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Assessment Of Ricinus Communis Ml Crude Extracts Towards Urinary Tract Infection Strains With Their Associated Ailments

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Abstract – Cases of synergic Urinary Tract Infections with its associated ailments has become a menace. Due to its high costs of treatment, unavailability of relevant antibiotics, misdiagnosis and recurrence, most patients within Africa Sub-Sahara regions prefer ethnopharmacological ways of taking boiled and sieved liquid concoction made from mature leaves (ML) of ricinus communis to clear the urinary tract system from these microbes with their associated ailments. Crude extracts of methanol, chloroform, hexane, and Diethylether prepared from ricinus communis dried powered mature leaves were subjected to phytochemical screening, antibacterial, GC/MS and antioxidant assays. Crude methanolic and chloroform extracts prepared from a mixture of coarsely powdered dried twigs and mature leaves were evaluated for cytotoxic activity on cancer cell line (HCT-116 and K-562). Antibacterial assay of the crude extracts reveals recommendable range of values categorized as moderate and high sensitive with respect to the standard measure. Methanol extract exhibited antibacterial activities against all gram-positive and gram-negative bacterial species while diethylether extract showed its potency against the gram-positive staph only among escherichia, klebsiella and pseudomonas species. Chloroform, diethylether and hexane crude extracts proven to be inert against pseudomonas strain. Highest scavenging capacity range obtained is 60.38 ± 0.034 percent while the lowest cytotoxicity range is 20.18 ± 1.37 µg/ml^{-1}revealing the medicinal value and safeness of the plant extract. Phytochemical analysis and GC/MS results revealed the presence of phytoconstituents and museum of bioactive agents with antibacterial, antifungal, antiviral, anti-inflammatory, anticancer, antidiarrheal, antimutagenic and anti-oxidant properties thus ascertaining its efficacy towards the infection with associated ailments.

Keywords – UTI strains, Phytoconstituents, Bioactive Ingredients, Antioxidant, Cytotoxicity, Ethnopharmacological

I. INTRODUCTION

Infections of the urinary tract have been known since antiquity implying that there were traditional ways of handling the infection varying with various communities. The Greeks thought it resulted from harmony while the Egyptians referred to it as 'sending forth heat from the bladder' [1]. Modern studies reveals that these infections are inflammatory disorders of the urinary tract caused by the abnormal growth of pathogens. It is a synergic infection caused by conglomeration of microorganisms that affects different parts of the urinary tract system. The commonest pathogenic organisms so far isolated in UTI are *Escherichia coli* followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus Vulgaris, Pseudomonas aeruginosa* and other species of *Enterococcus*, and *Enterobacter* [2]. The clinical spectrum of UTIs ranges from asymptomatic bacteriuria, to symptomatic and recurrent UTIs, uncomplicated to complicated, and acute to chronic. Short-term morbidity in terms of fever, dysuria, nausea, vomiting, headaches, etc. may be present as associated features [3]. Urinary tract infections may be contracted via sexual intercourse minus the staph, abnormal canal sexual practice, improper hygiene, unhygienic uncircumcised men, internal infection particularly with women due to structural propinquity of perineum and can be either community or nosocomial acquired. The clinical manifestation of UTIs includes fever, burning sensations while urinating, strong and persistent urge to urinate, passing frequent and small urine volume, strong foul-smelling urine, urine appearance as cloudy, red or bright pink, bladder

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catheterization, itching, formation of blisters and ulcers in the genital area, genital and suprapubic pain, and pyuria [4]. These depends on the portion of the urinary tract involved, the etiologic organisms, the severity of the infection, diabetics, age of the patient and the patient's ability to mount an immune response to it. In addition to the above factors, it also varies in expressed local symptoms, frequency of recurrence, extent of damage caused, presence of complicating factors and the risk from their reiterate incidence [5]. These factors are also supplemented by obstructions in the urinary tract system due to prostate enlargement, diabetes, genitourinary tract abnormalities, pregnancy, use of spermicidal agents, intake of immune-suppressive drugs for autoimmune diseases and prolonged hospitalization status, consequently leading to recurrent UTI [6]. The major ingredient that favors the growth of the microbes is the urine as it comprises of the essential factors to allow the augmentation of microbes and is considered as the best natural medium for the growth and establishment of the microorganisms. Urinary instrumentation such as catheters serves as one of the routes of the infection and in the absence of medical devices the microbes invade the urinary tract through the urinary stream via the bladder hence causing abdominal pains. They later on ascends to the parts of the upper urinary tract like ureter and kidney and may result in permanent scarring of the kidney [7, 8] consequently leading to blood borne infection and in severe circumstances can lead to dire consequences including death.

From the Global Disease Burden 2021 released by WHO reported cases of UTI have increased by 63.4% compared with 60.4% reported in 2019. The burden is mainly concentrated in South Asia followed by Latin America [9] while it is on the rise in Sub-Saharan African countries with Ghana at 55.4% and East African regions approximated at 48.5%. In the United States, approximately 10.50 million ambulatory visits have been reported and 5 million emergency department visits for UTI in 2021. Currently over seven million dollars have been spend compared with $3.50 billion was spent alone in 2015 [10] to fight the infection. Recurrent urinary tract infection has proven to be massive burden for the economy, for healthcare systems and for the patients who suffer from them.[11]. In East African regions, the current prevalence rate varies depending on age, and around 68% sex workers are victims. Infection rates are higher in fast growing urban centers due to the life styles, socioeconomic status and do occasional rise during festive seasons. It accounts for nearly 25% of all infections in the elderly [12]. An average of 54% between 24 – 35 years old and average of 15% children below six years have been infected while uncircumcised infants younger than 6 months have a higher incidence of gram-negative [10, 13, 14,15] according to hospitals reports. Fifty percent of elderly women are affected by asymptomatic bacteriuria while simple uncomplicated UTIs are quite common in women ages 20 to 50 and with one third of women will have had at least one UTI by age 24 years [16].

The pathogens associated with UTI are known to exhibit a property called bio film formation which is responsible for causing these infections [17]. The more prevalent *Klebsiella pneumoniae* and *E. coli* varies depending on the life style and socioeconomic status of a given community [18]. Higher rates of recurring UTIs are found in post-menopausal women due to the lack of estrogen, pelvic prolapse, and increased peri-urethral colonization by *Escherichia coli*, loss of H_2O_2-producing Lactobacilli sp. in the vaginal flora particularly with sexual workers, and diabetes mellitus [15, 19]. *The gram-positive* cocci *Staphylococcus* species with its sub species saprophyticus is a causative agent in young girls has a complex genetic makeup responsible for its pathogenicity and toxicity in conferring UTI. While in elderly age women, the causative organisms of UTI in most cases are *Enterococcus faecalis* and *Proteus mirabilis* [20]. *Klebsiella pneumoniae* and *Proteus mirabilis* are related with increased frequency in complicated UTI [18] while *Klebsiella pneumoniae* is an important etiological factor in non- nosocomial and nosocomial infection [21].

Currently, the synthetic antibiotics are generally preferred for the treatment of patients of symptomatic UTI which result in long-term alteration of the normal microbial flora of gastrointestinal tract, female vagina and ultimately resistance development in micro-organisms [22]. The most common etiological bacterial pathogens are *E coli* and *K pneumoniae*, have shown low susceptibility to ampicillin, co-trimoxazole, clindamycin, amoxicillin, amoxicillin/clavulanic and ceftazidime/clavulanic but to some extent sensitive to ciprofloxacin, ceftriaxone, nitrofurantoin, cefotaxime and gentamicin for empiric treatment. So far *Proteus* species and *Enterobacter* species have been found resistant to nitrofurantoin [23,24]. In addition, antibiotics like cefotaxime and ciprofloxacin weren't effective against *P. aeruginosa* which is considered to be a sporadic pathogen when compared to etiological agents like *E. coli* and *Staphylococcus species* [24, 25]. Currently, the combination of trimethoprim and sulfamethoxazole (TMP-SMX) is the standard treatment of UTI but the bacteria have become resistant to these drugs. Quinolone antibiotics have now overtaken TMP-SMX as the choice to treat UTIs but researchers predicted that resistance might develop to these drugs as well [26]. This is an indication of the development of surfacing resistance among major pathogens conferring UTI and has made them resilient towards the commonly employed antimicrobial agents [22, 26, 27]. High resistance to antibiotics

calls for antimicrobial stewardship and surveillance to preserve antibiotics' effectiveness in treating uropathogens [28]. Therefore, understanding the extent of antibiotic resistance is of vital importance as the changing rate of antibiotic resistance and misdiagnosis has a great impact on the management of UTI. [29,30]. So, it can be argued that the 'golden era' of antibiotics is waning hence the need for rational and alternative treatments [31,32] before resistance to UTI synthetic antibiotic medicines becomes intolerable. Due to fewer reported side effects, cost effectiveness, easy availability, easy concoction preparations and lack of bacterial resistance so far have been reported, medicinal plants have gained more and more popularity as well as reliability worldwide [32]. Traditionally, most inhabitants from Sub-Saharan regions of Africa have been taking boiled mature leaves from *Ricinus communis* mixed with a little lemon juice for the treatment up to date. To add, antibiotic drugs are normally prescribed to a patient for 7 – 14 days but it is a two to three days of the herb concoction depending on the patient's age in case of the herbal concoction to clear these microbes from the system with their associate ailments, restore the damaged tissues and natural ingredients within the urinary tract system. This has motivated us to give this plant a scientific approach in assessing and ascertaining the ethnopharmacological claims towards the microbial disease with its associated ailments as an alternative treatment.

II. Materials And Methods

2.1 Plant Authentication

The *Ricinus communis* is a plant species of the family Euphorbiaceous, sole member of the genus *Ricinus* and of the tribe *Acalypheae*, subtribe *Ricininae*. (Wikipedia, 2008). It is a fast- growing, suckering perennial shrub which can reach the size of a small tree (around 12 meters /39feet). It has glossy leaves which is 15- 45 centimeters in length, long - stalked, alternate and palmate with 5-12 deep lobes with coarsely toothed segments. Their color varies from dark green, sometimes with a reddish tinge, to dark reddish purple or bronze with the tips of the spikes, have prominent red stigmas. The stems (and the spherical, spiny seed pods) also vary in pigmentation. The plant grows wildly but can also be cultivated. The plant was then identified and authenticated by a plant taxonomist Prof. Ahmed El Bakri at Sumait university, Zanzibar

2.2 Plant material collection

Plant material, *ricinus communis* mature leaves and twigs were collected from Chukwani regions of Unguja Islands, Zanzibar. They were transported in sample bags to the laboratory for analysis.

2.3 Preparation of plant material

Plant material, *ricinus communis* mature leaves and twigs were washed with distilled water and air dried at room temperature for 2 weeks then ground into uniform powder and divided into two parts. One part is the powdered dried mature leaves while other part is the mixture of both powdered dried mature leaves and twigs, and finally stored at room temperatures.

2.4 Extraction

The methanol, chloroform, n-hexane and diethylether extracts were prepared by socking 200 g of the powdered dried mature leaves in 1 litre of each extractant and kept in a cupboard maintained at room temperature for 48 hrs. The extracts were then filtered and concentrated using rotary evaporator (Heidoph 4001 efficient), warmed on the bath at temperature of 40 °C for the methanol, chloroform, n-hexane and Diethylether. These extractions were done at the Chemistry laboratory, Department of Chemistry, University of Dar-es-salaam, Tanzania.

2.5 Qualitative Phytochemical screening

Phytochemical screening for the plant extracts were performed to determine the presence of phytoconstituents by using following standard methods described in the literature protocols (33,34) with minor modifications.

2.6 Microbial assay

The microbial assays were done with all procedural protocols and regulations observed as per the Clinical and laboratory Standards Institute [35].

2.6.1 Microorganisms

Among the ITU pathogens, *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were used in the antimicrobial assay since they were locally available and conforms with our available research funds. These clinical isolate strains were obtained from the Tanzania National Health laboratory, Mabibo, Dar es salaam. Cultures of each bacterial strain were maintained on Luria–Bertani (LB) agar medium at 4°C [36].

2.6.2 Minimum Inhibition Zone Concentration

The antibacterial test for the pathogens was carried out by agar disc diffusion method shown elsewhere [36] using 100µL of standardized inoculum suspension containing 107 CFU/mL of bacteria. Whatman No. 1 sterile filter paper discs (6mm diameter) were impregnated with 10µL of the extracts 30mg/ mL categorically and placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the samples. Standard antibiotic, gentamycin (20µg/disc), was used as positive control for the tested bacteria. The plates were incubated micro-aerobically at 37°C for 24h. Antibacterial activity were evaluated by measuring the diameter of the zones of inhibition.

2.6.3 Preparation of McFarland standard (Turbidity standard) for MIC and MBC inoculum

Barium sulphate turbidity standard equivalent to a 0.5 McFarland was used to standardize the inoculum density for susceptibility test. To make the turbidity standard 0.5 McFarland, Barium chloride solution, 200ml, 175% w/v, was added to 99.5ml 1% sulphuric acid solution, mixed well and stored in the dark at room temperature.

2.6.4 Inoculum preparation for minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC)

Inoculum was obtained from an overnight agar culture of the test organism. Inoculum for the MIC and MBC test was prepared by taking at least three to five well isolated colonies of the same morphology from agar plate culture. The top of each colony was touched with a sterile loop and the loop was transferred into a tube containing 5 ml of normal saline and then vortexed. The broth culture was incubated at 37 °C and monitored for approximately 4 h until it achieved the turbidity of 0.5 McFarland standard (1.5×10^8 cfu/ml).

2.6.5 Minimum inhibitory concentration (MIC)

The MIC of each extract was determined by using concentrations (10, 5, 2.5, 1.25 and 0.625 mg/ml) of the plant extracts by using the broth dilution method [37]. 4 ml of the nutrient broth was pipetted into each of 6 test tubes. 0.1 ml of the prepared successive two-fold serial dilutions of the crude extract concentrations were mixed with the nutrient broth. Thereafter, 0.1 ml of the standardized inoculum of test pathogens was dispensed into each of the test tubes containing the suspension of nutrient broth and the crude extract. Then, all test tubes were properly corked and incubated at 37 °C for 24 h. The MICs were read as least concentration that inhibited any visible growth (absence or turbidity) of the test organism.

2.6.6 Determination of Minimum Bactericidal Concentration (MBC)

To determine minimum bactericidal concentration (MBC) as mentioned [38] ,100 µl of the culture from each well of the micro-broth assay was sub-cultured on Mueller Hinton Agar plates after 24 hours of the incubation. MH plates were further incubated for 24 hours. The lowest concentration of extracts which exhibited no bacterial growth was deliberated as MBC. The experiment was repeated in triplicate for each strain.

III. Antioxidant Capacity Test

The free radical scavenging activity of the methanol and chloroform extracts of Ricinus communis was determined using a 1,1-diphenyl2-picryl hydrazyl (DPPH) according to [39] through free radical assay. A total of 24 milligrams of DPPH were dissolved in 100 mL of methanol for making the stock solution. Filtration of DPPH stock solution using methanol yielded a usable mixture with an absorbance of around 0.972 at 517 nm determined using u UV-VIS spectrophotometer (Shimandzu double-beam spectrophotometer). In a test tube, 3 mL DPPH workable solutions were combined with 100 µL of the extract. Three milliliters of solution containing DPPH in 100 µL of methanol is often given as a standard. After that, the tubes were kept in complete darkness for 30 min. The absorbance was determined at 517 nm using the same spectrophotometer. The assay was carried in triplicate and statistical significant test done at 95% confidence level using student t-test to ascertain the accepted value.

The following formula was used to compute the percentage of antioxidants or RSA: % of antioxidant activity= [(Ao−As) ÷Ao] × 100 where: Ao—Control absorbance; As—sample absorbance.

IV. Cytotoxicity assessment method

The Cytotoxicity assays were performed according to MTT method [40,41]. The crude methanolic and chloroform extracts prepared from a mixture of coarsely powdered dried twigs and mature leaves were evaluated for their cytotoxic activity on cancer cell line (HCT-116 and K-562). The methanol extract was tested at the range (25 - 800 µg /ml after 72 hrs treatment. The human colon tumor cells (HCT-116) and Leukemia cells (K-562) were used for the MTT assay. The cells were harvested and inoculated in 96 well microtiter plates. The cells were washed with phosphate buffered saline (PBS) and the cultured cell were than incubated for 72 hrs with the test sample and the final concentration of 25- 800µg/ ml. 100 µl of MTT solution (5mg ml^{-1} in PBS, PH 7.2) is added to each well and plates are incubated for 4 h at 37 ^{o}C, after incubation 200µl DMSO was added to each well and the absorbance of each well was measured at 570 nm using a micro plate reader and surviving cell fraction was calculated. The inhibition of cell viability was calculated by means of formula % inhibition = (1- absorbency of treated cells / absorbance of untreated cells) × 100.

In case of chloroform extract, the test solution was prepared as a series concentration of 10 µg / ml to 2000 µg /ml from the stock solution. The treatment was done by adding 100 µl of test solution into each well containing confluent cells, except the wells that would be used as cell control, media control, and positive control (doxorubicin). Treatments were done nine replicates. The 96 wells cassette was then incubated in a CO_2 incubator for 24 hrs. After 24 hrs, the culture medium was removed and replaced with 100 µl of MTT solution. The MTT solution was prepared by diluting 1 ml of MTT stock in PBS adding with the culture media to 10 ml then incubated in the CO_2 incubator for 4 hrs. After the incubation, each well was added with 100 µl 10 % SDS in 0.1 N HCl for dissolving the formed formazan crystals. The plates were further wrapped with aluminum foil and placed at room temperature overnight. The absorbance value was read by microplate ELISA reader at λ 595 nm. The absorbance results were used for calculating the cell viability using this formula: ((sample absorbance – control media absorbance) / (control cell absorbance – control media absorbance)) × 100%. The absorbance values are used to estimate the cytotoxic concentration of plant extract which reduces at 50% the cells (IC_{50} value).

V. GC-MS Assay

The samples were diluted in ethyl acetate spectroscopic grade. They were filtered through PTFE 0.45 µm syringe filters and transferred into autosampler vials for GC-MS analysis. A Shimadzu QP 2010-SE GCMS with an auto-sampler was used for the analysis. Ultrapure Helium was used as the carrier gas at a flow rate of 1 mL/ minute. A BPX5 non-polar column, 30 m; 0.25 mm ID; 0.25 µm film thickness, was used for separation. The GC was programmed as follows: 50°C (1 min); 5°C/min to 250°C (4 min). Only 1 µL of the sample was injected. The injection was done at 200°C in split mode, with a split ratio set to 10:1. The interface temperature was set at 250°C. The electron-ion (EI) source was set at 200°C. Mass analysis was done in full scan mode, 50 – 600 m/z, ionization voltage set at 70eV. a scan interval of 0.5 s, fragments from 45 to 450 Da. and the solvent delay was 0 to 2 min. The total run-time was 45 min. Detected peaks were matched against the National Institute of Standards and Technology (NIST 2014 MS library) for possible identification**.**

VI. Results

The results obtained according to the above methods and procedures for the assessment assays are reported categorically in tabular forms as shown below.

6.1 Phytochemical assay results

The results from phytochemical analysis of the extracts are shown in table 1 below.

Table 1: Showing the results for Phytochemical screening

Phytochemical Constituents	**Methanol**	**Chloroform**	**Diethylether**	**n-hexane**
Steroids	++	+	+	-
Terpenoids	++	+	+	-
Tannins	++	++	-	+
Alkaloids	+	+	+	+
Carbohydrates	-	+	+	-
Glycosides	+	-	+	-
Saponins	+	++	++	-
Flavonoids	++	++	++	+
Anthraquinones	+	-	++	+

Key: ++ highly positive, + moderately positive, -- negative

6.2 Microbial assay results

The result from determination of inhibition zones, minimum inhibition concentrations and minimum bacterial concentrations are tabulated in tables 2,3 and 4 respectively shown below.

Table 2: Showing the results for Inhibition Zones

Extract	**E. Coli**	**Staphylococcus`**	**Klebsiella**	**Pseudomonas**
Methanol	33 – 28 mm	32 – 20mm	8 – 6mm	13 – 7 mm
Chloroform	20 - 17 mm	16 – 12 mm	8- 5 mm	00mm
Hexane	7 - 4 mm	17 – 6 mm	6 – 3 mm	00 mm
Diethylether	00 mm	14 – 19mm	00 mm	00 mm
Imipenem(-ve control)	00 mm	00 mm	00 mm	00 mm
Gentamycin (+ve control)	00 mm	33 – 30 mm	13 – 9 mm	28 – 26 mm

Table 3: Showing the results for Minimum Inhibition Concentration (MIC) in mg/ml

Extract	**E. coli**	**Pseudomonas**	**Klebsiella**	**Staphylococcus**
Methanol	1.75 ± 0.12	4.5 ± 0.72	6.3 5 ± 0.45	2.25 ± 0.24
Chloroform	3.25 ± 0.65	10 ± 1.24	7.0 ± 1.56	4.85 ± 0.55
Hexane	8.2 ± 1.24	5.4 ± 1.25	8.7± 0.75	5.4 ± 0. 85
Diethlyether	Not identified	Not identified	Not identified	4.2 ± 0.15

Table 4: Showing the results for Minimum Bactericidal Concentration (MBC) in mg/ml

Extract	E. coli	Pseudomonas	Klebsiella	Stapylococcus
Methanol	5.35 ± 1.15	13.5 ± 0.75	21.28 ± 1.25	7.83 ± 1.54
Chloroform	11.45 ± 0.55	36.2 ± 1.78	23.0 ± 1.78	13.85 ± 2.05
Hexane	30.27 ± 3.18	21.4 ± 1.85	31.35 ± 2.45	19.8 ± 1. 34
Diethlyether	Not identified	Not identified	Not identified	16.28 ± 1.12

6.3 GC/MS results

The gc/ms spectra data for chloroform and methanol crude extracts are shown below categorically in tabular form. .

Table 5: Showing the GC-MS data of Chloroform extract

NO	NAME OF THE COMPOUND	RETANTION TIME	m/z	AREA	HEIGHT	% COMP
1.	Dodecane, 4,6-dimethyl-	4.924	57.05	656916	251014	4.064765
2.	Hexadecane	5.376	57.05	83787	45475	0.736394
3.	1-Tetradecene	5.996	55	145160	73186	1.185129
4.	Nonadecane	6.067	57.05	98839	53577	0.867593
5.	Heptadecane	6.271	57	158807	86338	1.398104
6.	Octadecane	6.541	57	73493	53917	0.873098
7.	Heptadecane	6.624	57.05	207878	120918	1.958071
8.	2,3-Dimethyldodecane	0	57	0	0	0
9.	Eicosane	6.926	57.05	855300	456273	7.640913
10.	Phenol, 2,4-bis(1,1-dimethylethyl)-	7.086	191.1	806156	465471	7.825953
11.	Cetene	7.716	57.05	214125	137024	2.218882
12.	Heneicosane	8.357	57	149719	101903	1.650154
13.	2-methyloctacosane	8.633	57.05	1197440	725183	11.74316
14.	Dotriacontane	8.947	57	133536	113221	1.833431
15.	1-Nonadecene	9.222	57.05	231352	165923	2.686854
16.	Phytol, acetate	0	57.05	0	0	0
17.	2-Pentadecanone, 6,10,14 trimethyl-	0	57	0	0	0
18.	Phytol	9.982	57.05	12322	63456	1.04283
19.	Tetrapentacontane, 1,54-dibromo-	0	57.05	0	0	0
20.	2-Bromotetradecane	0	57.05	0	0	0
21.	Tridecanoic acid, 4,8,12-trimethyl-, methyl ester	10.139	57.05	1094723	661675	9.556902

22.	Tetracosane	0	57.05	0	0	0
23.	Dichloroacetic acid, heptadecyl ester	10.576	57.05	214456	170161	2.755482
24.	Dotriacontane	11.01	57	177545	138533	2.243318
25.	Linolenic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (Z,Z,Z)-	0	79.05	0	0	0
26.	Citronellol	11.352	71.05	1572528	1066644	16.22994
27.	2-methylhexacosane	0	57.05	0	0	0
28.	Octacosanol	0	57.05	0	0	0
29.	5,5-Diethylpentadecane	12.718	57.05	597539	408645	6.617344
30.	Octatriacontylpentafluoropropionate	0	57.05	0	0	0
31.	Bis(tridecyl) phthalate	0	57.05	0	0	0
32.	Hexatriacontane	14.503	57.05	286647	199809	3.235583
33.	Benzyl isothiocyanate-	14.724	57.05	11121	60989	0.421133
34.	Hexatriacontane	14.85	57	399310	214552	3.474322
35.	2-Piperidinone, N-[4-bromo-n-butyl]-	0	69.05	0	0	0
36.	Nonacosane	15.562	57.05	544330	253011	4.097103
37.	Tetracontane	16.086	57.05	233510	110724	1.792996
38.	Pentatriacontane	16.214	57.05	228701	102186	1.654737
39.	1-Chloroeicosane	0	57.05	0	0	0
40.	Vitamin E	0	165.1	0	0	0.
					6299808	**100**

Table 6: showing the GC/MS data of methanol extract

NO	**NAME OF THE COMPOUND**	**RETANTION TIME**	**m/z**	**AREA**	**HEIGHT**	**% COMP**
1.	1-Dodecene	4.021	55	122379	26196	0.7267
2.	1-Pentadecene	5.996	55	193328	87324	2.422445
3.	Phenol, 2,4-bis(1,1-dimethylethyl)-	7.086	191.1	857864	484493	11.98741
4.	1-Pentadecene	7.717	55	248035	157336	4.364641
5.	1-Pentadecene	7.717	55	248035	157336	4.364641
6.	1-Heptadecene	9.222	57.05	278931	196385	5.447895
7.	Hexadecanoic acid, methyl ester	10.134	74	361685	261751	6.261206
8.	n-Hexadecanoic acid	0	149.05	0	0	0
9.	Behenic alcohol	10.575	57.05	273956	206369	5.72486

10.	3-Pyridinecarbonitrile, 1,2-dihydro-4-methoxy-1-methyl-2-oxo-	10.856	164.05	970829	340774	9.453374
11.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	11.222	67	69463	52265	1.449878
12.	Methyl 8,11,14-heptadecatrienoate	11.266	79.05	132805	100291	2.782162
13.	Phytol	11.345	71.05	812996	599057	16.61837
14.	1-Heptacosanol	11.807	57.05	262649	176458	3.895102
15.	[1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)-	12.798	57.05	87017	60647	1.682402
16.	Oxiraneoctanoic acid, 3-octyl-, 2-ethylhexyl ester	13.523	57	36125	24819	0.688501
17.	Bis(2-ethylhexyl) phthalate	13.781	149.05	672252	465931	12.92534
18.	Docosyl heptafluorobutyrate	13.985	57.05	125567	80407	2.230562
19.	Sulfurous acid, octadecyl 2-propyl ester	14.531	57.05	34281	17007	0.471789
20	2,5 dimethoxy-6-(14-nonadecenyl)-1,4-benzoquinone	14.775	57.05	231869	173228	3.45876
20.	Heneicosyl trifluoroacetate	14.974	57.05	68470	41135	1.141122
21.	Heptafluorobutyric acid, n-octadecyl ester	16.186	57.05	28629	12190	0.338161
22.	1-Chloroeicosane	17.089	57.05	102473	23977	0.665143
23.	Vitamin E	17.439	165.05	105607	32639	1.008436
					3778013	**100**

6.4 Antioxidant Capacity results

The experimental results for percentage scavenging capacity of the solvents crude plant extracts are tabulated in table 7 shown below.

Table 7: Showing Antioxidant Capacity results of the extracts

Plant extract	Sample absorbance at 517 nm	Control absorbance	Scavenging capacity (Percentage)
Methanol Ext	0.3851 ± 0.034	0.972	60.38 ± 0.034
Chloroform	0.4482 ± 0.051	0.972	53.91 ± 0.051
Diethylether	0.687± 0.042	0.972	29.32 ± 0.042
Hexane	0.571 ± 0.023	0.972	41.26 ± 0.023

6.5 Cytotoxicity results

The results obtain from cytotoxicity studies of methanol and chloroform crude extracts on colon tumor and leukemia cells are tabulated in table 8 shown below.

Table 8: Showing Cytotoxicity evaluation results of the methanol and chloroform crude extracts on Colon tumor and Leukemia cells

Plant Extract	Cell	IC_{50} (µg mL^{-1})/Percent at 800 µg
Methanol	colon tumor cells (HCT-116)	44.28 ± 0.85 %
	Leukemia cells (K-562)	26.67 ± 0.54 %
Chloroform	colon tumor cells (HCT-116)	31.44 ± 1.65 µg/ml^{-1}
	Leukemia cells (K-562)	20.18 ± 1.37 µg/ml^{-1}
Doxorubicin		0.41 ± 2.86 µg/ml^{-1}

VII. Discussion

7.1. Phytochemical data assessment

The results obtained from qualitative phytochemical analysis (table 1) of the plant extracts reveal the presence of phytochemical constituents such as steroids, terpenoids, tannins, alkaloids, saponins, glycosides, anthraquinones and flavonoids which are considered to be responsible for the biological and pharmacological activity of the plant [42,43]. From the obtained experimental data (table 1), the secondary metabolite terpenoids are known for dissolution of the cell wall of microorganisms by weakening the membranous tissue thus facilitates antimicrobial activities [44, 45] of the plant extract. Terpenoids are also found to provide the provitamin A, influencing the human immune function, act as potential antioxidant and anti-inflammatory [46] thus aids against the associated ailments. Plant alkaloids are known to bind microtubule proteins during metaphase causing mitotic arrest and finally the cell dies thus promoting the antimicrobial properties [47]. Saponins are normally inhibitory to the growth of the gram-positive bacteria *staphylococcus aureus* [48]. Anthraquinones are known to be active against gram positive bacteria [49]. Saponins and anthraquinones compounds are known to penetrate the peptidoglycan cell wall of gram-positive bacteria consequently inhibits the bacterial multiplication [48.49]. Flavonoid compounds are known to be a good source of antimicrobial activities besides being found to be effective as hepatoprotective, anti-inflammatory, antioxidant, antidiabetic and anticancer [50]. They have the ability of complexing with the bacterial cell wall to suppresses cytoplasmic membrane functions, inhibits the synthesis of nucleic acid and energy metabolism consequently discouraging microbial growth [51, 52]. This indeed is a great pharmacological property. The plant steroids are also found to possess antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* [53] besides from being an effective anti-inflammatory, anti-cancer, cytotoxic, and antiproliferative [54]. The plant extract was also observed to have significant glycosides which are known for antimicrobial properties that inhibits the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* species [55]. The presence of tannins also facilitates the plant's effectiveness toward antimicrobial activities [56] specifically against *Staphylococcus aureus, Shigella sonnei, Shigella flexneri, Escherichia coli* and *Pseudomonas aeruginosa* [57]. Tannins are also found to be preventive against chronic diseases [56], effective antioxidant, enzyme-inhibitor, anti-hemorrhoidal, anti-inflammatory, antidiarrheal and antimutagenic [57, 58]. Thus, the presence of these antimicrobial phytoconstituents promotes the natural medicinal synergisms against these UTI bacterial strains. Since these phytochemicals also poses anti-inflammatory, antifungal, hepatoprotection, antioxidant, anticancer, antidiarrheal, antidiabetic and antimutagenic properties signifies the associated ailments with the infection including fungal infections like candidiasis can also be managed. Therefore, the management of UTI pathogenic strains and other ailments associated with the infections can also be accosted to the presence of these phytoconstituents which does not only aim at a particular infection [59] but also, they improve the human health defense mechanism. Therefore, with these plethora characteristics of the phytoconstituents, this plant extract is of great phytopharmaceutical importance and auger well with the claimed medicinal properties.

7.2. Bacterial data assessment

From the invitro bacterial analysis results tabulated in table 2, the solvent extracts of *ricinus communis mature leaves* have exhibited recommendable variation in antibacterial activity against the UTI strains. According to Balouiri and Eloff, the range of inhibition diameter of the circles including the disc in millimeter break points for anti-bacterial activity may be divided into the following categories as; >12mm Zone of inhibition – High sensitive, 9-12mm Zones of inhibition –Moderate sensitive, 6-9mm Zone of inhibition – less sensitive and <6mm Zone of inhibition – Resistant [36,60]. The present study reveals that high sensitivity >12mm zone of inhibition was observed in methanol and chloroform crude extracts against, *E. coli* and *Staphylococcus*

spp while the methanol crude extract recorded moderate sensitivity against *Pseudomonas spp* and *klebsiella spp*. Chloroform extract was less sensitive to *klebsiella spp* and resistant to *Pseudomonas spp*. Diethylether was found to be highly sensitive to the gram-positive staphylococcus can be attributed to the presence of saponins and anthraquinones [48,49] but resistant to the gram-negative microbes. The n-hexane extract found also to show recommendable antibacterial activity towards the gram-positive *staphylococcus spp* [55,56] but somehow less sensitive and resistant towards the gram-negative microbes. From the data (table 2), the failed inhibition of growth experienced in case of the chloroform extract against *pseudomonas spp* and diethylether extracts against the gram-negative microbes can either be due to the microbe's effective permeability barrier composed of a thin lipopolysaccharide exterior membrane that could restrict permeation of the plant extract or the bacteria were more resistant to the plant-origin antimicrobials agents extracted by the solvent. This can also be strongly related to extracellular polymeric substances (Eps) inhibitors in combination with plant antimicrobials leading to a striking increase in antimicrobial activity [61]. Efflux pumps and β-lactamase production have been reported to cause the inhibition growth resistance [62]. Inhibition zone diameter of extracts were significantly ($p < 0.05$) lower than the mean of inhibition zone diameter of positive control. Reason could be that the extracts may possess both pharmacologically active and non-active substances whereas the control positive possessed purified and concentrated active ingredient. From table 3 and 4, The methanol extract recorded the lowest MBC_{50} (4.20 mg/ml) against *E .coli* and MIC_{50} value of 3.78, and have shown recommendable antibacterial activity against both gram negative and gram-positive bacteria. The MIC_{50} of the crude extracts ranged from 1.75 mg/ml to 10 mg/ml where they experienced bacterial effects compared with gentamycin ≤4 mg/L and ≥16 mg/L as discussed elsewhere [35, 63]. This signifies the crude extracts effectiveness towards antibacterial activities. From the micro bioassay data, we can conclude that, lower the MIC larger the inhibition zone while higher MIC smaller the inhibition zone signifies more resistance the microorganism. The MBC_{50} values obtained (table 4) are approximately threefold higher or approaching fourfold than the MIC_{50} values determined signifying that these extracts exhibit bactericidal activity against pathogens [63] at a higher concentration and bacteriostatic at lower concentrations. It also signifies a dose dependent increase and high potent activities even at low concentration suggesting that the extracts could be developed further into UTI drugs. Therefore, the results obtained for MIC and MBC determinations might be considered sufficient for further investigation aimed at isolating and identifying the active phytocompound(s) and evaluating their possible antimicrobial synergism.

7.3. GC/MS spectral data assessment

From the Phytochemical screening and microbial assay experimental data [table1,2,3,4], chloroform and methanolic extracts reveal remarkable antimicrobial results, therefore their GC/MS spectral analysis were considered for evaluating qualitative natural antibacterial agents. Their GC/MS spectrogram data (table 5 and 6) reveals that these two extracts are a museum of bioactive ingredients which poses antibacterial, antifungal, anti-parasitic, anti-inflammatory and antioxidant activities as per documented pharmacognosy and pharmacology research articles and reviews [64]. Both the extracts have in common phenol 2,4-bis(1,1-dimethylethyl) which poses recommendable antibacterial and anti-inflammatory activities [65] and phytol which is antibacterial, anti-inflammatory, anticancer, diuretic, antifungal against S. typhi, resistant gonorrhea and headache [64,66]. From methanolic extract GC/MS data, researches have shown that hexadecanoic acid methyl ester is an antifungal, antioxidant and antibacterial, pesticide, lubricant, chemo-preventive and lipoxygenase inhibitor [67]. 1-Dodecene is antibacterial, [68], 9,12-Octadecadienoic acid (Z, Z)-, methyl ester has antibacterial, antiviral and antioxidant properties [69] and sulfurous acid octadenyl1-propyl ester poses antibacterial properties [70] respectively. Vitamin E supplementation has been found to ameliorate the symptoms of urinary tract infection [71] and ailments associated with the infection. Hexadecane obtained from chloroform extract has antibacterial and antioxidant activities [64]. Octadecane, Nonadecane and Heptadecane have antimicrobial activity [72,73], Eicosane has antibacterial activity [74] while Tetracosane and nonadecene have both anti-inflammatory, antioxidant and antimicrobial activities [75, 76] respectively. Tetracontane poses anti-inflammatory properties [77]. Heneicosane (antioxidant and antimicrobial, urine acidifiers [78] while both 1-Octadecane and Citronella are both antifungal and antiseptic [79]. The presence of bioactive ingredients such as phytol, sulphuryl, isothiocyanate and quinone derivatives in addition to phytochemicals such as saponins, flavonoids, terpenoids, anthraquinones and hardens the plant extract antimicrobial activities [tables 1,5,6}. This could be the among the reasons behind the methanol extract is active against the resistant *pseudomonas species*. Since these extracts also contains bioactive ingredients which possess anti-inflammatory and antifungal activities signifies that they can deal with the overgrowth of microbes in the vaginal area causing vaginitis (inflammation) issues. With the presence of urine acidifier and antiseptic activities among the realized bioactive ingredients also signifies that the infected urine (urine being the main vehicle) can be cleansed from these microbes and disrupted natural vaginal pH levels can be restored. Behenic acids are known restores

the natural ingredients of the affected tissues. Thus, the presence of these natural antimicrobial bioactive compounds rejuvenates the plant extract activity against these UTI strains including associated fungal infections within the urinary tract system.

7.4. Antioxidant assessment

Studies done on medicinal plants strongly supports the idea that the constituents with natural antioxidant activity have the capability of exerting protective effects against oxidative stress in biological systems [80]. The chloroform and methanolic extracts have registered an average value of 53.91% and 60.38% respectively for anti-oxidant activity (table 7). These values are attributed to a recommendable concentration of phenolic compounds, cetenes and terpenoids which have been found in the plant extracts. The presence of phytochemicals such as tannins, saponin, flavonoids, and other secondary metabolites with lower molecular weights possessing redox properties, conjugated ring structures and carboxylic groups are known to scavenge the free radicals [81] thus adds to the antioxidant activity values of the plant extract towards its medicinal achievements. Therefore, diseases that are associated with free radicals, cell damages, damages to biological molecules such as DNA, proteins, lipids, carbohydrates leading to tumor development and progression [82] are reduced.

7.5. Cytotoxicity assessment

Cytotoxicity assessment is a very critical aspect in medicinal acceptance of a plant extract. This is mainly due to the fact that it elucidates information about the extract safety, as well as precautionary measures that should be considered while administering it to a patient in need. The MTT-based assay and both colon tumor cells (HCT-116) and Leukemia cells (K-562) were used to assess the cytotoxicity of the methanol and chloroform plant extracts since they prove to contain high content of medicinal values. In HCT-116 cell lines, the percentage of cytotoxicity of the methanol extract is 42.1 % and that in K-562 cell lines is 28.4% at the concentration of 800 µg. The IC_{50} values of the bioactive components from chloroform plant extract ranged from 31.44 ± 1.65 $\mu g/ml^{-1}$and 20.18 ± 1.37 $\mu g/ml^{-1}$ respectively as presented in table 8. According to Zirihi et al [83] and Kigondu et al [84], the plant extracts can be considered non-cytotoxic when their IC_{50} values > 20 µg/ml. This plant extract showed low, moderate or no cytotoxicity activity, in other words, they did not have any significant cytotoxicity against both of the cell lines since the average lowest value is 20.18 µg/ml(table7). So far, a number of invitro cytotoxicity and mutagenicity studies have been done on different parts of the plant using various solvents on different cells and using different techniques revealing the plant safeness [85,86] towards drug discovery and precautionary measures. This signifies that the toxicity of plant extract is not life threatening. This is supported by high antioxidant values obtained and the presence of terpenoids which are known to play functional roles in defense, antibiotics and toxins [50].

7.6. Additive antimicrobial assessment

In addition to *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the plant extracts have also been proven active against *Proteus Vulgaris*, *Enterococcus faecalis*, *Enterobacter spp*, gram positive *bacillus subtilis* and fungal strains such as *Candida albicans*, *Aspergillus fumigatus* and *Aspergillus flavus* [87,88]. The antimicrobial activities of medicinal plants have been found to be influenced by other factors such as altitude, temperature, age, illumination and moisture [89,90]. These factors regulate bioactive ingredients variation, accumulation, presence of different active ingredients and metabolism of secondary metabolites in medicinal plants [89,90] thus influence its efficacy towards microbial infections. Studies too have shown that the concentrations these phytoconstituents are more on mature tissues and differ depending on the location of the medicinal plants [89]. Working too with clinical isolate or laboratory strains and crude extracts do hinders effectiveness [46]. This means that we cannot get the same results similar to other studies done which proven the plant's efficacy towards antimicrobial activities.

VIII. CONCLUSION

The research has realized that the plant mature leaves extract poses diverse phytochemicals and bioactive ingredients which halts proliferation or multiplication of the pathogen strains. These natural antimicrobial agents also focus on associated ailments and potent too on fungal infections within the urinary tract systems including gonorrhea. This signifies that it is active against the synergistic pathogens of urinary tract infection and their effects. The research has also realized from various reviews that these isolated antimicrobial agents and phytoconstituents also improve the entire health of an individual since they boost body oxidant status and act as immunomodulators. The findings also shows that the plant is medicinally safe. The research findings conform well with the ethnopharmacological claims.

IX. Recommendations

Since the findings are based on crude extracts, the results obtained are qualitative thus, quantitative data for these natural antimicrobial data is highly recommended. Therefore, mechanistic studies on isolated, purified and identified bioactive ingredients against the bacterial pathogens should be conducted. In vivo antimicrobial studies should be conducted to confirm the invitro antimicrobial activities of the plant extracts against the bacterial strains. There is the need to carry out hemolysis to evaluate toxicity of normal erythrocyte cells. A conventional drug from this plant needs to be designed embracing all these medicinal features against the UTI strains with their associated illness including fungal infections within the urinary tract system. Development of regional surveillance programs is necessary for implementation of UTI guidelines.

X. Research Protocol

Author Contributions

All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The data presented in this study are available at W.H.O, C.D.C., East Africa Community Health Secretariat and www.healthdata.org/gbd.IHME.

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