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Growth Inhibitory Effect of *Datura stramonium* and *Tamarindus indica* on Resistant Malaria Mosquito Body Surface and Gut Microbiota

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Abstract

Bacteria mediated resistance which possibly is initiated when the vectors pick insecticides through feeding, inhalation or contact of their cuticle is threatening effective malaria vector control. This study tested growth inhibitory effectiveness of crude *Datura stramonium* leaf and seed extracts, it's seed isolate F7, *Tamarindus indica* fruit pulp extracts on bacteria isolated from midgut and cuticle of *Anopheles gambiae* resistant to deltamethrin. Data was analyzed by R version 4.2.3. Seed extracts isolate F7' had superior growth inhibition efficacy comparable to only lincomycin on gram-negative bacteria of genus *Elizabethkingia, Aeromonas, Pseudomonas, Klebsiella, Proteus, Serratia, Shigella, Acinetobacter, salmonella Enterobacter*, and *Citrobacter,* bacteria isolated from the mosquito. F7 can be considered for control of microbiota induced deltamethrin resistance.

Keywords: *Datura stramonium, Tamarindus indica*, Growth inhibitor, *Anopheles gambiae*

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Introduction

Malaria elimination is yet to be achieved in Sub-Saharan African countries including Kenya where the economic burden is huge and adversely affecting the country development goals. (Hallowell *et al*., 2023, Elnour *et al*., 2022, WHO, 2023). Successful and sustainable malaria control and elimination is currently under serious challenge due to malaria parasite's resistance to antimalarial drugs and the situation is further compounded by the malaria vectors resistance to chemical insecticides which have been used in internal residual spraying and long-lasting insecticide treated nets (Omoke *et al*., 2021, Ondeto *et al*., 2021, Orondo *et al*., 2021; Owuor *et al*., 2021). A new vector resistance mechanism mediated by microbiota is a current concern threatening malaria vector control (Dada *et al*., 2018 and 2019; Barnard *et al*., 2019; Muturi *et al*., 2021, Omoke *et al.,* 2021; Xia *et al*., 2013). The aforementioned studies have reported that microbiota which are able to degrade insecticides affect vector competence of *Anopheles* mosquitoes. Some bacteria associated with resistance enhance the development of plasmodium and increase sporozoite infection of the vectors (Kabula *et al*., 2016; Minard *et al*., 2013). Malaria vector control tools targeting microbiota have been suggested Dada *et al*., 2021). Microbiota commonly isolated from gut, salivary glands, reproductive organs, exoskeleton and haemocoel have been associated with physiological changes on *Anopheles* and resistance of the vectors to insecticides (Douglas *et al*., 2015; Guegan *et al*., 2018; Wang *et al*., 2017). *Anopheles gambiae* microbiota has not been fully explored (Dada *et al*., 2018; Omondi *et al*., 2017).

Almeida and others, (2017) documented that *Streptoccocus pyrogenes* reduced deltamethrin induced mortality in *Anopheles* mosquitoes. They concluded that the gram-negative bacteria are able to protect mosquito from insecticide by synthesizing some particular toxins. Omoke *et al*., (2021), agreed with Almeida when their study associated *Streptococcus*, *Sphingobacterium*, *Lysinobacillus* and *Rubrobacter* to resistant *Anopheles gambiae*. Bacteria ecology on vectors therefore cannot be ignored if effective control tools are to be recommended (Grant *et al*., 2022; Minard *et al*., 2013; Banard *et al*., 2018; Dada *et al*., 2018 and 2019).

Ecology of bacteria on the *Anopheles* vectors is very important in resistance profiling of different species and for case filing of vector resistance studies. There is need for information on resistant mosquitoes and their microbiota. Different pyrethroid detoxifying taxa of bacteria have been identified in different studies. *Sphingobacterium*, *Lysinobacillus* and *Streptococcus* and radio-tolerant taxa *Rubrobacter* were isolated from resistant *An. gambiae* species resistant to fivefold diagnostic does of permethrin (Bonizoni *et al*., 2012; Wang *et al.,* 2021; US Presidential Initiative 2024). This study found significant differences in species composition of bacterial community of *An. gambiae* with krd-east alleles (L1014S) to those of susceptible ones. Omoke *et al*., (2021) reported 21 bacteria specific to *An. gambiae* with krd-east alleles and 16 others specific to susceptible ones with *Myxococcus* species most abundant in susceptible samples and not found in the resistant groups (Omoke *et al*., 2021). In a different study, *An. coluzzii* resistant to deltamethrin had a number of gram-

negative bacteria genera such as *Onchrobactrum*, *Stenostrophonomas* and Lysinibacillum unlike their susceptible counterparts which had less bacteria diversity and with *Asaia* and S*erratia* as the dominant genera (Pelloquin *et al*., 2021). *Lysinibacillus sphaericus* was able to detoxify 83%of cyfluthrin while using the insecticide as a source of nitrogen or carbon (Bando *et al*., 2013). From the gut of fourth instar larvae of multiple-resistant *Anopheles arabiensis*, bacteria of genus *Klebsiella*, *Staphylococcus*, *Enterobacter* and *Aeromonas* were isolated (Barnard *et al*., 2019). *An. albimanus* resistant to organophosphate insecticide (fenitrothion) were dominated by insecticide degrading bacteria of the genus, *Klebsiella*, *Acinetobacter*, *Escherichia*, and *Salmonella* (Dada *et al*., 2019). *An. albimanus* larvae and adults, exposed to pyrethroid insecticides indicated alterations in their cuticular and internal microbiota (Dada *et al*., 2019; Dada, *et al*., 2018). The study exposed *An. albimanus* adults to alphacypermethrin and permethrin and found *Pseudomonas fragi* and *Pseudomonas agglomerans* abundant in the treated samples as compared to the non-treated samples that had large numbers of *Acinetobacter* and *Serratia* (Dada *et al*., 2018). Mid gut of *An. stephensi* resistant to temephos had *Pseudomonas*, *Aeromonas*, *Exiguobactrium* and *microbactrium* (Soltani, *et al*., 2017).

In the family *Enterobacteriaceae*, the gram-negative bacteria are genera *Serratia*, *Ewingella*, *Enterobacter,* and *Klebsiella*. *Actenobacteriae* family are genera *Actenobacter* and *Asaia*. *Flavobacteriacea* are genera *Elizabethkingia* and *Chryseobacterium*. Mid-gut of *Anopheles*, *Serratia*, *Asaia*, *Acinetobacter*, *Aeromonas*, and *Pantoea* bacteria have been isolated. In the salivary glands and reproductive tissue, *Asaia*, *Serratia*, *Acinetobacter*, *Pseudomonas*, and *Klebsiella* which are associated with insecticide detoxification have been isolated (Wu *et al*., 2006). These studies have documented evidence of bacteria mediated resistance which possibly is initiated when the vectors pick insecticides through feeding, inhalation or contact of their cuticle. The insecticide degrading bacteria make the vectors non-responsive to insecticides adversely affecting malaria vector control. It has been speculated by researcher that this type of resistance is metabolic in nature (Barnard, *et al*., 2019; Dada *et al*., 2019). Significant microbial diversity had been associated with susceptible mosquito (Ochomo *et al*., 2023; Dada *et al*., 2019; Barnard, *et al*., 2019) than resistant ones. Changes in susceptibility to insecticides therefore can be improved by increasing bacteria diversity on the vectors (Djihinto, *et al*., 2022). Vector control tools targeting mid mosquito microbiota are recommended.

Datura stramonium has been researched in the laboratory for medicinal considerations for its antibacterial activity. In Pakistan, chloroform extracts from fruits impeded the growth of *P. aeruginosa* and leaf extracts inhibited the growth of *K. pneumoniae* (Bhakta *et al*., 2013). Banso and others documented the inhibitory effect of *D. stramonium* on gram-positive bacteria. Ethanolic, leaf extracts at 25% W/V obstructed the growth of *P. aeruginosa*, *Klebsiella pneumoniae,* and *E. coli*. (Hu *et al*., 2014). In other studies (Al-Snafi, 2017; Saab, *et al*., 2020; Gupta *et al*., 2021), 2.5, 1.25, and 0.75 mg/l concentrations of *D. stramonium* extracts inhibited the growth of *S. haemolytus*, *S. aureus*, *Shyngella dysentriae*, *Bacillus cereus*, *P. aeruginosa*, *K. pneumonia,* and *Escherichia coli*. *Enterobacter* and *Micrococcus lutius* have also been inhibited (Al-Snafi, 2017; Rehman, *et al*., 2022). Even though *D. stramonium* has dose-dependent antibacterial properties on

human disease-causing bacteria, further research needs to be done to establish the correct human dosage to avoid human poisoning as the plant is highly poisonous. Information on the effects of ethyl acetate extracts on deltamethrin resistant mosquito cuticle and mid gut bacteria is not well understood so as to confirm its effectiveness on bacteria-mediated resistance in malaria vectors.

Tamarindus indica also known as tamarind is a leguminous indigenous tree that grows in the tropics (ICRAF 2019). *Tamarindus indica* seeds and leaves are both edible and medicinal (Arora, 2017; Mbunde, 2013). Sesquiterpenes from the plant have been documented to exhibit significant toxicity against mosquito larvae (Adebote, 2015) while flavonoids and triterpenes have adulticidal activity and oviposition deterrence. According a study (Arora *et al*., 2017), methanolic leaf extracts of *T. indica* contain alkaloids, terpenoids, flavonoids, phenols and saponins that have insecticidal activity. There is scarcity of data in mosquitocidal potential of T. indica. A lot of research has focused on the medicinal potential of the fruit pulp (Fagbemi *et al*., 2022; Abukakar *et al*., 2008; Adeola *et al*., 2010; Bhadoriya *et al*., 2011). Most of the studies with T. indica extracts used leaves (Sooyking *et al*., 2022). Different solvents extract different quantities and qualities of compounds of *T. indica* (Kaewnarin *et al.,* 2014) while pre-treatment before extraction determine phenolic contents of the extracts (Leng *et al*.,2017). Most phenolic contents of *T. indica* are extracted with methanol while both phenols and flavonoids are better extracted using ethyl acetate (Kaewnarin *et al*., 2014) The extracts are antimicrobial (Abubakar *et al., 2008*). Data on effectiveness on deltamethrin resistant *Anopheles gambiae* mosquitoes is scarce.

Material and Methods

Plant material collection and preparation

Datura stramonium seeds and leaves used in this study were collected between June and August 2019, from Marura open-air market in Kimumu Eldoret, and around also Milimani Hospital is Maili Nne within the environs of Eldoret town in Uasin-Gishu county in Kenya. Eldoret is 2090 meters above sea level. Maili Nne is located latitude 0.5182 and longitude 35.2717. The fruits and leaves of *Datura stramonium* were collected from the roadsides and farms one of which seemed to be a grazing field for livestock, typical with cow-dung all over. The plants were identified by experts of the Department of Botany, University of Eldoret, Kenya. Only the mature fruits of *Datura stramonium*, coloured between yellow and yellow-green were collected with an assumption of them being matured. Tamarind fruit was bought from Eldoret at Marura market. The fruits and leaves were dried under shade for 7 days at the University of Eldoret in Chemistry Laboratory 3. The fruits of *Datura stramonium* were then pounded with a wooden rod to split them open so the seeds could be separated. Chaff was removed from the seeds using a sieve and the seeds dried under shade for another 4 days. The fruits were packed and kept in a paper box and sealed. One kilogram of the dried seeds, leaves and fruit pulp was ground into powder using an electric blender, sieved, and stored in the same laboratory ready for extraction.

Extraction of secondary plant metabolites from the crushed *Datura stramonium* seeds, leaves and *Tamarindus indica* pulp.

Extraction of phytochemicals was done using standard maceration method with slight modification (Nortje *et al*.,

2022) at the University of Eldoret Chemistry department laboratory 3 using standard procedures. Ethyl acetate was considered for its moderate polarity. I kilogram of crushed *D. stramonium* leaves, seeds powder or *Tamarindus indica* each was soaked in ethyl acetate in a 5-liter volumetric flask for 72 hours to achieve maximum extraction. The extracts were then filtered using filter paper, and a funnel, into another volumetric flask. Filtrate was concentrated using a rotary evaporator at 40 degrees centigrade. Crude extracts in liquid form were kept in a dark cupboard for 12 hours to evaporate the solvent completely. The liquid seed extracts were transferred into amber bottles well capped, covered with aluminium foil, and kept in a dark place inside a cupboard. The crude extracts were used in bio-assays on deltamethrin-resistant adult *Anopheles gambiae* mosquitoes, compound isolation through column chromatography and for phytochemical screening.

Plant secondary metabolites' screening from the crude extracts

Crude extracts were extracted sung standard procedures (Nortje *et al*., 2022). A small amount of the crude extracts was individually screened for tannin, phenol, saponin, terpenoid, alkaloid, steroid, and flavonoid. To test for saponin, 5 ml of individual extracts were diluted in 10 ml of water and agitated for 10 minutes by hand. The formation of a layer of foam that lasted for over 5 minutes indicated the presence of saponin. For tannin test, 2g of each of the crude extracts were mixed with 2.5 ml water, heated in a water bath, and filtered using a filter paper. 2 drops of ferric chloride solution were added to the filtrate. A dark green or blue-black colour was to indicate the presence of tannin. Phenol test procedure used was; 2 ml of each of extracts one at a time, was mixed with 2 ml of ferric chloride. Colour change to dark green was to be observed as positive. For terpenoid test, 2ml chloroform was added to 0.5g of extracts and 3 ml concentrated sulphuric acid. A reddish-brown colour between inter-phase showed the presence of terpenoid. For steroid test; 1 g of each of the extracts was added 2 ml of acetic anhydride then 2 drops of sulphuric acid. Colour changed to violet or blue or red or green indicating presence of steroids. To test for alkaloids, 3 ml of 2% sulphuric acid was added to crude extracts and warmed for 3 minutes. Solution was filtered using a filter paper. Two drops of, Mayer's reagent was added to the filtrate. Cream precipitate indicated the presence of alkaloids. To test for flavonoid, 10% hydroxide was used to dissolve 0.5 g of extracts and 2 ml hydrochloric acid was added. A yellow solution that turns colorless meant positive results.

Isolation of bio-active compounds in the extract solutions

Datura stramonium seeds as crude extracts were taken through column chromatography with silica gel to isolate active compounds using a procedure described by Kowalska and Sejewicz, (2022) with some modifications (Kowalska and Sejewicz, 2022). Column was run using Hexane and ethyl acetate using gradient purification. Silica gel absorbs less polar compounds first then highly polar compounds and so separate them. The slurry of crude extracts in the test tube was poured onto the column and allowed to equilibrate at room temperature.

The sample was then filtered with a 0.45 µm nylon membrane before it is loaded into the column to ensure a longer column life. The column was regenerated by washing the Sephadex LH-20 gel with 2-3 column volumes of eluent, followed by re-equilibration. All fractions that looked

alike were put together and the eluates were checked for differences using thin layer chromatography plates (TLC). Further purification of the collected eluates was done using comparative TLC. When seven spots were yielded, all the 7 different eluates labeled F1- F7 were kept in amber bottles covered with aluminum foil used in bio-assays.

Bio-assays with plant extracts and dissection of mosquitoes

Mosquitoes body parts to be used for bacterial cultures were dissected from the midgut, wings and legs (cuticle) of randomly sampled deltamethrin resistant colony. During dissection, the mosquitoes were surface sterilized with 70% ethanol solution then rinsed three times in sterile phosphate buffered saline (PBS). Midgut were dissected under stereo microscope at X10 magnification. Sterile forceps were used and each mosquito was dissected one at a time in 50 micro-liters of sterile PBS, whole knocked down mosquitoes were stored in labeled vials and stored at -20 degrees centigrade for processing (CDC, 2018).

To culture mid gut and cuticular bacteria isolated from *Anopheles gambiae* mosquito for bio-chemical and molecular identification. Samples of mosquitoes were transported to University of Eldoret in dry ice from KEMRI Kisian Kisumu for bacterial culturing in Botany lab 1 and for molecular work at the Fish Genetics and genomics laboratory. Culturing of the sample were done using standard procedure for MaCckonkey agar, which is selective to gram-negative bacteria especially *Enterobacteriae.*

Samples were inoculated one at a time on the plated Macckonkey agar using streak plate dilution method to isolate colonies. Culture was kept at 28^0C under aerobic conditions for 24 hours. Further sub-culturing and streaking was done three times to ensure single colonies. Macckonkey was preferred for its ability to differentiate lactose fermenting and non-lactose fermenting bacteria species. The expected results were pink to rose- red colour would suggest *E. coli, Enterobacter* while *Klebsiella* bacteria would be mucoid pink. *Salmonella, Elizabethkingia* and *Shigella* species would be colourless, media colour or even orange to amber and *Pseudomonas* would have irregular colourless to pink colonies.

Bio-chemical tests of cultured bacteria isolated from deltamethrin resistant *An. gambiae*.

Standard procedure for catalase test using hydrogen peroxide, Sulphur indole motility test, triple sugar iron agar test, and citrate tests were used to identify the genus of the bacteria (Bettey *et al*., 2007). Catalase tests identify *Enterobacteriae*. A drop of hydrogen peroxide was added to the bacteria on a slide and bubble showed oxygen is produced and the bacteria was catalase positive. *Staphylococcus, Bacillus, Enterobacter, Klebsiella, Shigella, Proteus*, *Salmonella, Serratia, E. coli* and *Pseudomonas* would be positive while *Streptococcus* and *Enterococcu*s would be negative. Triple sugar iron agar test was done using standard procedures to test for glucose fermentation, hydrogen sulphide and gas production. Splitting of the agar or agar pushed to the top showed gas (carbon dioxide and oxygen production). *Enterobacter, Salmonella, Klebsiella* and *E. coli* would produce gas. Glucose fermentation would cause the change on agar yellow to red. Black precipitate would show production of hydrogen sulphide (H2S). *Salmonella* would have red slant, yellow butt, gas and black butt. Yellow slant and black, gas positive, blackening would suggest *Citrobacter*, a red slant and yellow butt without blackening but with gad

produced would be *Proteu*s were expected to be black. Shigella would have gas produced, red slant, yellow butt and no blackening while *Enterobacter* would have yellowing of agar, gas and no blackening. Escherichia coli would have yellow agar, gas and no blackening. Elizabethkingia would also show blackening, gas positive. Simmons citrate agar test turning blue would mean no *E. coli* and *Shigella*.

Molecular confirmation of bacteria isolated from deltamethrin resistant *Anopheles gambiae* mosquito mid gut and cuticle

ZymoResearch® Quick-DNA Fungal/Bacteria miniprep kit for fifty bacteria samples was used. The kit had fifty ZR BashingBead™ lysis tubes (0.1 and 0.5mm), genomic lysis buffer¹ 100 mls, DNA pre-wash buffer² 15ml, g-DNA wash buffer 50mls, DNA elution buffer 10mls, fifty Zymo-Spin™ III filters, fifty Zymo-Spin™ IICR columns and 150 collection tubes. The kit was used alongside a Thermofisher equipment: microcentrifuge, vortex, Bead-mill 24 (cell disrupter). The manufactures protocol was used with some modifications.

First 100g of bacteria was weighed and suspended in 200ml of nuclease free water then added to the a ZR Bashing-beads lysis tubes (0.1 and 0.5mm). The tubes with the samples were labeled both on the lid and sides to denoted the actual samples they were carrying. The samples were processed at a high speed for 3 minutes. The procedure was repeated. Secondly the ZR BashingBead™ lysis tube (0.1 and 0.5mm) were centrifuged at 10000 x g for 2 minutes. 400 µl of supernatant was transferred to a corresponding labeled Zymo-Spin™ III filters in a collection tube with the same corresponding label and was centrifuged for 8000 x g for 1 minute. 1200 µl of Genomic lysis buffer¹ was added to the filtrate in each of the collection tubes in the previous step. Then, 800 µl of each of the mixtures from the collection tubes in the previous procedure was added to labeled Zymo-Spin™ IICR columns in corresponding labeled collection tubes and centrifuged at 10000 x g for 2 minutes. the flow was discarded and the process repeated. To clean the DNA, 200ul of DNA pre-wash buffer was added to each of the Zymo-Spin™ IICR columns with samples in new labeled corresponding collection tubes and centrifuged 10000 x g for 1 minute. Further purification was done by adding 500ul of g-DNA wash buffer to each of the Zymo-Spin™ IICR columns with samples and centrifuged for 1 minute. To get eluate with DNA, the Zymo-Spin™ IICR columns with samples were transferred to labeled sterile 1.5 ml micro-centrifuge tubes (eppendoff tubes) and 100ul of DNA elution buffer ² warmed at 65 ℃ for 15 minutes was added directly to the column matrix and allowed to cool for 5 minutes. The mixture was centrifuged at 1000 x g for 5 minutes then 10000 x g for 30 seconds to elute DNA into the micro-centrifuge tubes. Pure DNA for each sample collected in the Micro-centrifuge tubes were placed in tube racks and place on ice for agarose gel electrophoresis and all samples positive or negative were kept at -20^{0}C and used in polymerase chain reaction (PCR) processing.

Polymerase chain reaction primers used in this study was 27F forward primer of length 20 and moles 37.24, AGAGTTTGATCMTGGCTCAG. Forward primer mix was prepared following the manufactures instruction to add 372.4 µl of nuclease free water to make 100 µm stock solution. The reverse primer was 1492R with length of 22 mole 31.89 and sequence TACGGYTACCTTGTTACGACTT. The reverse primer mix was prepared by adding 318.94µl of nuclease free water. Before mixing the pellet, primer tubes were

centrifuged at 10000xg for 1 minute to settles pellets at the bottom for the pellets can be displaced. Woking solution was diluted from the stock solution using nuclease free water and he concentrated stock kept at -20°C. Polymerase chain reaction mixture (PCR) mixture 25 µl was prepared by pipetting 11. 75 µl nuclease free water into a sterile micro-centrifuge tube, 6.25 µl of master mix was added, then 1 µl of forward primer then 1 µl of reverse primer to make 20 µl. For many samples the quantities were multiplied by the number of samples to be processed per experiment. 5 µl of individual bacteria DNA sample was added to the 20 µl of PCR mix in a corresponding labelled PCR tube. The mixture was then placed in the PCR machine and PCR performed using the following program of initial denaturation at 96°C for 10 minutes, 35 cycles of further denaturation at 94°C for 4 minutes, annealing temperature of 58°C for 30 seconds, elongation at 72°C for 45seconds and a final expansion at 72°C for 10minute and hold at 4°C for 10 minutes. To confirm amplification, Agarose gel was prepared using, agarose powder and Tris -borate-Edta (TBE) buffer (P.H 8.3) was used to separate nucleic acids. 1xTBE buffer was reconstituted from 10x TBE by measuring 100ml of 10xTBE which was then added to 900mls of distilled water to make 1000ml of 1x TBE buffer. 2 g of agarose was added to 200 mls 1xTBE, the mixture was heated in a microwave for 3 minutes to completely dissolve the agarose. Pre-cast dies of 30ul of safe-view was added. The mixture was allowed to cool for five minutes and poured into gel trays fitted with gel combs and allowed to solidify for 20 minutes. Gel trays were placed into the gel tanks with well at the starts of the arrows in the tank. 1xTBE was filled into the gel tank up to the recommended level. The set up was checked and corrected for any bubbles. Positive control, 3ul of ladder was mixed with 1 µl of loading die. Samples were prepared by first labeling all the tubes to denote respective sample codes. 2ul of die was pipetted into each of the tubes. 4ul of each of the samples were picked one at a time and added to the die in the respective tubes and mixed thoroughly. The well combs were then pulled out and 1ul of each sample was loaded unto the well using a pipette each at time. A sterile pipette tip was used to load each of the samples. Once fully loaded, the electrophoresis machine was set at 100 voltage, 150 current and 60 minutes time. The gel was them observed in the DNA gel electrophoresis UV ultraviolet transilluminator and photos of results saved. Polymerase Chain Reaction (PCR) for the bacteria DNA Then 5µl portion of the amplicons were mixed with 3µl of loading die and tested for DNA amplification using the gel electrophoresis at 2% for 1and ½ hours and amplification confirmed on the gel analyzer and photo taken and saved. All positive amplicons were kept at -20°C for sequencing.

Bacteria growth inhibitory tests.

Mueller Hinton agar (M173-500G) was used in this experiment. The agar contains300gms of Hm beef infusion, 17.5 gms acicase, 1.5 gms starch and 17gms agar with PH of 7.3-+ 0.1. Mueller Hinto agar was recommended for bacteria growth inhibition tests. To prepare the agar, thirty-eight grams of Mueller Hinton agar (M173-500G) was suspended in 1000ml distilled water. The mixture was heated to boiling on a hot place to completely dissolve the media. The mixture was then autoclaved at 121°C for 15 minutes to ensure it is sterile. The mixture was allowed to cool 45-50°C and poured into respective labelled petri dishes. 50gms of each sample of bacteria cultures were suspended in 50mls of distilled water in sterile test tubes. Once

the agar had solidified 20mls of Bactris suspended in water was inoculated onto the agar and spread evening using a plastic streaking stick. Ampicillin 10mcg (AMP), Oxacillin 1mcg (OX), Amoxyclav or Amoxycillin/clavulenic acid 30mcg or 20/10 mcg (AMC), vancomycin 30mcg (VA), erythromycin 15mcg (E), doxycycline hydrochloride 30mcg (DO), Ceflazidime 30mcg (Caz), co-trimazole or sulphur-trimethropin 25mcg (COT), lincomycin 2mcg (L), penicillin-G 1 unit (P), minocycline 30mcg (MI), methicillin 5mcg (MET), lincomycin 2mcg (L), chloramphenicol 30mcg (C), *T. indica* fruit pulp extracts (TP), *D. stramonium* see extracts (DS), *D. stramonium* seed isolate (F7) and leaf extracts and all extracts mixed together at 1000ppm (A) were tested for inhibition effectiveness. The bacteria were chosen for their effectiveness in increasing mortality in deltamethrin resistant anopheles arabiensis mosquitos (Banard *et al*., 2019). Paper punch was used to make small round pieces of filter papers to be treated with the extracts. The petri dishes were labelled with corresponding treatments except for commercial antibiotics which had been labelled. The round filter papers were place in labelled petri dishes with F7 (F), *D. stramonium* seed (DS) Leaf (DL) and *T. indica* (T) fruit pulp extracts. Inoculated petri- were zoned for plant extracts using the same labels and incubated at 28°C for 24 hours. Antibiotics were placed in different inoculated petri -dishes with corresponding to the bacteria labels already treated with extracts and incubated for 24hours at 28°C. Inhibition diameter (areas clear of bacteria of had no bacterial growth) was measured for each sample using a 30 cm ruler and recorded for each bacteria sample. Zero clear area meant no inhibition and so resistance to the treatment.

Data analysis

Data collected was stored in Microsoft Excel Spread sheet programme, flash disc and hard copy in a laboratory book. Stored data was organized and analysed by R application version (4.3.2).
To analyze secondary metabolites of the extracts, descriptive statistics was used to analyze phytochemical data.

Bacteria inhibition diameters data were recorded in millimetres. Means of inhibition diameter were computed. The analysis of variance (ANOVA) and Tukey's honesty significance difference (HSD) test was used to analyse and compare the mean inhibition diameter of effectiveness of different treatments in the dataset. Inhibition means were compared using box plot. Gel photos of confirmed PCR amplicons were saved in a flash disk.

Ethical procedures

Research permit was acquired from National Commission for Science Technology and Innovation (NACOSTI) and ethical approval reviewed Baraton University, Scientific Ethical Review Committee. Informed verbal consent was solicited as explained by WHO, 2013 from local authority (chiefs, sub-chiefs and village elders) and from all individual participants and field owners of farms from which larvae were collected.

Results

Effect of *D. stramonium* and *T. indica* extracts on bacteria isolated deltamethrin resistant *Anopheles gambaie* mosquitoes.

The biochemical test results for bacteria culture showed different bacteria species. MacConkey agar which allows for the growth of gram-negative *Enterobacteriae* showed both lactose fermenting which were pink or red and

non-lactose which were greenish gold, Cream, pale and translucent. Of all the bacteria cultured, 32 samples were non-lactose fermenters and had red slant yellow butt, 22 were glucose fermenters with red slant and yellow butt while yellow slant and yellow but had 6 samples that fermented all the sugars. Wings and legs treated with F7 were not positive for bacterial cultures. The cultured bacteria were all positive for citrate test.

The bacteria from microscopy were rode like *(Bacillus*) and some were oval (*coccobacilli).* Catalase test positive confirmed presence of all genus *Bacillus*. *Elizabethkingia, Aeromonas, Pseudomonas, Klebsiella, Shigella*, *Proteus, Enterobacter, Serratia, Acinetobacter, Citrobacter* and *Salmonella* were identified using bio-chemical tests.

Elizabethkingia rode shaped under microscope showed cream colour in macckonkey to imply it is non lactose fermenting, catalase, indole and citrate test positive while negative for hydrogen sulphide, gas and motility. *Aeromonas* was rode-shaped under the microscope, catalase, indole and hydrogen sulphide positive. *Pseudomonas* had red slant and red butt, gas and hydrogen sulphide negative. *Klebsiella w*as pink on Macckonkey, had non-motile and indole negative, had red slant, red butt, catalase, hydrogen sulphide, and gas positive. *Proteus* had red slant and yellow butt, gas positive and hydrogen sulphide negative. *Shigella*, had red slant and -yellow butt, gas positive and hydrogen sulphide negative. *Enterobacter* had yellow slant and yellow butt, no hydrogen sulphide and gas positive.

Table 1: Resistant malaria mosquito body surface and internal microbiota isolated from deltamethrin resistant *Anopheles gambiae* mosquitoes.

Cat	TSI	H2S	Gas	Citrate	Indole	Motility	MaCckonkey	GENUS
+	-	-	-	+	+	-	Cream	*Elizabethkingia*
+	-	-	-	+	-	+	Amber	*Aeromonas*
+	+	+	+	+	-	-	Pink/Red	*Klebsiella*
+	+	+	-	+	+	-	Amber	*Proteus*
+	-	-	+	+	-	+	Pink	*Enterobacter*
+	+	-	-	+	+	-	Pale pink	*Shigella*
+	+	-	+	+	-	+	Red or Pink	*Serratia*
+	+	-	+	+	-	+	Pink	*Actinetobacter*
+	-	-	-	+	-	-	Green-gold	*Psedunomas*
+	+	+	-	+	-	+	Pale	*Salmonella*
+	-	+	+	+	+	+	Pale	*Citrobacter*
Cat (Catalase test), TSI(Tripple sugar Iron agar test) H2S (hydrogen sulphide)								

Serratia showed red slant and yellow butt, no hydrogen sulphide, gas was produced. *Acinetobacter,* looked oval on microscopy and pale coloured on Macckonkey agar, had yellow slant and yellow butt, catalase and citrate test positive, no hydrogen sulphide, no indole non-glucose fermenter.

Citrobacter were pale and smelly yellow slant and yellow butt, hydrogen sulphide, gas, indole, catalase and citrate tests positive, and motile. *Salmonella* was positive for citrate, catalase, triple sugar iron test, motile but indole negative (Table). From the cuticle were

Enterobacter, Klebsiella, Aeromonas and *Shigella* only while were all were identified from mid-guts.

Figure 1: (a) Triple Sugar Iron Test (b) Citrate test

Figure 2: Sulphide Indole Motility test

DNA by ZYMORESEARCH kit extraction was confirmed using 1% agarose gel electrophoresis (Figure 3)

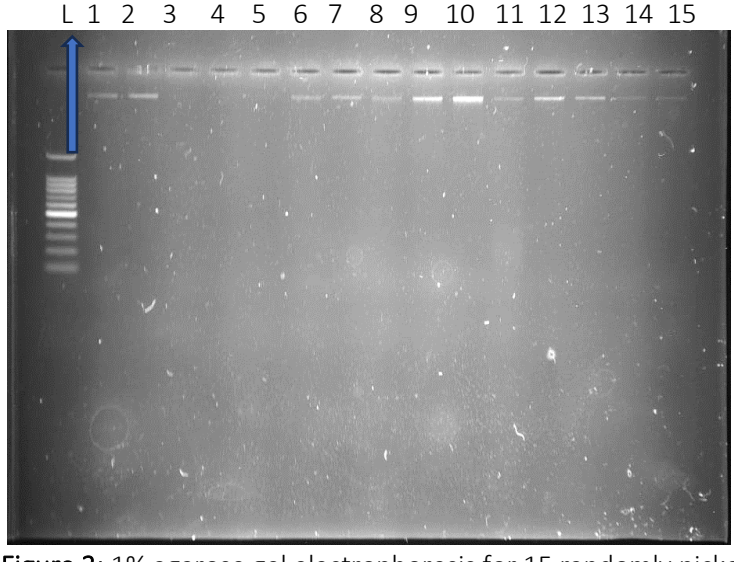

Figure 3: 1% agarose gel electrophoresis for 15 randomly picked samples

Bacteria gene confirmatory test by polymerase chain reaction (PCR) for four samples using a universal primer 27f and 1492r showed 1.5 kb amplified 16s rDNA of two samples DNA clearly positives and faintly positives.

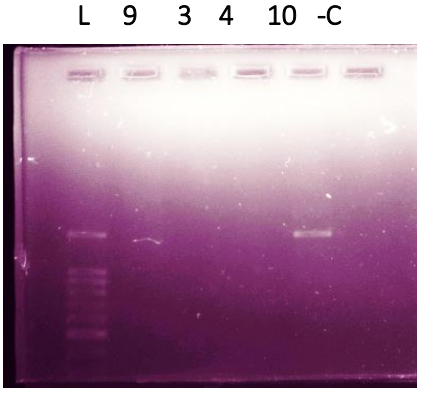

Figure 4: PCR amplicon gel electrophoresis results for 3, 4, 9 and 10

The PCR done for samples in figure 4.26 labeled 3, 4, 9 and 10 had PCR amplicon gel electrophoresis results in figure 4.26 confirming amplification of bacteria genes in the samples. Sample 3 and 4 were cuticles treated with F7 extracts and did not show growth of bacteria.

Comparison of extracts' F7, DL, DS and TP effectiveness in inhibiting growth of bacteria isolated from deltamethrin resistant *Anopheles gambiae* mosquitoes.

The average effectiveness of the extracts at 1g/100ml (F7, TP, DS, and DL) was analyzed. The highest mean diameters for each extract were as follows: F7: 10.78, A: 10, TP: 9.8, DS: 7.95 and DL: 8.8. From the means, F7 was the most effective extract, followed by a mixture of all extracts at 1g/100ml water, TP, DL, and DS. The higher mean diameter for F7 indicates its superior ability to inhibit bacterial growth across the extracted samples (Figure 5).

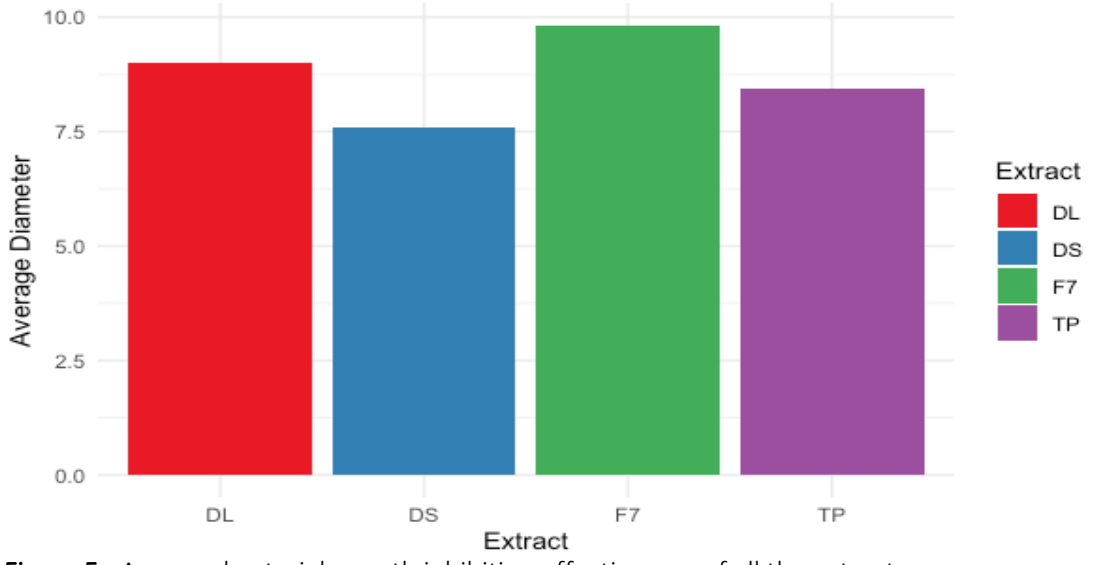

Figure 5: Average bacterial growth inhibition effectiveness of all the extracts

Comparison of Antibiotics Effectiveness on bacteria isolated from deltamethrin resistant *Anopheles gambiae* cuticle and mid gut.

The average effectiveness of the antibiotics used when analyzed based of the diameters of their area of inhibition. Antibiotics inhibition ability rated Lincomycin as the most effective, followed by co-trimazole, ceflazidime, and doxycycline (Figure 6). The effectiveness of these antibiotics in inhibiting the growth of cuticular and mid gut *Anopheles gambiae* bacteria is significantly higher compared to others. This suggests their potent antibacterial properties on mosquito microbiota associated with *Anopheles gambiae* from Chwele in Bungoma.

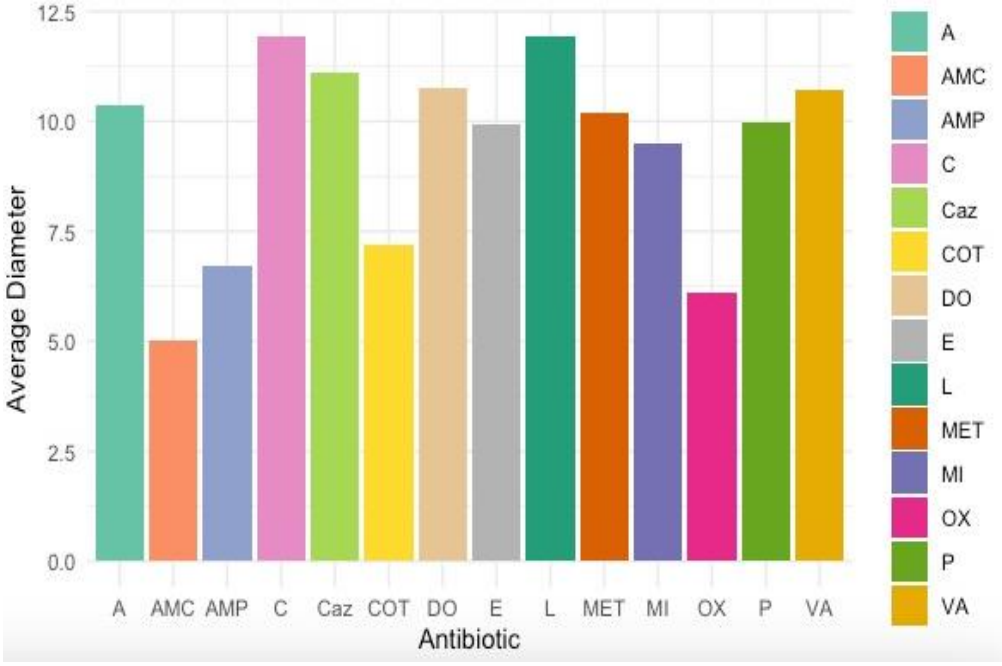

Figure 6: Mean growth inhibition effectiveness of antibiotics on bacteria isolated from deltamethrin resistant *Anopheles gambiae* mosquitoes

Ampicillin 10mcg (AMP), Oxacillin 1mcg (OX), Amoxyclav or Amoxycillin/clavulenic acid 30mcg or 20/10 mcg (AMC), vancomycin 30mcg (VA), erythromycin 15mcg (E), doxycycline hydrochloride 30mcg (DO), Ceflazidime 30mcg (Caz), co-trimazole or sulphur-trimethropin 25mcg (COT), lincomycin 2mcg (L), penicillin-G 1 unit (P), minocycline 30mcg (MI), methicillin 5mcg (MET), lincomycin 2mcg (L), chloramphenicol 30mcg (C), *T. indica* fruit pulp extracts (TP), *D. stramonium* see extracts (DS), *D. stramonium* seed isolate (F7) and leaf extracts and all extracts mixed together at 1000ppm (A) .

Comparison of growth inhibition effectiveness between Extracts and Antibiotics on bacteria isolated from deltamethrin resistant *Anopheles gambiae* mosquitoes

Comparison of mean growth inhibition diameters of the different bacteria revealed that antibiotics, with mean effectiveness of 10.54, outperformed extracts which had a mean of 9.11.

Resistant of bacteria isolated from deltamethrin resistant *Anopheles gambiae* mosquitoes to Treatment with extracts and antibiotics.

From the analysis, the study identified bacteria isolated from deltamethrin resistant *Anopheles gambiae* mosquitoes which were resistant to treatments and had no inhibition zone. In such samples all treatments resulted in a diameter of 0, indicating no inhibition of bacterial growth. The resistant samples were twenty-seven, Bact 3 (a, b), Bact 6 (a, b), Bact ,7a, 7b, 7c, Bact10b, Bact 11(a, b, c), Bact 12(a, b), Bact 13 (ss), Bact 14 (a, b, c), Bact 15, Bact16 (a, b, c), Bact 18 and Bact 19 (a, b, c). Summary of resistant species identified were *Elizabethkingia (10b), Aeromonas (*19b,11*) Pseudomonas* (10), *Klebsiella* (16C), *Shigella* (16b, 10c,

11a), *Proteus* (8), *Enterobacter* (11b), *Serratia (*7,11), *Salmonella (13), Enterobacter (3, 18), Acinetobacter (17)* and *Citrobacter* (9) (Table 1). These samples showed complete resistance to a number of tested extracts and antibiotics. *Elizabethkingia* (15*)* was resistant to ampicillin, amoxicillin, vancomycin and the extracts of *D. stramonium* seed, leaves and *T. Indica* fruit pulp. *Aeromonas (*19b,11*)* was resistant to lincomycin, metronidazole and extracts of *D. stramonium* seed, leaves and *T. Indica* fruit pulp. *Pseudomonas* (10) was resistant to ampicillin, oxacillin, erythromycin and extracts of *D. stramonium* seed, leaves and *T. Indica* fruit pulp. *Klebsiella* (16C) was resistant to ampicillin, oxacillin, erythromycin and extracts of *D. stramonium* seed and *T. Indica* fruit pulp, *Shigella* (3b) and *Enterobacter* (11b), were resistant to doxycycline and ceflazidime only. *Proteus* (8), was resistant to erythromycin and *T. indica* fruit pulp extracts. *Serratia (*7,11c) was resistant the largest number of treatments ampicillin, oxacillin, erythromycin, vancomycin, doxycycline, erythromycin and ceflazidime and all the extracts except F7. *Salmonella (*13), was resistant to clotrimazole. *Acinetobacter (17)* and *Citrobacter* (9) were to ampicillin and to leaves and *T. indica* fruit pulp extracts.

Among the extracts, *D. stramonium* seed oil isolate (F7) was the most effective, while *D. stramonium* seed extract was the least, highlighting the variability in their chemical composition and effectiveness. For antibiotics, lincomycin stands out as the most effective, followed by chloramphenicol, co-trimazole and ceflazidime, underscoring their potency and potential as first-line treatments (Figure 7). Overall, antibiotics generally had better inhibitory effectiveness on the bacteria than extracts, confirming their targeted and potent action against bacterial infections.

Figure 7: Comparison of inhibition differences of the sample number 14

Box plot comparison of inhibition differences of the samples

Ampicillin, chloramphenicol, ceflazidime, doxycycline, lincomycin and vancomycin had larger inhibition diameter with some bacteria species. F7 seven clearly shows larger inhibition area diameter of above 10 effectiveness comparable to many commercial antibiotics and larger than some as well. (Figure 8).

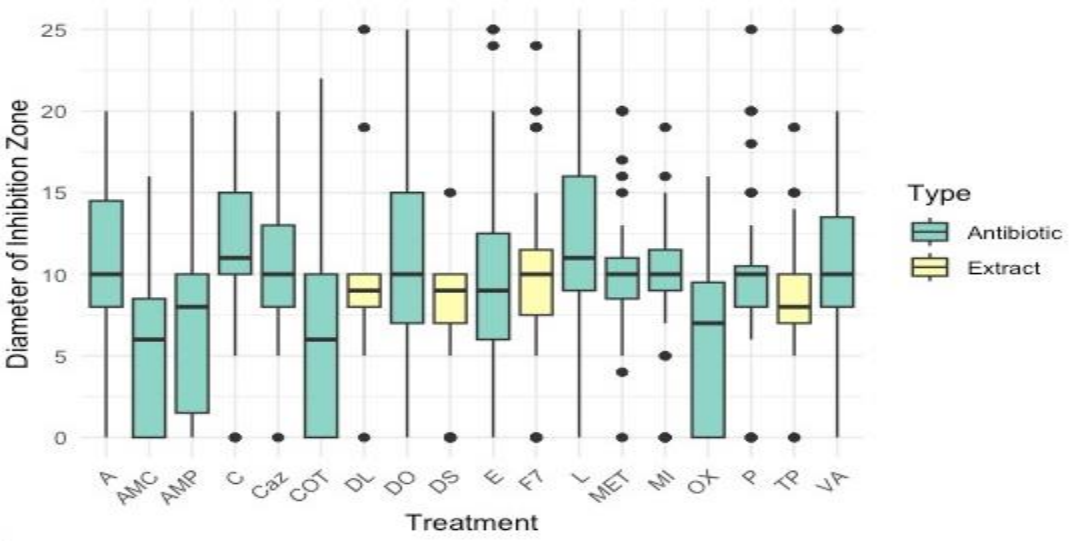

Figure 8: Box Plot Visualization of extracts and all antibiotics

Tukey's HSD Test Results for means of inhibition area diameters

The results for each pairwise comparison included the differences in means (diff), the lower and upper bounds of the 95% confidence interval (lwr and upr), and the adjusted p-value (p adj) (Table 2). When bacterial growth inhibition effectiveness of amoxicillin was compared for antibiotics and extracts it was noted that ampicillin (diff 1.710, p<0.05), co-trimazole (diff=2.197, p=0.917), oxacillin (diff=1.095, p= 0.999) Significant differences were noted with chloramphenicol (diff=6.915, p=0.000), Ceflazidime (diff 6.095, p=0.000), doxycycline- hydrochloride (diff=5.762, p=0.000), metronidazole (diff=5.172, p=0.001), lincomycin (diff=6.6941, p= 0.000, minocycline (diff 4.505, p=0.0146), penicillin-G (diff=4.967, p=0. 003), erythromycin (diff=4.941, p= 0. 003), and vancomycin (diff=5.7, p<0.05). Amoxicillin was the most ineffective as compared to all the treatments.

However, when compared to the plant extracts, *D. stramonium* leaves extracts (diff=3.922, p=0.064), *D. stramonium* seed extracts (diff =2.582, p= 0. 744) and *T. indica* pulp (diff=3.428, p=0.234) had not significant relationship. Significant differences were noted with F7 (diff 4.812, p= 0. 005) and all the extracts mixed together at 1000ppm (diff=5.351, p= 0.000) (Table 2).

Comparison of mean inhibition diameter of ampicillin and all antibiotics showed significant differences with chloramphenicol (diff=5.205, p=0.001**), ceflazidime (diff=4.385, p=0.0211352*), doxycycline -hydrochloride (diff=4.051, p=0.0548349 and lincomycin (diff=5.231, p=0.001**). All the rest had no significant mean differences; erythromycin (diff=3.231, p=0.335), metronidazole (diff=3.462, p=0.219), Oxacillin (diff=0.615, p=1), vancomycin (diff=4.01, p=0.061), minocycline (diff=2.795, p=0. 611), minocycline (diff=2.795, p=0. 611), co-trimazole (diff=0.487, p=1), and penicillin -G (diff=3.256, p=0.320). The observation was same for all the extracts *D. stramonium* leaf *(*diff *=2.282, p=0.8885401),*), *D. stramonium* seed (diff=0.872, p=0.999), F7 (diff =3.103, p=0.411), all extracts at 1000ppm (diff=3.641, p=0.502), and *T. indica* fruit pulp (diff=1.718, p=0.992) (Table 2).

Effectiveness of chloramphenicol (C) against other antibiotics showed no significant difference with ceflazidime (Caz) (diff= -0.821, p= 0.999), doxycycline hydrochloride (DO) (diff=-1.154, p =0.999), erythromycin (diff=-1.974, p=0.968), metronidazole (p=-1.744 and p=0.990), minocycline (diff=-2.410, p=0.835), Penicillin-G (diff=-1.949,p=0.971), lincomycin (diff=0.0256, p=1), and vancomycin (diff=-1.1945, p=0.999). Co-trimazole (diff= - 4.718, p=0.007**) and Oxacillin (p=-5.821, p=0.000), had significant differences. While amongst the extracts, there was no significant difference. *D. stramonium* leaf (diff=-0.633, p=0.527) *D. stramonium* seed extracts (diff=-4.333, p=.0246), F7 (diff=-2.102, p=0.942), all extracts mixed together at 1000ppm (diff= -1.564, p=0.977) and *T. Indica* pulp (diff=-3.487, p=0.208), Table 2.

Ceflazidime mean inhibition diameter mean was compared with all the other treatments and there was no significant differences with co-trimazole (diff= -3.897, p=0.082), doxycycline hydrochloride (diff= -0.33 p= 1), erythromycin (diff= -1.974, p =0.999), Lincomycin (diff =-0.026, p= 0.999), methicillin (diff= -1.744, p=0.999, minocycline (diff=-2.41, p=0.997), doxycycline hydrochloride (diff=-5.821, p=0.999), vancomycin (diff=-1.195, p=1) and extracts *D. stramonium* leaf (diff =-2.102, p= 0.942), seed (diff= -3.512, p=0.198), F7 isolate (diff= -2.102, p = 0.999),), all extracts mixed together (diff= -1.564, p=0.999), and *T. indica* pulp (diff=-3.487, p= 0.693). Only oxacillin had significant (diff=-5.821, p= 0.002*) (Table 2).

When the mean inhibition diameter of co-trimazole was compared to other extracts the results reveal that there were no significant differences in their effectiveness with all the extracts *D. stramonium* leaves (diff =1.795, p= 0.988), *D. stramonium* seed oil (diff= 0.385, p>0.05), F7 (diff= 2.615, p= 0.725), mixture of all extracts (diff =3.154, p=0.379) and *T. indica* (diff =1.231, p=0.999), and most antibiotics doxycycline hydrochloride (diff =3.564, p=1), erythromycin (diff= 2.744, p=0.644), minocycline (diff =2.30, p= 0.878), oxacillin (diff=-1.103, p= 0.999), penicillin-G (diff= 2.769, p=0.628), and vancomycin (diff =3.523, p=0.193). Lincomycin (diff= 4.744, p=0.007**) and methicillin (diff =2.974, p=0.493) had significance difference (Table 4.21). Doxycycline hydrochloride (DO) also had no significant mean difference except with oxacillin (OX) with p =0.008* and mean difference of - 4.667 (Table 4.21). Erythromycin mean diameter of inhibition was rated with that of F7 (diff=-0.128, p=1), lincomycin (diff= 2, p=0.963), metronidazole (diff= 0.231, p= 1), minocycline (diff= -0.436, p=1), all extracts mixed at 1000ppm (diff=0.41, p=1), oxacillin (diff=-3.846, p=0. 092), penicillin – G (diff=0.026, p=1), *T. indica* fruit pulp (diff= -1.513, p=0.998), vancomycin (diff=0.779, p=0.999) (Table 4.21). There was no significant inhibition mean differences between areas inhibited by erythromycin (E) and all the other treatments. When mean diameters of inhibition of lincomycin (L) was compared to methicillin (diff=-1.769, p=0.989), minocycline (diff =-2.436, p=0.822), all extracts mixed together (diff -=1.590, p= 0.997), *T. indica* pulp (diff =-3.513, p=0.198), penicillin -G (diff -=1.974, p= 0.968) and vancomycin (diff =-1.221, p=0.999) and no significant difference in effectiveness was observed (Table 4.21). Only oxacillin had a significant difference of (diff=-5.846, p=0.000***). Minocycline (MI) mean diameters of inhibition compared to that a mixture of all extracts at 1000ppm (A) (diff=0.846, p= 0.999), oxacillin (OX) (diff=-3.410 p= 0.2423186),

penicillin-G (P) (diff= 0.462, p= 1), *T. indica* pulp extracts (TP) (diff =-1.077, p= 0.999), and vancomycin (VA) (diff=1.215, p= 0.999) (Table 4.21) and all had no significant difference. Metronidazole means inhibition diameter compared to that of other had no difference with minocycline (diff =-0.667, p=1), all extracts mixed together at 1000ppm (diff=0.179, p=1), penicillin-G (diff=0.462, p=1), *T. indica* fruit pulp extracts (diff= -1.077, P=0.991) and vancomycin (diff= 0.549, p=1) (Table 4.21). but only Oxacillin had a slightly significant superior effectiveness (diff=-4.077, p=0.051) to metronidazole in inhibiting bacteria growth. When oxacillin mean inhibition diameter (table 4.21) was compared to that of penicillin-G (diff =3.872, p=0.087), *T. indica* fruit pulp (diff =2.333, p=0.868) and vancomycin (diff =4.625, p=009*), only vancomycin inhibition effectiveness had a significant difference. Penicillin-G inhibition diameter when compared to *T. indica* (diff =-1.538, p=0.998) and vancomycin (diff =0.754, p=0.999) had no mean differences. Vancomycin compared to *T. indica* extracts (diff =2.929, p=0.885) (Table 2) and all other treatments had no significant differences in effectiveness $p<0.05$.

T. indica fruit pulp extracts, vancomycin, and penicillin -G and vancomycin and. *T. indica* fruit pulp compared showed no significant differences in their bacterial growth inhibition ability.

Effectiveness of D. *stramonium* leaf extracts when compared to all the treatments showed no significant difference with all treatments; doxycycline hydrochloride (diff=1.769, p =0.989), Erythromycin (Diff=0.949, p=0.9999971), Lincomycin (diff= 2.949, p=0.5097502, methicillin (diff=1.179, p=0.999 and minocycline (diff=0.513, p=10). Compared to extracts, *D. stramonium seed* (diff= -1.410, p= 0.999), F7, *D. stramonium* seed oil isolates, (diff=0.821, p =0.999). Generally, there was no significant differences between the diameters of inhibition areas of antibiotics and leaf extracts (Table 4.21). There were no significant differences in the means of inhibition diameters of all the treatment compared to those inhibited by *D. stramonium* seed extract except for lincomycin which had p value of 0.022*(diff=4.359) (Table 4.21). This implies that there are no differences in the effectiveness of this extracts and all the other treatments but only for lincomycin. *D. stramonium* essential oil isolate (F7) mean inhibition diameter was compared to that of lincomycin (diff= 2.128, p=0.936), metronidazole (diff =0.359, p=1), minocycline (diff= -0.308, p=1), mixture of all extracts at 1000ppm (diff =0.538, p=1), oxacillin (diff= -3.718, p=0.126), penicillin -G (diff =0.154, p=1), *T. indica* fruit pulp (diff =-1.385, p=1), vancomycin (diff=0.908, p=0.999) (Table 4.21). F7 and all the treatments had no difference in bacteria growth inhibition ability. The potential of F7 to inhibit bacterial growth was as good as the medically approved and commercially sold antibiotics. When all extracts mixed together at 1000ppm was compared with that of Oxacillin (diff =-4.256, p=0.030), penicillin -G (diff= -0.385, p=1), *T. indica* pulp extracts (diff= -1.923, p=0.974) and vancomycin (diff=0.369, p= 1) (Table 2), there was no significant difference in their effectiveness to inhibit bacterial growth.

Table 2: Antibiotics and extracts inhibition bioassays
p>0.05 *, p>0.01**, p>0.0001***

Treatment	AMC	AMP	C	Caz	COT	DL	DO	DS	E	F7	L2	MET	MI	A	OX	P	TP
AMP	1.71																
C	6.915***	5.205**															
Caz	6.095***	4.385	-0.821														
COT	2.197	0.487	-4.718**	-3.897													
DL	3.992	2.282	-0.633	-2.102	1.795												
DO	5.761**	4.051	-1.154	-0.333	3.564	1.769											
DS	2.582	0.872	-4.333	-3.512	0.385	-1.410	-3.179										
E	4.941**	3.231	-1.974	-1.154	2.744	0.949	-0.821	2.359									
F7	4.812**	3.103	-2.102	-1.282	2.615	0.821	-0.949	2.231	-0.128								

L2	6.941**	5.231**	0.026	0.846	4.744*	2.949	1.179	4.359*	2.000	2.128							
MET	5.172**	3.462	-1.744	-0.923	2.974	1.179	-0.590	2.590	0.231	0.359	-1.769						
MI	4.505*	2.795	-2.41	-1.589	2.308	0.513	-1.256	1.923	-0.436	-0.308	-2.436	-0.667					
A	5.351*	3.641	-1.564	-0.744	3.154	1.359	-0.410	2.769	0.410	0.538	-1.590	0.179	0.846				
OX	1.095	-0.615	-5.821***	-5*	-1.103	-2.897	-4.667*	-1.487	-3.846	-3.718	-5.846***	-4.077	-3.410	-4.256			
P	4.967*	3.256	-1.949	-1.128	2.769	0.974	-0.795	2.385	0.026	0.154	-1.974	-0.205	0.462	-0.385	3.872		
TP	3.428	1.718	-3.487	-2.667	1.231	-0.564	-2.333	0.846	-1.513	-1.385	-3.513	-1.744	-1.077	-1.923	2.333	-1.538	
Av	5.72***	4.01	-1.195	-0.374	3.523	1.728	-0.041	3.138	0.779	0.908	-1.221	0.549	1.215	0.369	4.625*	0.754	2.292

Ampicillin 10mcg (AMP), Oxacillin 1mcg (OX), Amoxyclav or Amoxycillin/clavulenic acid 30mcg or 20/10 mcg (AMC), vancomycin 30mcg (VA), erythromycin 15mcg (E), doxycycline hydrochloride 30mcg (DO), Ceflazidime 30mcg (Caz), co-trimazole or sulphur-trimethropin 25mcg (COT), lincomycin 2mcg (L), penicillin-G 1 unit (P), minocycline 30mcg (MI), methicillin 5mcg (MET), lincomycin 2mcg (L), chloramphenicol 30mcg (C), *T. indica* fruit pulp extracts (TP), *D. stramonium* see extracts (DS), *D. stramonium* seed isolate (F7) and leaf extracts and all extracts mixed together at 1000ppm (A)

Discussion

Similar studies but with different plants extracts have been done by (Omoke *et al*., 2021; Berhanu *et al*., 2019). The results of this study showed that the commercial antibiotics with a mean inhibition diameter of 10.54, had a general better inhibition ability than the extracts at 1g/100ml with a mean inhibition diameter of 9.11 (Figure 8). While the present study experimented with biochemically identified bacteria genus, other similar studies have been done with bacteria of known species (Asrar *et al*., 2020; Komakech *et al*., 2019; Fagbemi *et al*., 2022). In the study by Asrar and others (2020) just like the present study amoxiclav 30mcg was the least effective followed by oxacillin 1mcg and amoxicillin 20 mcg. Fagbemi *et al*., 2019 experimented with methanolic extracts of *T. indica* fruit of low phenolic content and reported inhibition of Plesmionas *shigella* ATTCC 15903 and *Bacillus pulmillus* ATTCC which were the most sensitive. Another study reported best inhibition of *Klebsiella pneumoniae* (18.75mg/ml) followed by *S. aerius* (9.38mg/ml) (Goanar *et al*., 2024). These bacteria have been associated with resistant *Anopheles gambiae* mosquitoes in other studies (Ondeto *et al*., 2021; Berhanu *et al*., 2019). This present study confirms that 1g/100ml of the *D. stramonium* seed isolate F7 had better inhibition effectiveness than amoxyclav $p>0.005$ followed by the mixture of all extracts which had $p>0.0007$. Amoxiclav had the least inhibition effectives (Table 2). There is scarcity information about such comparative studies of the crude extracts even though their effectiveness in inhibition of both gram-negative and gram-positive bacteria have been reported (Komakech *et al*., 2019; Pu *et al* 2010). This study report for the first time that the *D. stramonium* isolated essential oil F7 had the best effectiveness with no significant differences with those of commercial antibiotic and all the other extracts. The results from the experiments can inform the nullification of the assumption that the extracts of D. *stramonium* seed and leaf, F7 and *T. indica* fruit pulp extracts has no inhibitory effect on microbiota of deltamethrin resistant Anopheles gambiae mosquitoes. Banard *et al.,* (2019) study on the contribution of gut bacteria to insecticide resistance and the life history of *Anopheles arabiensis* reported that gram-negative bacteria *Streptococcus* pyrogenes reduced deltamethrin-induced mortality. The study exposed the mosquitoes to antibiotics and reported that antibiotic treatment increased deltamethrin-induced mortality in the treated samples. The effectiveness of F7 can be attributed to its ability to negate deltamethrin resistance in *An. gambiae* from Chwele. F7 possibly changed bacterial community and diversity in the treated samples making them weak and consequently knockdown.

There was interest in knowing the bacteria community on the mosquitoes. The biochemical results on the bacteria identified 11 genera: *Elizabethkingia, Aeromonas, Proteus, Enterobacter, shigella, Klebsiella, Salmonella, Acinetobacter, Citrobacter, Pseudomonas* and *Serratia* were isolated from midgut and cuticle of *Anopheles gambiae* from Chwele. These bacteria have been isolated from *An. gambiae by* other researchers who associated them with insecticide resistance (Ondeto *et al.,* 2021; Banard *et al.,* 2019; Dada *et al*., 2019, Berhanu *et al*., 2019). The present study agrees with earlier studies which reported that these genera of bacteria are resistant to commercial grade antibiotics (Kamfer *et al*., 2011; Betty *et al*., 2007). Elizabethkingia for instance has been reported to be resistant to ampicillin, streptomycin, chloramphenicol and tetracycline (Kamfer *et al*., 2011). The

present study reports not only its resistance to the mentioned antibiotics but also vancomycin. The growth of number of bacteria isolated in the present study has been inhibited by antibiotics and plant extracts. This study therefore agrees with earlier reports that *Elizabethkingia, Aeromonas, Proteus, Enterobacter, shigella, Klebsiella, Pseudomonas, Acinetobacter, Salmonella, Citrobacter and Serratia* are associated with resistant strains of *An. gambiae* mosquitoes (Ondeto *et al.,* 2021; Banard *et al.,* 2019; Dada *et al*., 2019). Both extracts and commercial grade antibiotics have been used to inhibit growth of *Elizabethkingia* (Wong *et al*., 2020; Baldini *et al*., 2014; Gomes *et al*., 2017; Cirimotich *et al*., 2011; Bahia *et al*., 2014; Diamini *et al*., 2020; Mesfin *et al*., 2012; Fagbemi *et al*., 2019); *Aeromonas (*Soltani *et* al., 2017); *Klebsiella (*Diamini *et al*., 2020; Dada *et al*.,2018 and 2019, Wu *et al*., 2006; Gendrin *et al*., 2013; Wong *et al*., 2020; Al-Snafi ,2017; Rehman *et al*., 2022; Bhakta *et al*., 2013; Arruda et al., 2021); *Pseudomonas (*Hu *et al*., 2014; Arruda *et al*., 2021; Wu *et al*., 2006; Cirimotich *et al*., 2011; Soltani *et al*., 2017; Bahia *et al*., 2014; Feng *et al*., 2021; Soni *et al*., 2012; Takhi and Quinten, 2011; Dada *et al*., 2018 and 2019; Mesfin *et al*., 2012; Fagbemi *et al*., 2019) and *Shigella* (Banard *et al.,* 2019; Dada *et al.,* 2019).

Although the role of the isolated bacteria from deltamethrin the resistance of *Anopheles gambiae* from Chwele has not been fully explored for control purposes. Many studies have associated the bacteria identified in the present study with insecticide resistance in *Anopheles* mosquitoes. *Serratia, Aeromonas, Pseudomonas, Actenobacte*r and *Klebsiella* have been associated with insecticide detoxification (Wong *et al.,* 2020; Diamini *et al.,* 2020; Soltani *et al.,* 2017; Arruda *et al.,* 2021; Feng *et al.,* 2020; Dada *et al.,* 2018; Wang *et al.,* 2021). In this study *Serratia* species was the most resistant to antibiotics even though its growth was inhibited by F7. Owing to its being associated with resistance, there is a possibility of this bacteria contributing to resistance to deltamethrin in Chwele. Effectiveness of *D. stramonium* and *T. Indica* extracts to inhibit the growth of *Proteus, Enterobacter, Acinetobacter* and *Serratia* has not been fully understood. *(*Wu *et al*., 2006; Gendrin *et al*., 2013; Diamini *et al*., 2020; Sharma *et* al., 2021; Bahia *et al*., 2014; Cirimotich *et al*., 2011; Diamini *et al*., 2020; Pelloquin *et al*., 2021; Wu *et al*., 2006; Gendrin *et al*., 2013; Wong *et al*., 2020; Fagbemi *et al*., 2019; Komakech *et al.,* 2017). , This study reported the inhibition effectiveness of ethyl acetate extracts of *T. indica* fruit pulp, *D. stramonium* seed, leaves and novel isolate F7 against commercial antibiotics resistant strains of bacteria *Proteus , Enterobacter, Acinetobacter* and *Serratia* isolated from mid gut and cuticle of deltamethrin resistant *An. gambiae* mosquitoes. F7 therefore is a potential bio-control tool for deltamethrin resistant *Anopheles gambiae* with both knock down and bacteria mediated resistance that when fully explored can supplement other control measures in an integrated vector management set up.

Conclusion and Recommendation

Gram-negative bacteria of the genera *Elizabethkingia, Salmonella, Aeromonas, Proteus, Enterobacter, shigella, Klebsiella, Pseudomonas, Actenobacter, Citrobacter* and *Serratia*, which were isolated from the mid-gut and cuticle of deltamethrin-resistant *Anopheles gambiae*, have been identified as insecticide-detoxifying genera and exhibited resistance to commercial standard antibiotics, with the exception of the F7 isolate from *Datura stramonium*

seed. The F7 isolate demonstrated high effectiveness in inhibiting bacterial growth, suggesting its potential to manage deltamethrin resistance mediated by microbiota in the mid-gut and cuticle of resistant *An. gambiae*. Hence, there is need for further research on the mosquito microbiome, particularly species identification through sequencing.

References

Abukakar, M. G., Ukwuani, A. N., & Shehu, R. A. (2008). Phytochemical screening and antibacterial activity of Tamarindus indica pulp extract. *Asian Journal of Biochemistry*, *3*(2), 134-138.

Adeola, A. A., Adeola, O. O., & Dosumu, O. O. (2010). Comparative analyses of phytochemicals and antimicrobial properties of extracts of wild *Tamarindus indica* pulps. *African Journal of Microbiology Research*, *4*(24), 2769-2779

Almeida, L. G., Moraes, L. A., Trigo, J. R., Omoto, C. & Consoli, F. L. (2017). The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation. *PloS one* 12, e0174754.

Al-Snafi E.A. (2017). Medical importance of *D. fastuosa* (syn: *D. metel*) and *D. stramonium* – A review. *IOSR Journal of pharmacy* (e) - *ISSN*: 2250-3013, (p) - *ISSN*: *2319-4219*. *Volume 7*, *Issue 2 Verson*. *1* (*Feb 2017*), *PP*.43-58

Arruda, A., Ferreira, G., Júnior, A., Matos, N., Carvalho, T., Ozaki, L., Stabeli, R. and Batool, Aamana & Batool, Zahra & Qureshi, Rahmatullah & Raja, NaveeD. (2021). Phytochemicals, Pharmacological Properties and Biotechnological Aspects of Highly Medicinal Plant: *Datura stramonium*. *Journal of Plant Sciences.* 8. 29.

Asrar, M., Bakht, I., Rasool, B., Hussain, S., Hussain, D. and Javed, Z. (2023). Efficacy and toxicity of different plant extracts over the period of time in *Bracon hebetor* (Say) (*Hymenoptera*: *Braconidae*). Heliyon. 2023 Oct 26;9(11):e21631.

Baldini, F., Segata, N., Pompon, J., Marcenac, P., Shaw, W. R., Dabiré, R. K., Diabaté, A., Levashina, E.A., & Catteruccia, F. (2014). Evidence of natural *Wolbachia* infections in field populations of *Anopheles gambiae*. *Nat Commun*. Jun 6;5: 3985.

Bando, H., Okado, K., Guelbeogo, W., Badolo, A., Aonuma, H., Nelson, B., Fukumoto, S., Xuan, X., Sagnon, N., & Kanuka, H. (2013). Intra-specific diversity of *Serratia marcescens* in *Anopheles* mosquito mid-gut defines *Plasmodium* transmission capacity. *Scientific reports*. 3. 1641. 10.1038/srep01641

Barnard, K., Jeanrenaud, A. C. S. N., Brooke, B. D., & Oliver, S. V. (2019). The contribution of gut bacteria to insecticide resistance and the life histories of the major malaria vector *Anopheles arabiensis* (*Diptera*: *Culicidae*). Sci. Rep. 2019; 9:9117.

Batool, A., Batool, Z., & Qureshi, R., & Raja, N. (2020). Phytochemicals, Pharmacological Properties and Biotechnological Aspects of Highly Medicinal Plant: *Datura stramonium*. *Journal of Plant Sciences*. 8. 29. 216637269

Bazaoui, A., Bellimam, M. & Soulaymani, A. (2012). Tropane Alkaloids of *Datura innoxia* from Morocco. *Zeitschrift für Naturforschung C*, *67*(1-2), 8-14.

Berry, C. (2012). The bacterium, *Lysinibacillus sphaericus*, as an insect pathogen. *J. Invertebr. Pathol*, 109.

Bettey A. F., Daniel, F. S., & Alice, S. (2007). Bailey and Scott's Diagnostic Microbiology. Weissfeld, 12th Ed 2007, Publisher Elsevier.

Bhadoriya, S. S, Ganeshpurkar, A, Narwaria, J., Rai G, & Jain, A.P. (2011). *Tamarindus indica*: extent of explored potential. *Pharmacogn. Rev.*;**5**(9):73– 81.

Bhakta, G. & Subedi, L. (2013). A review on the pharmacological and toxicological aspects of *Datura stramonium* L. *Journal of Integrative Medicine*. 11. 73-79. 10.3736/jintegrmed2013016.

Bharadraj, R. & Kamble, D. (2017). To study Extraction and Characterization of plant Poison Alkaloid. Atropine. *Forensic Science*, 7(3)

Bharti, V. & Ibrahim, S. (2020). Bio-pesticides production, production and application systems. 9 (10):3931-3946.

Bonin, E., Avila, V. D., Carvalho, V. M., Cardoso, M. A. P., Matos, A. M., Ramos, A. V. G., Cabral, M. R. P., BaLCoqui, D. C., Sarragiotto, M. H., Filho, B. A. A., & Prado, I. N. D. (2023). Study of chemical constituents, antioxidants and antimicrobial activities of *Tamarindus indica* L. seed. *Journal of food science*, *88*(11), 4639–4652.

Bonizzoni, M., Afrane, Y., Dunnn, W.A., Atieli F.K. & Zhou, G. (2012). Comparative transcriptome analyses of deltamethrin-resistant and -susceptible *An. gambiae* mosquitoes from Kenya by RNA-Seq. *PLoS ONE*. 7: e44607

Bussmann, R.W., Paniagua-Zambrana, N.Y. & Njoroge, G. N. (2020). *Datura stramonium* L. *SOLANACEAE*. In: Bussmann, R.W. (eds) Ethnobotany of the Mountain Regions of Africa. Ethnobotany of Mountain Regions. *Springer*, Cham.

Centre for Disease Control and Prevention (CDC) (2018). Malaria Surveillance United States.

Cirimotich, C. M., Dong, Y., Clayton, A. M., Sandiford, S. L., Souza-Neto, J. A., Mulenga, M. & Dimopoulos, G. (2011). Natural microbe-mediated refractoriness to *Plasmodium* infection in *Anopheles gambiae*. *Science* 332, 855, 858.

Coetzee, M. (2020). Key to the females of Afrotropical *Anopheles* mosquitoes (Diptera: Culicidae). *Malaria journal*, *19*(1), 70.

Dada, N., Jupatanakul, N., Minard, G., Short, M. S., Akorli, J., & Villegas, M. L., (2021) Considerations for mosquito microbiome research from the Mosquito Microbiome Consortium. *Microbiome* **9**, 36.

Dada, N., Lol C. J., Benedict C. A., Lopez, F., Sheth, M., Dzuris, N., Padilla, N. & Lenhart, A. (2019). Pyrethroid exposure alters internal and cuticle surface bacterial communities in *An. albimanus. The ISME Journal.*

Dada, N., Sheth, M., Liebman, K., Pinto, J. & Lenhart, A. (2018). Whole metagenome sequencing reveals links between mosquito microbiota and insecticide resistance in malaria vectors. *Sci*. *Rep*. 8:2084.

Damergi, B., Essid, R., Fares, N., Khadraoui, N., Ageitos, L., Ben, A. A., Gharbi, D., Abid I., Rashed A. M., Limam, F., Rodríguez, J., Jiménez, C. & Tabbene, O. (2023). *Datura stramonium* Flowers as a Potential Natural Resource of Bioactive Molecules: Identification of Anti-Inflammatory Agents and Molecular Docking Analysis. Molecules. Jul 4;28(13):5195.

Damiani, C., Ricci, I., Crotti, E., Rossi, P., Rizzi, A., Scuppa, P., Capone, A., Ulissi, U., Epis, S., Genchi, M., Sagnon, N., Faye, I., Kang, A., Chouaia, B., Whitehorn, C., Moussa, G.W., Mandrioli, M., Esposito, F., Sacchi, L., Bandi, C., Daffonchio, D. & Favia, G. (2010). Mosquito-bacteria symbiosis: the case of *Anopheles gambiae* and *Asaia*. *Microb Ecol*. 60 (3): 644-54.

Debnah, T. & Chakraverty, R. (2017). Newer Insight into the Pharmacological Activities of *D. stramonium* Linn. A Review. *Indo Americal Journal of Phamaceutical Research*: 7(9), pg 441-444.

Demirak S. S.M., & Canpolat, E. (2022) Plant based bioinsecticides for Mosquito Control: Impact on Insecticide Resistance and Disease Transmission. *Insects*, *13*(2), 162.

Djihinto, O. Y., Medjigbodo, A. A., Gangbadja, A. R. A., Saizonou, H. M., Lagnika, H.O., Nanmede, D., Djossou, L., Bohounton, R., Sovegnon, P. M., Fanou, M. J., Agonhossou, R., Akoton, R., Mousse, W. & Djogbénou, L. S. (2022). Malaria- Transmitting Vectors Microbiota: Overview and Interactions with *Anopheles* Mosquito Biology. *Front Microbiol*. May 20;13: 891573.

Douglas, A. E. (2015). Multi-organismal insects: diversity and function of resident microorganisms. *Annu Rev Entomol*. 60:17–34.

E Silva, A. (2021). Diversity of Culturable Bacteria Isolated from the Faeces of Wild *Anopheles darlingi* (*Diptera*: *Culicidae*) Mosquitoes From the Brazilian Amazon. *Journal of Medical Entomology*. 58. 10.1093/jme/tjab028.

Elnour, Z., Grethe, H., Siddig, K. & Munga, S. (2023). Malaria control and elimination in Kenya: economy-wide benefits and regional disparities. *Malar J*,**22**,117 https://doi.org/10.1186/s12936-023-04505-6

Ezemuoka, L. C., Akorli, E. A., Aboagye-Antwi, F. & Akorli, J. (2020). Mosquito midgut *Enterobacter cloacae* and *Serratia marcescens* affect the fitness of adult female *Anopheles gambiae* s.l. *PLoS One* 15: e0238931.

Fagbemi, K. O., Aina, D. A., Adeoye-Isijola, M. O., Naidoo, K. K., Coopoosamy, R.M., & Olajuyigbe, O. O. (2022). Bioactive compounds, antibacterial and antioxidant activities of methanol extract of *Tamarindus indica* Linn. *Scientific reports*, *12*(1), 9432.

Feng, Y., Tang, J., Zhu, D., Zhang, Y., Zhu, G. & Akorli, W. J. (2021). The microbiota of three *Anopheles* species in China. *J. Am*. *Mosq*. *Control Assoc*. 37, 38–40.

Getachew, D., Balkew, M., & Tekie, H. (2020). Anopheles larval species composition and characterization of breeding habitats in two localities in the Ghibe River Basin,

southwestern Ethiopia. *Malaria journal*, *19*(1), 65.

Githure, J. I., Yewhalaw, D., Atieli, H., Hemming-Schroeder, E., Lee, M. C., Wang, X., Zhou, G., Zhong, D., King, C. L., Dent, A., Mukabana, W. R., Degefa, T., Hsu, K., Githeko, A. K., Okomo, G., Dayo, L., Tushune, K., Omondi, C. O., Taffese, H. S., Kazura, J. W., & Yan, G. (2022). Enhancing Malaria Research, Surveillance, and Control in Endemic Areas of Kenya and Ethiopia. *The American journal of tropical medicine and hygiene*, *107*(4_Suppl), 14–20.

Goanar, G., Tafesse, G., & Fereja, W. M. (2024). In vitro antibacterial activity of fruit pulp extracts of *Tamarindus indica* against *Staphylococcus aureus* and *Klebsiella pneumoniae*. *BMC complementary medicine and therapies*, *24*(1), 127.

Grant, R. J., Daniell, T. J. & Betts, W. B. (2002). Isolation and identification of synthetic pyrethroid-degrading bacteria. *J Appl Microbiol.* 2002; 92:534–40.

Guégan, M., Zouache, K., Démichel, C., Minard, G., Tran Van, V., Potier, P., Mavingui, P., & Valiente Moro, C. (2018). The mosquito holobiont: fresh insight into mosquito-microbiota interactions. *Microbiome*, *6*(1),

Gupta, C., Prakash, D. & Gupta, S. (2014).Studies on the antimicrobial activity of Tamarind (*Tamarindus indica*) and its potential as a food bio-preservative. *Int. Food Res. J.* 21(6), 2437–2441

Gupta, L., Molina-Cruz, A., Kumar, S., Rodrigues, J., Dixit, R., Zamora, R. & Barillas-Mury, C., (2009). The STAT pathway mediates late-phase immunity against *Plasmodium* in the mosquito *Anopheles gambiae*. Cell Host Microbe 5, 498–507.

Gupta, S., Chaubey, K. K., Khandelwal, V., Sharma, T. & Singh, S.V. (2021). *Datura Stramonium*: An Overview of Its Antioxidant System for Plant Benefits. In: Singh, H.B., Vaishnav, A., Sayyed, R. (eds) Antioxidants in Plant-Microbe Interaction. *Springer*, Singapore.

Gupta, V., Puri, R., Gupta, S., Jain, S., & Rao G. S. (2010). Tamarind kennel gum an upcoming natural polysaccharide. Syst. Rev. Pharm., 1:50-4

Hanif M.A., Nawaz H., Khan M. M, & Byrne H M.I., Ayub M.A. (2020). *Medicinal Plants of South Asia.* Elsevier; Amsterdam, The Netherlands: 2020. Datura; pp. 207–216.

Hollowell, T., Sewe, M.O., Rocklöv, J., Obor, D., Odhiambo, S., & Ahlm, C. (2023). Public health determinants of child malaria mortality: a surveillance study within Siaya County, Western Kenya. *Malar J* 22, 65.

Hu, G. P., Zhao, Y., Song, F. Q., Liu, B., Vasseur, L., Douglas, C., & You, M. S. (2014). Isolation, identification and cyfluthrin-degrading potential of a novel *Lysinibacillus sphaericus* strain, FLQ-11-1. *Res Microbiol*. Feb-Mar;165(2):110-8.

Ibrahim, M., Siddique, S., Rehman, K., Husnain, M., Hussain, A., Akash, M. S. H., & Azam, F. (2018). Comprehensive analysis of phytochemical constituents and ethnopharmacological investigation of genus Datura. *Critical Reviews™ in Eukaryotic Gene Expression*, *28*(3).

Iqbal, A. (2019). Effect of different solvent extracted samples from the leaves and fruits of *Datura stramonium* on the growth of bacteria and fungi. *Pakistan Journal of Pharmaceutical Sciences*.20.

Kabula, B., Tungu, P., Rippon, E.J., Steen, K., Kisinza, W., Magesa, S., Mosha, F & Donelly, M. J., (2016). A significant association between deltamethrin resistance, *Plasmodium falciparum* infection and the Vgsc-1014S resistance mutation in *Anopheles gambiae* highlights the epidemiological importance of resistance markers. Malar J. 2016;15:289.

Kämpfer, P., Matthews, H., Glaeser, S. P., Martin, K., Lodders, N., & Faye, I. (2011). Elizabethkingia anophelis sp. nov., isolated from the midgut of the mosquito Anopheles gambiae. *International journal of systematic and evolutionary microbiology*, *61*(Pt 11), 2670–2675.

Karunarathne, P., Pocquet, N., Labbé, P. & Milesi, P. (2022). BioRssay: an R package for analyses of bioassays and probit graphs. *Parasites Vectors* **15**, 35.

Kirar, M., Singh, H., Singh, S. P. & Neelam, H. (2023). Identification of Novel Protein from *Datura stramonium* Leaves with Bioinsecticide Potential Against *Anopheles Stephensi*. *Int J Pept Res Ther* 29, 49 (2023).

Kiuru, C.W., Oyieke, F. A., Mukabana,W. R., Mwangangi, J., Kamau, L. & Muhia-Matoke, D. (2018). Status of insecticide resistance in malaria vectors in Kwale County, Coastal Kenya. *Malar. J.* 17, 3.

Komakech, R., Kim, Y. G., Matsabisa, G. M. & Kang, Y. Anti-inflammatory and analgesic potential of *Tamarindus indica* Linn. (Fabaceae): a narrative review. *Integrat. Med. Res.* 8(3), 181–186.

Kowalska, T., & Sajewicz, M. (2022). Thin-Layer Chromatography (TLC) in the Screening of Botanicals-Its Versatile Potential and Selected Applications. *Molecules (Basel, Switzerland)*, *27*(19), 6607.

Krajacich, B. J., Huestis, D. L., Dao, A., Yaro, A. S., Diallo, M., Krishna, A., Xu, J., & Lehman, T. (2018). Investigation of the seasonal microbiome of *Anopheles coluzzii* mosquitoes in Mali. *PLoS One* 13:e0194899.

Kukutla, P., Lindberg, B. G., Pei, D., Rayl, M., Yu, W., Steritz, M., Faye, I., & Xu, J. (2014). Insights from the genome annotation of *Elizabethkingia anophelis* from the malaria vector Anopheles gambiae. *PloS one*, *9*(5), e97715.

Lanfranchi D. A., Tomi F. & Casanova J. (2010). Enantiomeric differentiation of atropine/hyoscyamine by ^{13}C N MR spectroscopy and its application to *Datura stramonium* extracts. *Phytochemical Analysis*/Vol. 21(6) p597-601

Leber, A. L., (2016). Clinical microbiology procedures handbook (Fourth edition). Washington, DC : *ASM Press* 1752

Leng, L. Y, Nadzri, N., Shaari, A. R., Razak, A. & Yee, K.C. (2017). Antioxidant capacity and total phenolic content of fresh, oven-dried and stir-fried tamarind leaves. *Curr Res Nutr Food Sci J*. 5:282–7. Unexpected diversity of *Anopheles* species in Eastern Zambia: Implications for evaluating vector behaviour and interventions using molecular tools. *Sci*. *Rep*. 5, 17952.

Maheshwari, N. O., Khan, A. & Chopade, B. A. (2013). Rediscovering the medicinal properties of *Datura* 440 sp.: A review. *Journal of Medicinal Plants Research*. 7(39):2885-2897

Mairura, F. (2014). *D. stramonium* L Protologue Sp. Pl. 1: 179 (1753).

Mathias D.K., Ochomo, E., Atieli, F., Ombok, M., Bayoh, M.N., Olang. G., Muhia, D. Kamau, L., Vulule, J.M, Hamel, M.J. Hawley, W.A., Wlaker, E.D. & Gimnig J.E. (2011) Spatial and temporal variation in the kdr allele L1014S in *An. gambiae s.s.* and phenotypic variability in susceptibility to insecticides in Western Kenya. *Malar J.;* 10:10. 102.

Maurawa, K., Makaza, K., Sakadzo, N., Mubvuma, M., Chidoko, P., & Nyamusamba, R. (2022). Bioactive leaf extracts of thorn apple (*Datura stramonium l*.) as an alternative to synthetic pesticides for fall armyworm (*Spodoptera frugiperda* (J.E Smith) management in maize (*Zea mays* L.). 9. 99-105.

Mesfin, A., Giday, M., Animut, A., & Teklehaymanot, T. (2012). Ethnobotanical study of antimalarial plants in Shinile District, Somali Region, Ethiopia, and in vivo evaluation of selected ones against Plasmodium berghei. *Journal of ethnopharmacology*, *139*(1), 221–227.

Minard G, Mavingui P, Moro C. V. (2013). Diversity and function of bacterial microbiota in the mosquito holobiont. Parasit Vectors. 2013;6:146.

Mohammed, B., Owaid, Z., Jasim, E., Adnan, W., & Alfraji, N. (2020). Local culture medium from the legumes mixture as a novel media for the growth and stimulation of prodigiosin pigment which production from *Serratia marcescens* that isolated environmentally. Plant Archives. Vol. 20. pp. 991-1000.

Moreno-Pedraza, A., Gabriel, J., Treutler, H., Winkler, R., & Vergara, F. (2019). Effects of Water Availability in the Soil on Tropane Alkaloid Production in Cultivated *Datura stramonium*. *Metabolites*, *9*(7), 131.

Nortjie, E., Basitere, M., Moyo, D., & Nyamukamba, P. (2022). Extraction Methods, Quantitative and Qualitative Phytochemical Screening of Medicinal Plants for Antimicrobial Textiles: A Review. *Plants (Basel, Switzerland)*, *11*(15),2011.

Nwodo, U. U., Obiiyeke, G. E., Chigor, V. N., & Okoh, A. I. (2011). Assessment of *Tamarindus indica* extracts for antibacterial activity. *International journal of molecular sciences*, *12*(10), 6385–6396.

Ochomo, E., Milanoi, S., Abongo, B., Onyango, B., Muchoki, M., Omoke, D., Olanga, E., Njoroge, L., Juma, E., Otieno, J., Muhia, D., Kamau, L., Rafferty, C., Gimnig, J., Shieshia, M., Wacira, D., Mwangangi, J., Maia, M., Chege, C. & Kariuki, L. (2023). Detection of *Anopheles stephensi* Mosquitoes by Molecular Surveillance, Kenya. Emerging infectious diseases.

Ondeto, B.M, C., Kamau, L., Muria, S.M. Mwangangi, J.M., Njagi, K., Mathenge, E.M., Ochanda, H. & Mbogo, C.M. (2017). Current status of insecticide resistance among malaria vectors in Kenya. *Parasites and Vectors* 10:429.

Ondiba, I.M., Oyieke, F.A., Nyamongo, I.K. & Estambale, B.B. (2017). Diversity, distribution and abundance of potential

rift valley fever vectors in Baringo County, Kenya. *Int*. *J*. *Mosq*. *Res*. 4, 42–48.

Orondo, P. W., Nyanjom, S. G., Atieli, H., Githure, J., Ondeto, B. M., Ochwedo, K. O., ... & Yan, G. (2021). Insecticide resistance status of Anopheles arabiensis in irrigated and non-irrigated areas in western Kenya. *Parasites & vectors*, *14*(1), 335.

Pelloquin, B., Kristan, M., Edi, C., Meiwald, A., Clark, E., Jeffries, C. L, Walker, T., Dada, N., & Messenger, L.A., (2021). Overabundance *Asaia* and *Serratia* Bacteria Is Associated with Deltamethrin Insecticide Susceptibility in *Anopheles coluzzii* from Agboville, Côte d'Ivoire. *Microbiol Spectr*. Oct 31;9(2).

Rani, A., Sharma, A., Rajagopal, R., Adak, T., & Bhatnagar, R.K (2009). Bacterial diversity analysis of larvae and adult midgut microflora using culture-dependent and culture- independent methods in lab-reared and field-collected *Anopheles stephensi*-an Asian malarial vector. *BMC Microbiol*. May 19;9:96.

Rehman, S., Ullah, N., Jaffer, D., Zaidi, R. & Anwar, H. (2022). Light and scanning electron microscopy of *Datura stramonium* L. extract and its biological applications. *Microscopy Research and Technique*. 85. 10.1002.

Saab, S. A., Dohna, H. Z., Nilsson, L. K. J., Onorati, P., Nakhleh, J., Terenius, O. & Osta, M. (2020). The environment and species affect gut bacteria composition in laboratory co-cultured *Anopheles gambiae* and *Aedes albopictus* mosquitoes. *Sci*. *Rep*. 10:3352.

Saberi, N., Raeisi, A., Gorouhi, M. A., Vatandoost, H., Mashayekhi, M., Nikpour, F., Omid, B. F., Foroushani,R. A., & Hanafi-Bojd, A. A. (2022). Current Situation of Malaria and Resistance of Main Vectors to WHO Recommended Insecticides in an Endemic Area, Southeastern Iran. *Journal of Medical Entomology*, *59*(2), 607-614.

Satish P. V. V., Santha K. D., Sree R. N. & Sunita K. (2017). *In vitro* and *in vivo* antimalarial screening of *Calotropis gigantea* against *Plasmodium falciparum* (3D7 Strain) and *Plasmodium berghei* (ANKA) along with cytotoxicity. *Ind J of Vec Bor Dis*., 2017; 53(4): 215-225.

Satish, P. V. V., Kumari, D. & Sunita, K. (2018). Antimalarial Efficacy of *Datura stramonium* against Chloroquine sensitive *Plasmodium falciparum* 3D7 strain. *ejpmr*, 5(3). 287-294.

Sayyed, A., & Shah, M. (2014). Phytochemistry, Pharmacological and Traditional Uses of *Datura stramonium* L. *Rev. J. Pharm. Phytochem. 2*, 123–125.

Sharma, M., Dhaliwal, I., Rana, K., Delta, A. K. & Kaushik, P. (2021). Phytochemistry, Pharmacology, and Toxicology of Datura Species—A Review. *Antioxidants.* 10:1291.

Sharma, M., Dhaliwal, I., Rana, K., Delta, A. K., & Kaushik, P. (2021). Phytochemistry, Pharmacology, and Toxicology of Datura Species-A Review. Antioxidants (*Basel*, Switzerland), 10(8), 1291.

Shin, S. Y., Bajpai, V. K., Kim, H. R. & Kang, S. C. Antibacterial activity of eicosapentaenoic acid (EPA) against foodborne and food spoilage microorganisms. *LWT-Food Sci. Technol.* 40, 1515–1519 (2007).

Soltani, A., Vatandoost, H., Oshaghi, M.A., Enayati, A.A. & Chavshin, A.R. (2017). The role of midgut symbiotic bacteria in resistance of *Anopheles stephensi* (*Diptera*: *Culicidae*) to organophosphate insecticides. *Pathog. Glob. Health* 111, 289–296. `

Sookying, S., Duangjai, A., Saokaew, S., & Phisalprapa, P. (2022). Botanical aspects, phytochemicals, and toxicity of *Tamarindus indica* leaf and a systematic review of antioxidant capacities of *T. indica* leaf extracts. *Frontiers in nutrition*, *9*, 977015.

Srivastava, N., Khandagle, A., Morey, R. & Raut, K. (2022). Characterization of Some Medicinal Plants and Their Role in Mosquito Control. *Egyptian A Journal of Biological Sciences, E. Medical Entomology and Parasitology*, *14*(2); 189-197.

Srivastava, N., Khandalgle, A., Morey, R. & Raut, K. (2023). CGMS analysis and mosquitocidal effects of petroleum ether extracts of *Datura stramonium* and *Morus alba* against *Aedes aegypti. Indian Journal of Natural Products and Resources, 14,(1), 1-124*

Swathi, s., Murugananthan, G., Ghosh S. K. & Pradeep, A. K. (2012). Larvicidal and repellent activities of ethanolic extracts of *D. stramonium* leaves against mosquitoes. *International Journal* of *Pharmacognosy and Phytochemical Research*; 4(1); 25-27.

Takhi, D. & Ouinten, M. (2011). Study of antimicrobial activity of secondary Metabolites extracted 425 from spontaneous plants from the area of Laghouat, Algeria. *Adv Environm* 426 Biol.;5(2):469–476.

Turek & Stintzing (2013). Stability of essential oils: A review Research Gatel comprehensive Reviews in Food and Science and Food Safety, 12(1). 10.1111/154-4337.12006. US President's Malaria Initiative FY 2024 Kenya MOP (pmi.gov)

Wang, M., An, Y., Gao, L., Dong, S., Zhou, X., Feng, Y., Wang, P., Dimopoulos, G., Tang, H. & Wang, J. (2021). Glucose-mediated proliferation of a gut commensal bacterium promotes Plasmodium infection by increasing mosquito mid-gut pH. Cell Rep. 35:108992.

Wang, Y., Gilbreath, T. M. III, Kukutla, P., Yan, G., & Xu, J. (2011). Dynamic gut microbiome across life history of the malaria mosquito *Anopheles gambiae* in Kenya. PLoS One 6:e24767.

Wong, M.L., Liew, J.W.K., Wong, W.K., Pramasivan, S., Hassan, N.M., Sulaiman, W.Y., N.K., Leong, C.H., Van Lun, L.L. & Indra, V. (2020). Natural *Wolbachia* infection in field-collected *Anopheles* and other mosquito species from Malaysia. *Parasites and Vectors*.13.

World Health Organization. (2023). WHO guidelines for malaria, 14 March 2023. World Health Organization. License: CC BY-NC-SA 3.0 IGO

Wu, P. W., Liu, Y., & Wang, Z. Y., Zhang, X. Y., Li, H., Liang, W. Q., Luo, N. Hu, J.M., Lu, J.Q., Luan, T. G. & Cao, L. X. (2006). Molecular Cloning, Purification, and Biochemical Characterization of a Novel Pyrethroid-Hydrolyzing Esterase from *Klebsiella sp*. Strain ZD112. Journal of agricultural and food chemistry. 54. 836-42.

Xia, X., Zhen, D., Zhong, H., Qin, B., Gur, M. G., Vasseur, L., Lin, H., Bai, J., Hi, W, & You, M. (2013) DNA Sequencing Reveals the Midgut Microbiota of Diamondback Moth, *Plutella xylostella* (L.) and a Possible Relationship with Insecticide Resistance. *PLOS ONE* 8, e68852 (2013).