Determination of Antimicrobial Activity of Mexican Sunflower (Tithonia diversifolia) Leaf Extract against Selected Gram-Negative Bacteria

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Abstract

Introduction: The global rise of antibiotic resistance among pathogenic bacteria has become a significant public health concern, necessitating the exploration of novel antimicrobial agents. *Tithonia diversifolia*, commonly known as Mexican sunflower, has been traditionally used in various cultures for its medicinal properties, including the treatment of infectious diseases. The aim of this study was to investigate the antimicrobial activity of *T. diversifolia* leaf extract against common gram-negative bacteria such as *Escherichia coli, Enterococcus faecalis,* and *Klebsiella pneumoniae.*

Methodology: Experimental research was conducted on fresh leaves of the *Tithonia diversifolia* extracts. The extracts were obtained by the maceration method, and the antibacterial activity was tested using the agar well diffusion method.

Result: The ethanoic extract shows a good inhibition on *E. coli* and *K. pneumoniae* with the largest inhibition zone diameter was at a 100% concentration of 19 mm for *E. coli* and 15mm for *K. pneumoniae*, while the diameter of the smallest inhibition zone was at a 20% concentration of 6 mm for *E. coli* and 8mm for *K. pneumoniae* with no and less inhibition at 20% on both *E. coli* and *K. pneumoniae* respectively. The Aqueous extra shows no inhibition on both the selected Gram Negative bacteria on both the concentration in which all the concentration shows an inhibition zone of 6mm on all the selected pathogens

Conclusions: *T. diversifolia* leaf extracts demonstrate varying antimicrobial efficacy, with ethanolic extracts showing greater antibacterial activity against selected Gram-negative bacteria (*E. coli* and *K. pneumoniae*) compared to aqueous extracts. Interestingly, *E. faecalis* exhibited growth around the aqueous extract discs, suggesting possible attraction rather than inhibition. While aqueous extracts showed no antimicrobial activity, the ethanolic extracts did demonstrate some inhibition, which was measurable through MIC testing.

Introduction

Antimicrobial resistance (AMR) is a global health problem threatening safe, effective healthcare delivery in all countries and settings (WHO, 2019). The ability of microorganisms to become resistant to the effects of antimicrobials is an inevitable evolutionary process (Davies & Davies, 2020). The misuse and overuse of antimicrobial agents have increased the importance of a global focus on antimicrobial stewardship (AMS) (Dyar et al., 2017). According to the WHO (2019), AMR causes an estimated 700,000 deaths annually worldwide. AMR affects countries in all regions and everyone in different social class (O'Neill, 2016). Though it affects all regions, but low- and middle-income countries (LMICs) are heavily impacted (Ayukekbong et al., 2017).

Key factors contributing to the global AMR include lack of new antibiotics in development, limited access to quality healthcare and diagnostics in many regions (WHO, 2020).Africa faces unique challenges with AMR. Estimates suggest that by 2050, AMR could cause up to 4.1 million deaths annually in Africa if not

addressed (O'Neill, 2016). In Africa there are counterfeit and sub-standard medicines contribute to AMR development (Tadesse *et al.*, 2017). Weak healthcare infrastructure in many countries hampers efforts to combat AMR (Essack et al., 2017). Lack of access to clean water, sanitation and hygiene (WASH) in addition there is also poor infection and disease prevention and control in homes, healthcare facilities (WHO, 2021).

Kenya like many African countries, faces significant AMR challenges. Studies have shown high rates of resistance to commonly used antibiotics (Kimang'a, 2018). The National Action Plan on Prevention and Containment of AMR (2017–2022) was developed to address this issue (Ministry of Health Kenya, 2017). Key priorities included improving surveillance, promoting appropriate use of antimicrobials, and enhancing infection prevention and control. This increasing prevalence of antibiotic-resistant gram-negative bacteria poses a significant threat to public health globally (Prestinaci et al., 2015).

As conventional antibiotics become less effective, there is an urgent need to explore alternative sources of antimicrobial compounds (Ventola, 2015). *Tithonia diversifolia*, commonly known as Mexican sunflower, has shown promising medicinal properties in traditional medicine (Olukunle & Abegunde, 2018). However, its potential as a source of novel antimicrobial activity against gram-negative bacteria remains largely unexplored.This study aims to determine the antimicrobial activity of *Tithonia diversifolia* extracts against selected gram-negative bacterial strains, with the goal of developing alternative forms of antibiotic.

Antibiotics are becoming increasingly ineffective as drug resistance spreads leading to more difficult -to-treat infections and death. New antimicrobials and alternative therapies are urgently needed (WHO, 2021). It will be key in managing antibiotic resistance crisis with the rise of antibiotic-resistant bacteria, particularly with the gram-negative strains. Traditional antibiotics often prove ineffective against these pathogens due to the intrinsic protective mechanism of gram-negative bacteria, such as their impermeable outer membrane and efflux pumps, which limit the penetration and accumulation of antimicrobial agents (Li et al., 2015). The scientific justification for this research is strongly supported by preliminary studies and theoretical frameworks. Previous research by Wang et al. (2021) demonstrated significant antimicrobial activity in crude extracts of *T. diversifolia* against selected pathogens, hence need for more detailed investigation. The plant's documented phytochemical profile, as analyzed by Rodriguez et al. (2022) includes several classes of compounds known to possess antimicrobial properties, including: Flavonoids, phenolic compounds, diterpenoids, sesquiterpene lactones. Natural products continue to play a crucial role in modern drug discovery by providing unique chemical structures and bio-activities unmatched by synthetic libraries. As Atanasov et al. (2021) emphasize, these compounds offer exceptional structural diversity and evolutionary-optimized bio-compatibility, presenting significant opportunities for pharmaceutical innovation despite challenges in isolation and characterization.The unique chemical diversity of *T. diversifolia* increases the likelihood of discovering novel antimicrobial compounds.

In addition, the research is substantial in an economic perspective. The World Bank (2017) estimates that drug-resistant infections could cause economic damage on par with the 2008 financial crisis, with healthcare costs potentially increasing by $1.2 trillion annually by 2050 if resistance continues to rise unchecked. This economic burden underscores the urgent need for innovative antimicrobial development strategies that can deliver effective treatments while remaining economically sustainable.The research is also practical in that the sample is readily available and easily cultivated. Previous studies also provide methodological frameworks. Environmental sustainability of this research is also key. Natural product-based drug discovery presents a compelling alternative to conventional synthetic methods, particularly from an environmental perspective. As highlighted by Sheldon (2016), the utilization of waste biomass and naturally derived compounds significantly reduces the environmental footprint associated with pharmaceutical development.

Investigating the antimicrobial efficacy of *Tithonia diversifolia* leaf extract against the selected gram-negative bacteria could provide critical insights into alternative treatment strategies. These bacteria are not only clinically significant but are also known to develop resistance to multiple antibiotics (Ventola, 2015). The ability to tap into the antimicrobial properties of the Mexican sunflower could be a stepping stone in reducing reliance on synthetic antibiotics and combating antibiotic resistance. The emergence and spread of AMR represent one of the most pressing public health challenges of our time. According to the WHO 2023 report on Antimicrobial Resistance, infections caused by resistant pathogens cause approximately 1.27 million deaths annually (WHO, 2023). Gram-negative bacteria are particularly concerning, as noted by Tacconelli et al. (2020) in The Lancet Infectious Diseases, with some strains showing resistance to nearly all available antibiotics.

Tithonia diversifolia has a rich history of traditional medicinal use across multiple continents. Ethnopharmacological studies by Oyewole et al. (2019) documented its use in treating various infections in West African traditional medicine systems. More research by Chen et al. (2021) in the Journal of Ethnopharmacology has demonstrated that traditional medicinal plants are twice as likely to yield novel bioactive compounds compared to randomly selected plants, supporting the value of investigating traditional used species such as *T. diversifolia*. In the Journal of Natural Products according to Newman and Cragg (2020), approximately 50% of all approved drugs between 1981 and 2019 were either natural products or directly derived from them.

The development of new antimicrobial compounds from locally available plants *T. diversifolia* could have significant socioeconomic implications, particularly for developing nations. Studies by Rahman et al. (2022) indicate that locally-sourced natural medicines can reduce healthcare costs by up to 60% compared to synthesis alternatives. While previous studies have examined various properties of *T. diversifolia*, systematic investigation of its activity against specific gram-negative bacteria remains limited. This research addresses a critical knowledge gap identified by Martinez et al. (2021) in their comprehensive review of natural products against gram-negative bacteria. Beyond human health applications, this research has significant agricultural and environmental implications. Studies by Kumar

and Sing (2021) demonstrate that plant-based antimicrobials can reduce reliance on synthetic pesticides in agriculture, contributing to more sustainable farming practices.

MATERIALS AND METHODS

Study site

This study was done in the JOOTRH microbiology laboratory for the analysis after the collection of plants samples in Ulalo village in Kisumu West sub-county, South Kapuonja sub-location within Maseno division. It is located on the North of Kigadahi, east of Kiboswa, south of Kanyawegi, west of Darajambili. South Kapuonja is located at an approximate latitude of -0.0550°S and a longitude of 34.6337°E. The study area was selected due to its unique geographical and environmental characteristics, making it ideal for the study as in Fig. 1. The Mexican sunflower (Tithonia *diversifolia*) leaves will be collected from Ulalo village which is popularly known to the locals as “Akech”. Ulalo village will be an ideal sample site for the collection due to several reasons. There is abundance of *Tithonia diversifolia* since it is naturally found in the area; the availability of fresh plant material makes it a convenient location for sourcing samples consistently throughout the research period. The community is already familiar with the potential benefits of the Mexican sunflower hence this context would add up to the relevance of the study. Being a semi-rural area, the site has less environmental pollution making it easier to obtain samples that are more representative of the natural state of the plant.

Sample target

The sample target was the leaf of Mexican sunflower (*Tithonia diversifolia)*

Sample size

Fifteen grams of fresh *Tithonia diversifolia*

Sampling criteria

Purposive sampling technique was employed. Based on the characteristics that will be required.

Inclusive criteria

- Mexican sunflower leaves that was collected was in good condition, free of insect damage, rust or disease.
- Mexican sunflower leaves that was collected was well developed and mature.
- Mexican sunflower leaves that was collected represent the range of variation in the population.

Plant extract preparation

Fresh leaves of *Tithonia diversifolia* were harvested and washed with distilled water to remove all the dirty particles. Then the leaves were put on the clean area to air dry for 30 days. After the leaves were well dried, it was mashed using a blender and weighed.

Ethanolic extraction

The four grams of powdered leaves of Mexican sunflower was measured and placed in a bijou bottle, then subjected to extraction by soaking in 20 ml of 95 % ethanol for 72 hours that ensure maximization of extraction. Filtration was done to separate the plant residue and ethanolic extract using a filter paper in a sterile bijou bottle. To obtain a concentrated extract, the ethanol was evaporated by placing bottle-containing extract in a water bath at 40- 50℃ to leave behind the concentrated ethanolic extract. The container was well labelled and wrapped using aluminium foil to avoid any light degradation of antimicrobial compounds that may be present in the extract.

Aqueous extraction

Aqueous extract was prepared by cold maceration of collected leaf powder in distilled water in a ratio of 1: 5 which was done by addition of 20 ml of distilled water to four grams of the Mexican sunflower leaves powder in a bijou bottle. The mixture was then macerated for 72 hours with vigorous shaking to increase the efficiency of extraction after which it was filtered then stored in a well labelled bottle that is then wrapped using aluminium foil at -4℃ in a refrigerator.

Isolates revival

The previously preserved known isolates (*Enterococcus faecalis* strain ATCC® 29212, *Escherichia coli* strain ATCC® 25922, and *Klebsiella pneumoniae* strain ATCC® 13883) was revived, in which cryonate tubes was opened in a sterile environment then using a sterile wire loop the frozen bacteria was scraped off to the top and streaked on the Nutrient Agar (NA) media then incubated in an aerobic incubator overnight at 37 ^{0}C for 24 hours.

Bacterial Suspension

The bacterial suspension was prepared by taking the bacteria from the bacterial culture using an inoculum needle. The bacteria were added to 5 mL 0.9% NaCl in a test tube; then, the solution was homogenized using a vortex. The suspension was equalized with a 0.5 McFarland standard solution ($1x10^{8}$ CFU/mL) using a spectrophotometer at a wavelength of 625 nm. The absorbance ranged from 0.08 to 0.1 (WHO, 2003).

Antimicrobial assay

Antibacterial activity testing was carried out by the agar well diffusion method, which was by making a well or hole in Mueller-Hinton Agar (MHA) media containing the test bacteria. A total of 25 mL MHA media was poured into a Petri dish. There are seven wells or holes in the media; the diameter for each hole is 4 mm. Then, each bottom of the well was dripped slightly with MHA media. Then were dropped 20 µl of an extract with a concentration of 10, 20, 30, 40, 50%, clindamycin 2 µg, and distilled water in each well. Then the Petri disk containing test media was incubated at 37°C for 24 hours. The treatment was carried out in triplicates.

Minimum Inhibitory Concentration (MIC) Test

Minimum Inhibitory Concentration (MIC) is the lowest concentration of the ethanolic and aqueous extracts which will inhibit growth of the microbes. The MIC of the extracts was determined using the two-fold serial dilution method. The initial concentration of each of the stock solution was 200 mg/mL, if the 4 g (4,000 mg) of extract dissolved in 20 mL. This was the starting concentration of the two- fold serial dilution. A standardized inoculum of 1.5×10^8 CFU/ml of each microbe was prepared to give a concentration equal to that of 0.5 McFarland turbidity standard of equivalence. For each selected bacteria, a 14-tube set was used when testing per leaf extract. Four tubes was set aside where one will contain only the broth, the extract being tested, another having broth inoculated with bacteria and finally one with test organism and solvent extract will be used as controls. 10 sterile test tubes labelled with desired concentrations (200 mg/mL, 100 mg/mL, 50.0mg/mL, 25.0mg/mL, 12.5mg/mL, 6.25mg/mL, 3.13mg/mL, 1.56 mg/mL, 0.78mg/mL, and 0.39mg/mL) of the extract contain the broth (0.5 ml per tube). The volume of the broth was 0.5 ml in all the first tubes. 0.5 ml of the leaf extract stock solution was added to the tube labelled 100 mg/ml and mixed well with the broth. 0.5ml of the diluted solution from the first tube was transferred to the second tube. This process was repeated for the remaining tubes to create a two-fold serial dilution series. At the 10^{th} tube, after pipetting out 0.5 ml of the diluted solution, the pipetted diluted solution was discarded. A standardized inoculum of the selected bacterial culture to each test tube exempting the control ones which needed no addition of bacterial culture. Incubation of the tubes was done at 37℃ for 24 hours. This was repeated for the remaining extracts and selected bacteria. The MIC was determined by visually inspecting the tubes for turbidity.

Results and Discussion

Antibacterial activity test

Antibacterial activity test was carried out on selected Gram Negative using variations in the concentration of Tithonia diversifolia leaves extract of ethanolic and aqueous of 20, 40, 60, 80 and 100%. The positive control used was Amikacine 30 µg. The method of testing antibacterial activity used Kirby-Bauer disc diffusion. In this procedure, a bacterial suspension (adjusted to match McFarland standard) is spread evenly across a Mueller-Hinton agar plate, then sterile filter paper discs impregnated with specific extract concentrations were placed on the surface. During incubation (typically 16-24 hours at 37°C), extracts diffuse into the medium creating concentration gradients. Clear zones of inhibition

formed around discs where bacterial growth was prevented, and these zones were measured and compared to established breakpoints to classify the organism as susceptible, intermediate, or resistant (Bauer et al., 1966). This method is widely performed because it is relatively inexpensive, technically straightforward, highly reproducible, and provides clinically relevant data that guides appropriate antibiotic therapy for infections, helping combat antimicrobial resistance (CLSI, 2024). Additionally, it can detect emerging resistance mechanisms and is adaptable for testing numerous antibiotics simultaneously (Jorgensen & Ferraro, 2009).

The ethanolic extract showed a good inhibition on *E. coli* and *K. pneumoniae* with the largest inhibition zone diameter was at a 100% concentration of 19 mm for *E. coli* and 15mm for *K. pneumoniae*, while the diameter of the smallest inhibition zone was at a 20% concentration of 6 mm for *E. coli* and 8mm for *K. pneumoniae* with no and less inhibition at 20% on both *E. coli* and *K. pneumoniae* respectively. The positive control used was 30µg Amikacine, forming a 22mm and 25mm inhibition zone on *E. coli* and *K. pneumoniae* correspondingly. For the E. faecalis bacteria no inhibition was observed on the different concentration, instead a better growth of the organism was observed along the disc than away from it. The negative control used was distilled water and did not show any inhibitory zones on both *E. coli* and *K. pneumoniae* as shown in Table 3. Amikacine is an antibacterial that inhibits protein synthesis by binding to the 50s ribosome. The bacteriostatic or bactericidal effect of Amikacine depends on drug concentration, type of organism, and the location of infection. The Amikacine inhibition zone formed (Tab. 1 and Fiq. 2) shows that selected Gram Negative bacteria are still sensitive to Amikacine. Both the *E. coli* and *K. pneumoniae* bacteria are sensitive to Amikacine if the inhibition zone is >15 mm.

The Aqueous extra shows no inhibition on both the selected Gram Negative bacteria on both the concentration in which all the concentration shows an inhibition zone of 6mm on all the selected pathogens as in Table 2 and Fiq. 3. This resistance pattern shows alarming and a future study is recommended on the Aqueous Extract which is commonly used within our community members.

Table 1. Antibacterial activity of extract of *Tithonia diversifolia* (Ethanolic Extract) against selected Gram negative Organisms.

Concentration (%)	*E. coli*	*K. pneumoniae*	*E. faecalis*
	Zone of Inhibition (mm) ± SD	Zone of Inhibition (mm) ± SD	Zone of Inhibition (mm) ± SD
20	6.0 ± 1.50	8.0 ± 1.82	6.0 ± 1.50
40	9.0 ± 0.34	9.0 ± 0.34	6.0 ± 1.50
60	12.0 ± 0.45	11.0 ± 0.47	6.0 ± 1.50
80	14.0 ± 0.81	13.0 ± 0.80	6.0 ± 1.50
100	19.0 ± 1.49	**15.0 ± 0.85**	6.0 ± 1.50
Positive Control	22.0 ± 0.76	25.0 ± 0.78	18.0 ± 0.72
Negative Control	0 ± 0	0 ± 0	0 ± 0

Table 2. Antibacterial activity of extract of Tithonia diversifolia (Aqueous Extract) against selected Gram negative Organisms.

Concentration (%)	*E. coli*	*K. pneumoniae*	*E. faecalis*
	Zone of Inhibition (mm) ± SD	Zone of Inhibition (mm) ± SD	Zone of Inhibition (mm) ± SD
20	6.0 ± 1.50	6.0 ± 1.50	6.0 ± 1.50
40	6.0 ± 1.50	6.0 ± 1.50	6.0 ± 1.50
60	6.0 ± 1.50	6.0 ± 1.50	6.0 ± 1.50
80	6.0 ± 1.50	6.0 ± 1.50	6.0 ± 1.50
100	6.0 ± 1.50	6.0 ± 1.50	6.0 ± 1.50
Positive Control	22.0 ± 0.76	25.0 ± 0.78	18.0 ± 0.72
Negative Control	0 ± 0	0 ± 0	0 ± 0

On comparative to the known commonly used antibiotics on the selected Gram Negative the ethanolic extract showed a good inhibition zones as those of the known antibiotic unlike the Aqueous which have poor inhibition zone as shown in Fig. 4, 5 and 6.

On Minimum Inhibitory Concentration (MIC) using the two-fold serial dilution method with a concentration of 100 mg/mL, 50.0mg/mL, 25.0mg/mL, 12.5mg/mL, 6.25mg/mL, 3.13mg/mL, 1.56 mg/mL, 0.78mg/mL, and 0.39mg/mL by use of visual inspection on the tubes for turbidity. Ethanolic

extract shows a clearance of turbidity at a concentration of 100 mg/ml on the *E. coli* pathogen, 50 mg/ml on *K. pneumoniae* and lastly 25 mg/ml on *E. faecalis* while the remaining concentration had a growth for the selected Negative Organism as shown in table 3. Aqueous extract shows turbidity in all the concentration for the selected Gram-Negative pathogens an indication of the possibility of the organism to grow as shown in table 4.

Table 3: Ethanolic extract minimum inhibitory concentration on Selected Gram-Negative Pathogens.

Concentration)	*E. coli*	*K. pneumoniae*	*E. faecalis*
	MIC RESULT	MIC RESULT	MIC RESULT
100 mg/mL	-	+	+
50.0mg/mL	+	-	+
25.0mg/mL	+	+	-
12.5mg/mL	+	+	+
6.25mg/mL	+	+	+
3.13mg/mL	+	+	+
1.56 mg/mL	+	+	+
0.78mg/mL	+	+	+
0.39mg/mL	+	+	+
Positive Control	-	-	-
Negative Control	+	+	+

Key: +; Turbidity formed, **-**; No turbidity formed

Table 4: Aqueous extract minimum inhibitory concentration on Selected Gram-Negative Pathogens.

Concentration)	*E. coli*	*K. pneumoniae*	*E. faecalis*
	MIC RESULT	MIC RESULT	MIC RESULT
100 mg/mL	+	+	+
50.0mg/mL	+	+	+
25.0mg/mL	+	+	+
12.5mg/mL	+	+	+
6.25mg/mL	+	+	+
3.13mg/mL	+	+	+
1.56 mg/mL	+	+	+
0.78mg/mL	+	+	+
0.39mg/mL	+	+	+
Positive Control	-	-	-
Negative Control	+	+	+

Key: +; Turbidity formed, **-**; No turbidity formed

From this study, secondary metabolite mixtures suspected of having an antibacterial role on the selected Gram Negative specifically the *E. coli* and *K. pneumoniae*. Ethanolic extra plays a role in antibacterial activity than Aqueous extract. Gram-Negative bacteria cell wall have a more complicated cell wall structure with two membrane layers, namely the outer membrane and the inner membrane consisting of the cytoplasmic membrane. Both membranes are separated by the periplasmic space, which also contains a degradative enzyme and as a protein transport. The Gram-negative peptidoglycan layer is thinner, causing its polarity to be lower (Dewi, 2013). The nature of the ethanolic-soluble teichoic acid shows that Gram-Negative is more polar. Therefore, polar compounds will enter the cell wall easily then destroy the polar peptidoglycan. From other study done Phytochemical test results showed that the extract of *Tithonia diversifolia* leaves contains secondary metabolites of alkaloids, flavonoids, tannins, and saponins (Karou et al., 2005). The previous research by Agidigbi & Odeyemi (2014) showed that the leave extracts of *Tithonia diversifolia* possess active components, alkaloids, flavonoids, phenols sesquiterpenes, monoterpenes, and diterpenes as active components. Alkaloids are suspected of having antibacterial activity by disrupting the constituent components of peptidoglycan in bacterial cells so that cells are not formed properly and will cause the cells death. Tannin is a polar secondary metabolite because there are hydroxyl groups and have antibacterial activity by inactivating enzymes and destruction or inactivation of the function of genetic material from bacteria. Flavonoid acts as an antibacterial by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes (Karou et al., 2005).

Conclusion and Recommendation

Based on the study results, it can be concluded that *T. diversifolia* leaf extracts demonstrate varying antimicrobial efficacy, with ethanolic extracts showing greater antibacterial activity against selected Gram-negative bacteria (*E. coli* and *K. pneumoniae*) compared to aqueous extracts. Interestingly, *E. faecalis* exhibited growth around the aqueous extract discs, suggesting possible attraction rather than inhibition. While aqueous extracts showed no antimicrobial activity, the ethanolic extracts did demonstrate some inhibition, which was measurable through MIC testing. For future research, it is recommended to investigate the specific secondary metabolites responsible for the antibacterial properties of ethanolic extracts, explore different extraction methods to enhance efficacy, test against a broader range of pathogenic bacteria, and conduct cytotoxicity studies to determine potential therapeutic applications while ensuring safety.

Declarations

Ethics approval and consent to participate: Not Applicable

Clinical Trial: Not Applicable

Consent for publication: Not Applicable

Availability of data and material

The datasets used in the current study are available upon reasonable request to the corresponding author (SOA).

Competing interests: The authors declare that there are no competing interests.

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Author Contributions

Conceptualization, MMEA and SOA; methodology, MMEA, SOA, JN and SD; resources, MMEA and SOA; writing—original draft preparation, MMEA and SOA; writing—review and editing, MMEA, SOA, JN and SD; Final editing, All authors; visualization, MMEA, SOA, JN and SD; supervision, SOA, JN and SD; project administration, SOA. All authors have read and agreed to the published version of the manuscript.

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Figures

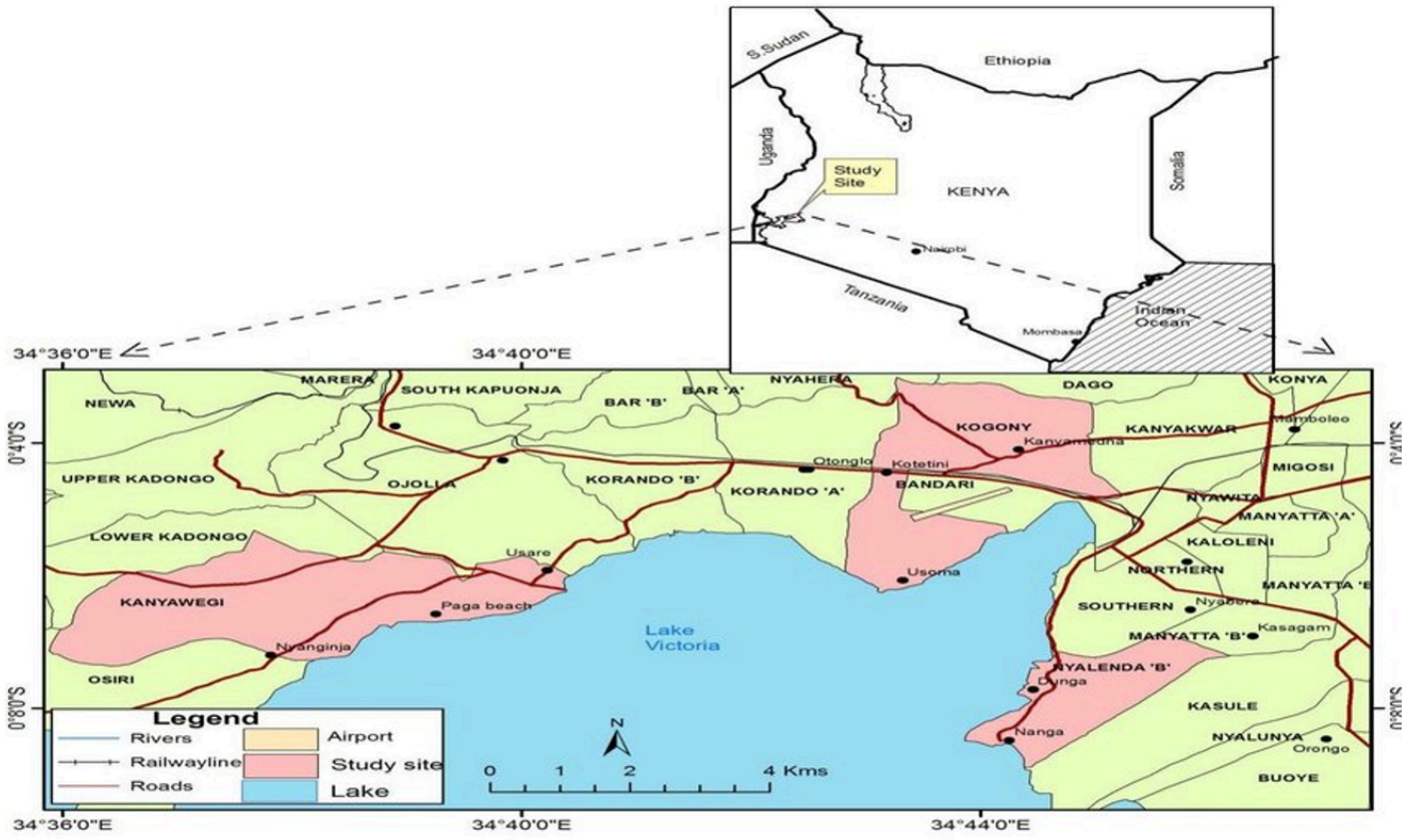

Figure 1

map of the study site (Ulalo village in Kisumu West sub-county) location.

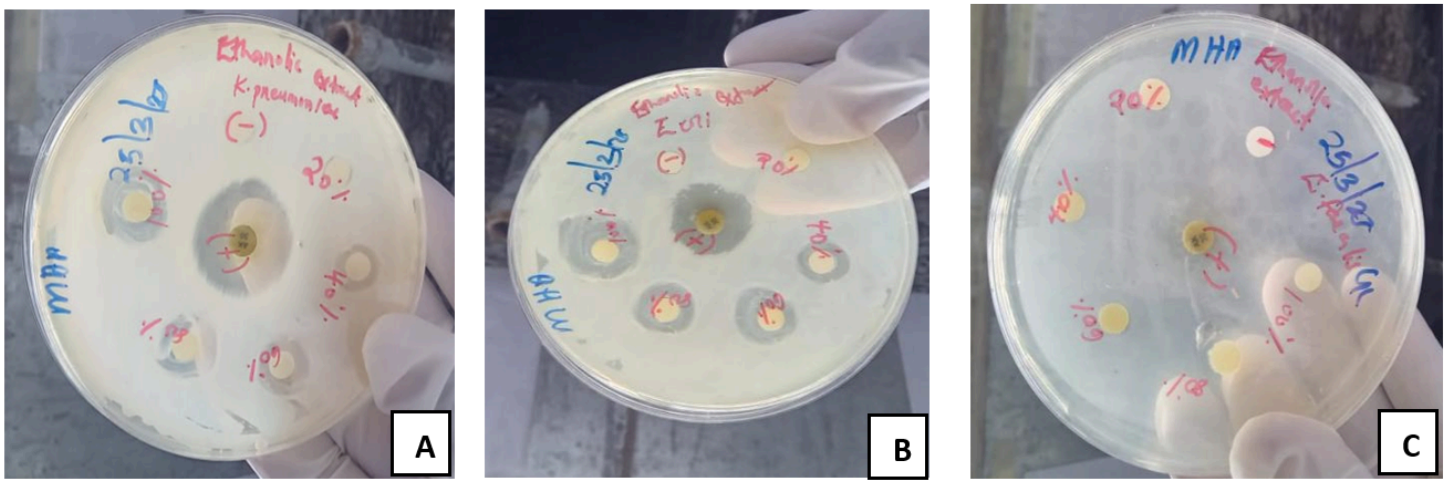

Figure 2

Antibacterial activity of extract of *Tithonia diversifolia*(Ethanolic Extract) against; **A**- *K. pneumoniae*; **B-** *E. coli* and **C**- *E. faecalis*

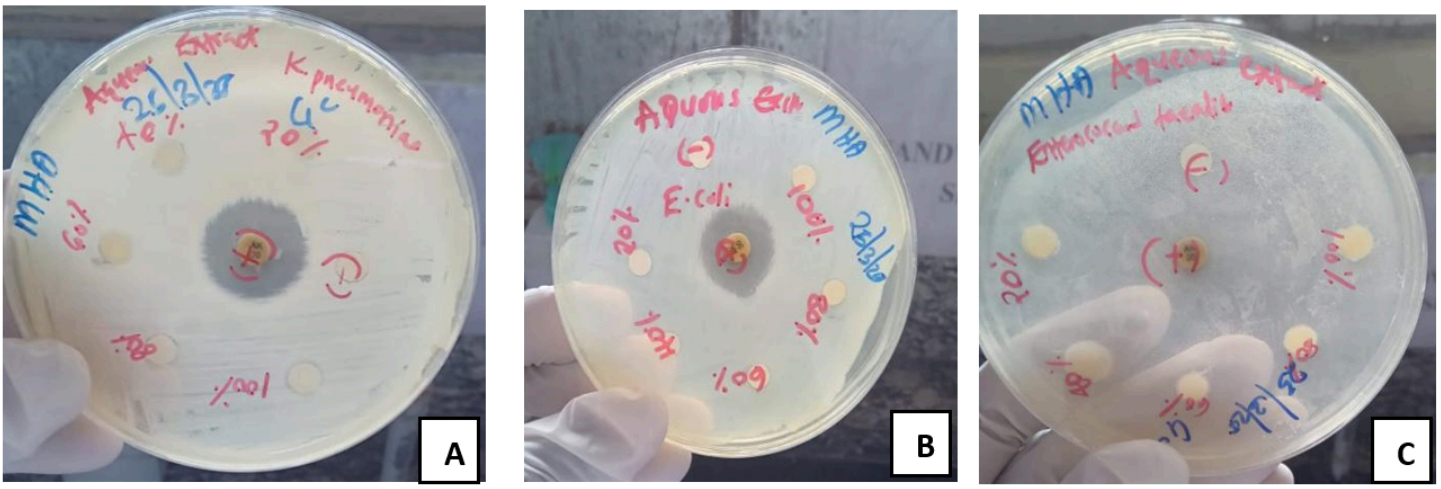

Figure 3

Antibacterial activity of extract of *Tithonia diversifolia* (Aqueous Extract) against; **A**- *K. pneumoniae*; **B**-*E. coli* and **C**-*E. faecalis*

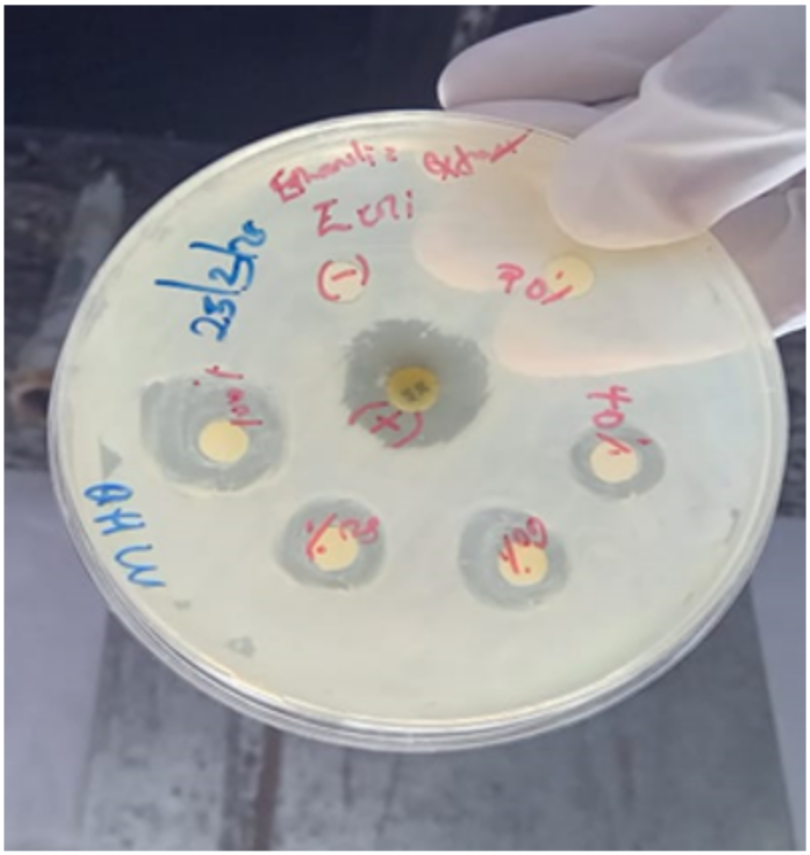

Figure 4

Ethanolic extract inhibition zone at different concentration zones.

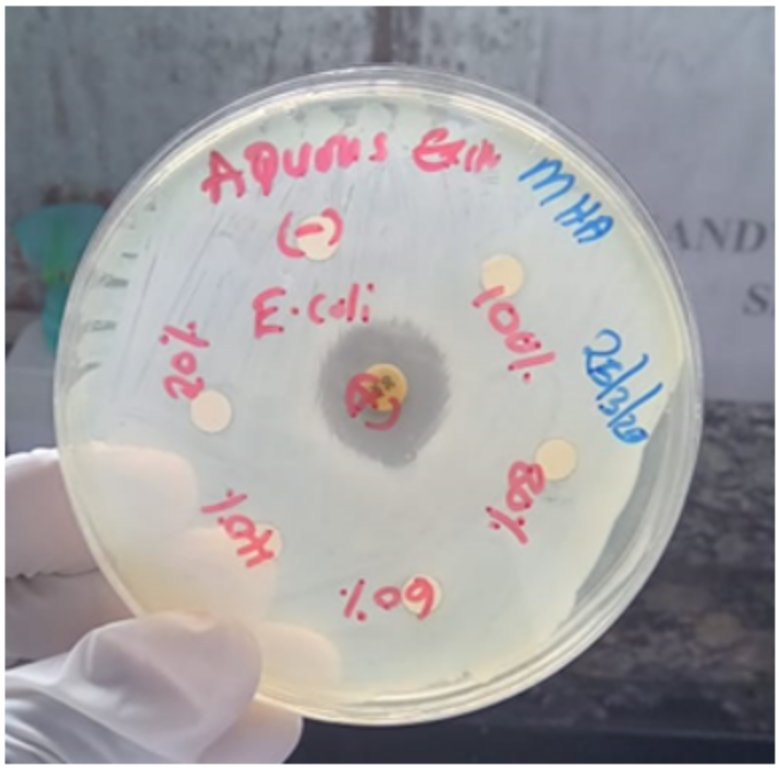

Figure 5

Aqueous extract inhibition zone at different concentration zones.

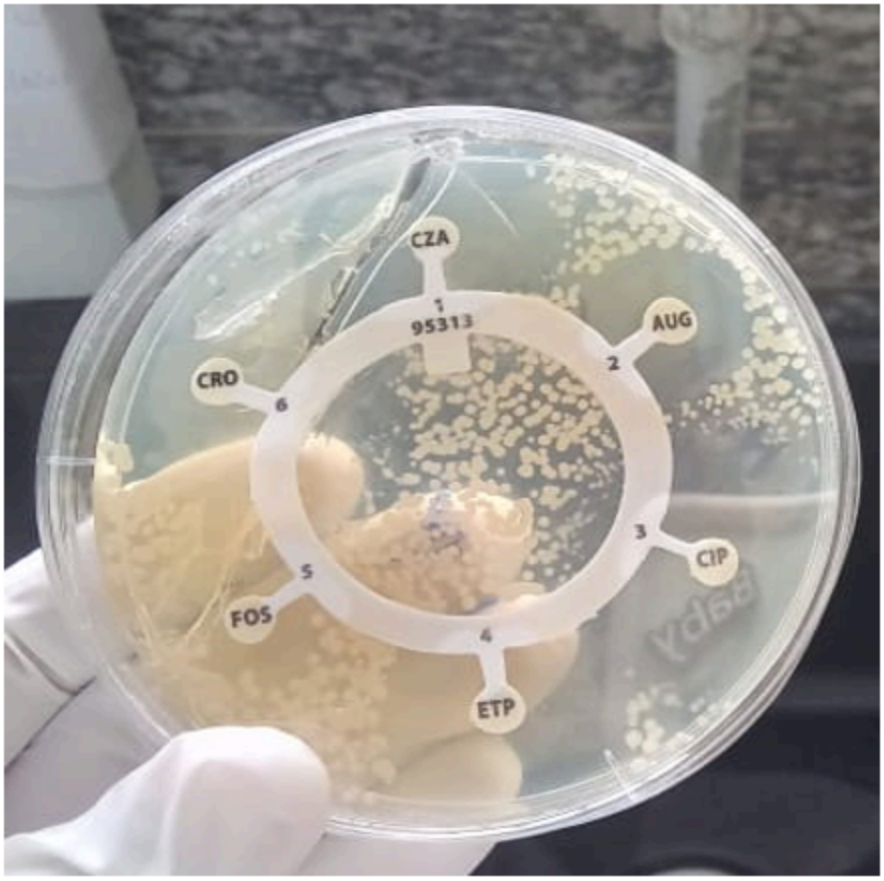

Figure 6

Known antibiotics inhibition zone at different concentration zones.