hospital Laboratory to Mount Kenya University for further analysis on 29th and 30th of January 2020.

Kindly accord her maximum cooperation.

Regards,

Malik Musembi 29/1/20

QA OFFICER

Kitui county Referral Hospital Laboratory

MEDICAL SUPERINTENDENT
KITUI COUNTY REFERRAL HOSPITAL
P. O. Box 22-90200, KITUI
29 JAN 2020
Tel: 044 4422665
Mobile: 0724 036822
Email: dmsokitui@gmail.com

Appendix X: Study site map, Kitui.

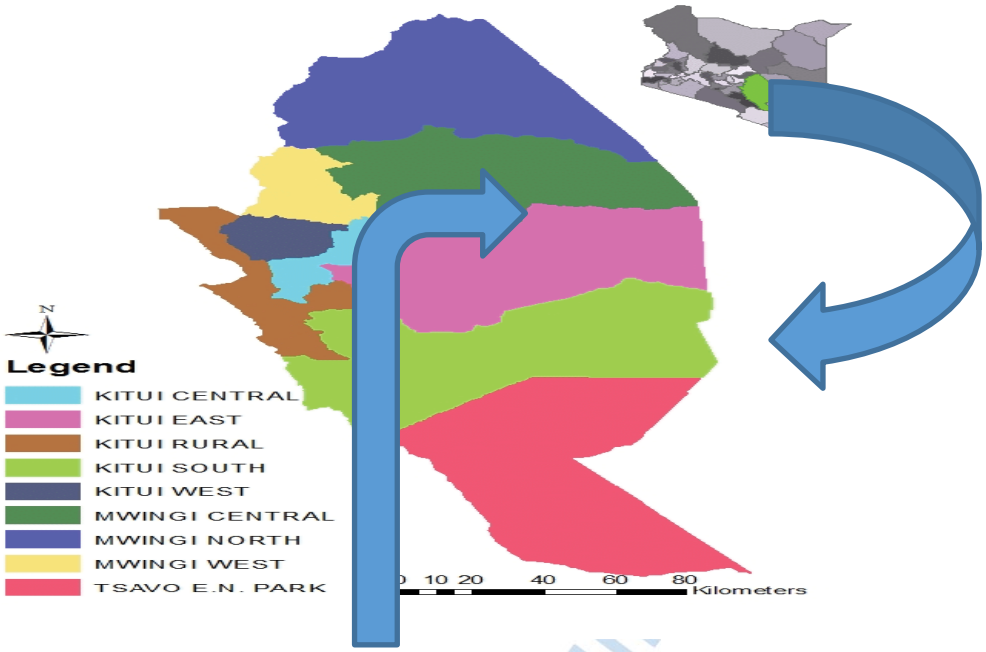
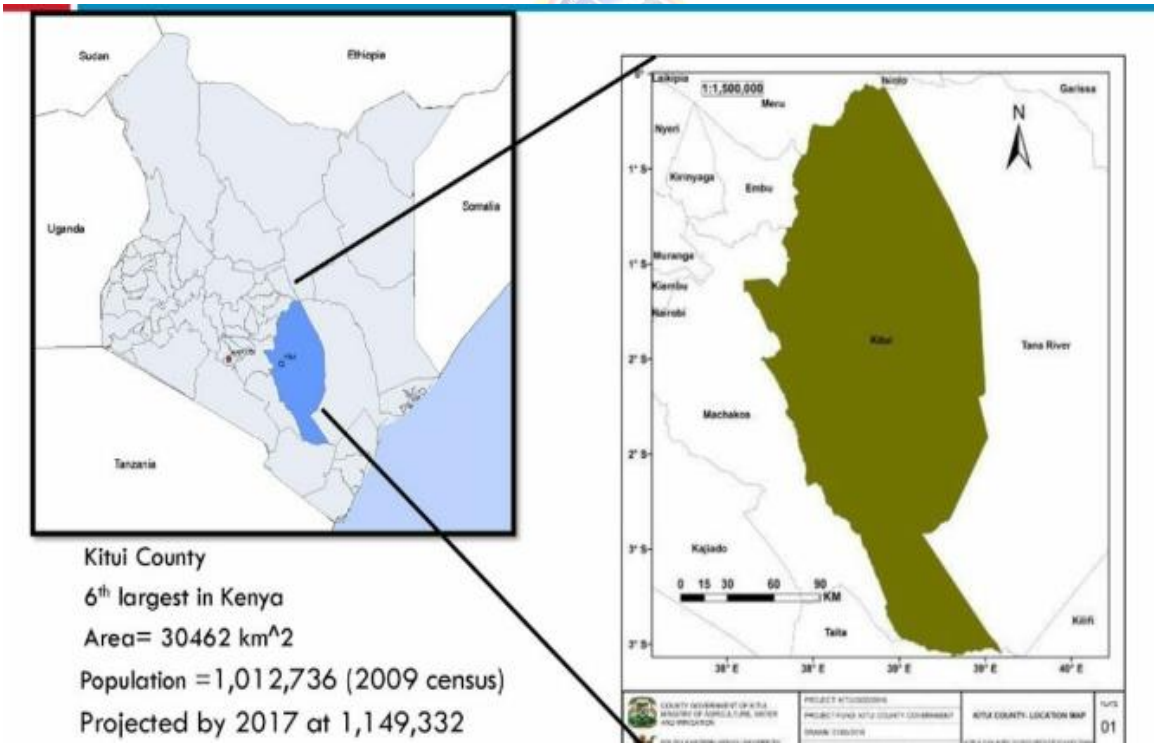


Photo Courtesy of Wikipedia the free encyclopedia



Appendix XI: Similarity Index

PHYTOCHEMICAL COMPOUNDS AND ANTIMICROBIAL EFFICACY OF JATROPHA CURCAS EXTRACTS ON ESCHERICHIA COLI FROM DIABETIC FEMALES IN KITUI COUNTY, KENYA

by Celestine Kanini Kyembeni

Submission date: 08-Apr-2023 03:11PM (UTC+0300)
Submission ID: 2058981365
File name: Celestine_BOE_inputs_THESIS.docx (10.58M)
Word count: 20055
Character count: 110619

PHYTOCHEMICAL COMPOUNDS AND ANTIMICROBIAL EFFICACY OF JATROPHA CURCAS EXTRACTS ON ESCHERICHIA COLI FROM DIABETIC FEMALES IN KITUI COUNTY, KENYA

ORIGINALITY REPORT

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