

EVALUATION OF ANTI-MICROBIAL ACTIVITY OF TAMARIND (*Tamarindus indica*) EXTRACTS COLLECTED IN FROM SEMI-ARID EASTERN PARTS OF KENYA AGAINST BACTERIA PATHOGENS.

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Abstract

Natural products are alternatively used in the control of pests and diseases because they are highly available, cheap and environmentally friendly. This study aimed at evaluating the antimicrobial activity of leaf and fruit extracts from tamarind trees growing in semi-arid Eastern Kenya. The extracts were tested for their activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Fruits and leaves of tamarind were sequentially extracted using methanol and water and evaporated using a rotary evaporator at 40 °C. The extracts were then reconstituted using the solvent and stored at 4 °C. The pathogenic bacteria were cultured on 28 g/l of nutrient agar and the extract-impregnated discs were inoculated on the plates and cultured at 37 °C for 96 hrs. The media were supplemented with chloramphenicol 500 mg/l. Sub-culturing was done to obtain pure isolates of the pathogens. The extracts were tested for their activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Data on bacterial inhibition zones were recorded after 24 hrs and analyzed using SPSS Version 12. The results of the study revealed that there was no significant difference in inhibition between the leaf and fruit extracts. However, there was a significant inhibition difference between the five study regions and a significant difference in water and methanol extracts against *B. subtilis*. There was significant inhibition of *P. aeruginosa* in the five study regions, fruits and leaves, and in water and methanol. Tamarind extracts were not effective against *S. aureus* and *E. coli*. When compared to common antibiotics Ampicillin, Methanol leaf extracts from accessions KT007, E017, and E020 had a higher inhibition to than *B. subtilis*; and also, water fruit extracts from accessions E008 and E014 had a higher inhibition to than *B. subtilis*. Additionally, Methanol leaf extracts from accessions KT012, E001, KB008, and KB011 had higher inhibition against *P. aeruginosa* compared to Streptomycin, Kanamycin, and Co-trimoxale. Water fruit extracts from the accession of KT012 had a higher inhibition of *P. aeruginosa* compared to Streptomycin, Kanamycin, and Co-trimoxale. Additionally, Water leaf extracts from accessions of KT001, KB004, KB005, KB011, KB012, KB014, and KB016 had a higher inhibition of *P. aeruginosa* compared to kKanamycin, gGentamycin, sStreptomycin, aAmpicillin, and Co-trimoxale. There was no significant inhibition of *S. aureus* and *E. coli* by tamarind extracts. The results of this study revealed that In conclusion, Eastern Kenyan tamarind had limited potential to be used as a biological control agent against *B. subtilis* and *P. aeruginosa*.

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Keywords: Anti-microbial, Eastern Kenya, tamarind, methanol, water

Introduction

Tamarind has been used for many years to control bacterial pathogens as different parts of tamarind contain different medicinal properties (Escalona--Arranz *et al.*, 2010). The increased antimicrobial properties of tamarind increased its ethnobotanical use in Latin America, Asia and Africa (Meléndez and Capriles, 2006). Tamarind fruit extracts are used as refrigerant in fever

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and as laxatives and carminatives alone or as a combination. In South East Asia the pulp has been used to cure sore throats (Du Preez, 2003). Tamarind pulp is composed of tartaric acid, malic acid, citric acid, pectin gum, potassium bitartrate and paranchymatous fibre (Nazir *et al.*, 2017).

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In West Africa, tamarind has been used as food and in herbal therapies (Nwodo *et al.*, 2011). In Nigeria, the pulp is used in production of local drink, preservation of food and general traditional medicine as a drug conveyor. A combination of tamarind with other herbs was reported to be effective against constipation, fever and sore throats (Abukakar *et al.*, 2008).

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Most rural communities worldwide depended on traditional medicines for health solutions (Nwodo *et al.*, 2011), which were more effective compared to the predominant synthetic drugs popularly found in urban areas where resistance to conventional medicine was a challenge. The resistance increased research in herbal medicine (Paul Das and Banerjee, 2014). Extracts of biologically active compounds were reported to offer a new source of anti-bacterial (Abukakar *et al.*, 2008). In Kenya, tamarind is present in the arid and semi-arid areas and there is limited information on its antimicrobial activity.

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Materials and methods

Sample preparation

Leaf and fruit samples were collected from tamarind trees in Embu, Kibwezi, Mwingi, Kitui and Masinga. Samples from Kitui were labelled as KT001-KT-025, Embu as E001-E021, Kibwezi as KB001-KB027, Mwingi as MW001-MW010, Masinga as MS001-MS006. The pods and the leaves were collected and dried under shade, the pods were dehusked and the pulp separated and the leaves and the fruits were pulverized separately and used for evaluation. Twenty grams of the pulverized leaves and fruits from each accession were weighed and each dissolved in 120 ml of solvent. This was extracted sequentially using methanol and water as described by Uthayarasa *et al.* (2010). The extract was dried using a rotary evaporator at 30-40 °C and 0.2 gms of the extract was dissolved in 1ml of the solvent as described by Predrag *et al.*, (2005) and stored at 4 °C.

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Pathogen inoculation

Two gram-positive bacteria (*B. subtilis* and *S. aureus*) and two gram-negative bacteria (*E. coli* and *P. aeruginosa*) were used. The micro-organisms were collected from National Public Health laboratories then preserved in nutrients broth and stored at 4 °C and cultured on 28 g/l of nutrient agar. Disc diffusion method was used to test the antimicrobial potential of the tamarind extracts against the selected bacteria pathogens as described by (Sandle, 2016). The pathogens were inoculated on nutrient agar media onto which extract impregnated discs were placed and incubated at 24 °C for 48 hrs. The antimicrobial potential of the tested extract was validated by measuring the magnitude of a clear zone of inhibition around the point of application of the disc with the extract. The solvents were used as negative control while streptomycin, kanamycin and co-trimoxazole, tetracycline, ampicillin, gentamycin, sulfamethoxazole used as control antibiotics.

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Data collection and analysis

The experiment was done in 3 replicates in a split-block design (two main block of leaves and fruits, each block divided into methanol and water as solvents, then solvent tested against the four pathogens. Data on inhibition zones collected as diameter of the zone in millimeters (mm)

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~~and were~~ analyzed using Two-way ANOVA followed by Post Hoc Test using Wald Chi square to compare mean inhibition zones of ~~the tamarind plant parts~~ and solvents ~~of extraction~~. Significance level was set at $p < 0.05$. This was done by SPSS Version 12.

Results

There was a significant difference in inhibition of tamarind extracts from ~~the~~ study regions; Kitui, Mwingi, Embu, Masinga and Kibwezi (Table 1-). Tamarind leaf and fruit extracts were not significantly different but there was significant difference in water and methanol ~~extracts~~ at $P_p < 0.05$.

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There was a significance in inhibition of *P. aeruginosa* by tamarind extracts from study regions; Kitui, Mwingi, Masinga, Embu and Kibwezi extracts (Table 2). Tamarind leaf and fruit extracts showed significant inhibition. The extraction solvents; water and methanol revealed significant inhibition.

The methanol leaf and fruits, and the water leaf and fruit extracts showed activity against *B. subtilis*. Also, the methanol leaf extracts, and the water leaf and fruit extracts showed activity against *P. aeruginosa*, *E. coli*, *S. aureus*, *Penicilium digitatum*, *Alternaria solani* and *Colletotrichum gloeosporioides* were not inhibited by tamarind extracts.

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The methanol leaf extracts that were active against *B. subtilis* included accessions of KT001, KT002, KT004, KT007, KT011, KT012, KT015, KT018, KT020, E001, E003, E004, E005, E008, E009, E010, E012, E014, E015, E016, E017, E018, E020, E021, MW002, MW005, MW006, MW010, MS004, KB002, KB004, KB006, KB009, KB010 and KB022 (Fig 1).

Tamarind methanol leaf extracts of accessions of KT007, E017 and E020 inhibited *B. subtilis* better than ampicillin (Table 3).

Tamarind methanol fruit extracts that were active against *B. subtilis* were from Kibwezi (KB001, KB002, KB003, KB004, KB005, KB006, KB007, KB008, KB009, KB011, KB012, KB013, KB014, KB015, KB016 and KB017) and Embu (E003 and E005) (Fig 2).

All fruit extracts from methanol as a solvent had inhibition zones less than the common antibiotics. Leaf extracts from methanol showed some inhibition against *B. subtilis* (Table 4).

Tamarind water leaf extracts that were active against *B. subtilis* were from Embu (E003, E005) and Kibwezi (KB001, KB002, KB003, KB004, KB005, KB006, KB007, KB008, KB009, KB011, KB012, KB013, KB014, KB015, KB016 and KB017) (Fig 3).

Almost all tamarind leaves extracted using water had less inhibition zones compared to common antibiotics (Table 5).

Tamarind fruit extracted using water that were active against *B. subtilis* were from Kitui (KT004, KT009, KT011, KT015, KT025), Embu (E003, E005, E006, E007, E008, E010, E013, E014, E017, E018, E019, E021) and Kibwezi (KB007 and KB022) (Fig 4).

Fruits extracted using water had less inhibition compared to common antibiotics except for extracts from accessions E008, E014 that performed better than ampicillin (Table 6).

Tamarind methanol leaf extracts from Kitui (KT005, KT007, KT0012, KT013, KT016, KT022, KT023), Embu (E001, E002, E005, E006, E009, E010, E015, E016 and E020) and Kibwezi

(KB008, KB011, KB016 and KB017) were active against *P. aeruginosa* accessions (Fig 6). Tamarind fruits extracted using methanol were not active against *P. aeruginosa*.

Methanol leaf extracts of accessions KT012, E001, KB008 and KB011 had inhibitions greater than streptomycin, kanamycin and co-trimoxale (Table 7).

Leaves extracted using water that were active against *P. aeruginosa* included extracts of accessions; KT001, KT002, KT003, KT004,KT005, KT008, KT009, KT011, KT012, KT013, KT014, KT015, KT016, KT017, KT018, KT019,KT020, KT022, KT023, KT024, KT025, E001, E002, E003, E004, E005, E006, E007, E008,E009, E010, E011, E012, E013, E015, E016, E019, E020, E021, MW001, MW003, MW004, MW006, MW007, MW008, MS001, MS002, MS003, MS004, MS005, MS006, KB001, KB002, KB003, KB004, KB005, KB006, KB008, KB009 KB010, KB011,KB012, KB013, KB015, KB014, KB016, KB017, KB018, KB019, KB020, KB021, KB022, KB023,KB024, KB025, KB026 and KB0027 (Fig 6).

Leaf Extracts of accessions KT001, KB004, KB005, KB011, KB012, KB014 and KB016 had inhibition zones greater than kanamycin, gentamycin, streptomycin, ampicillin, and co-trimoxale (Table 8).

Tamarind fruits extracted using water that were active against *P. aeruginosa* were from Kitui, Embu and Kibwezi (Fig7).

Fruits extracted using water had less inhibition zones compared to the common antibiotics except for extract of accession KT012 that performed better than streptomycin, kanamycin and co-trimoxale (Table 9).

Inhibition zones by all extracts from the different regions (Kitui, Mwingi, Embu, Kibwezi and Masinga) were not significantly different. The inhibition zones for the leaves and fruits were not significantly different. In addition, extracts from the different extraction solvents were not significantly different. *E. coli* was not inhibited by tamarind extracts.

Inhibition zones by all extracts from different for the regions (Kitui, Mwingi, Embu, Kibwezi and Masinga) were not significantly different. The inhibition zones for the leaves and fruits were not significantly different. In addition, the extracts from different solvents were not significantly different. Tamarind extracts were not active against *S. aureus*.

Tamarind extracts from Embu had high inhibition while the least inhibition was from Mwingi (Fig. 8A). Water extracts had higher inhibition than methanol (Fig. 8B). The leaves had higher inhibition than the fruits (Fig. 8C). Tamarind extracts inhibited *P. aeruginosa* highly followed by *B. subtilis* while there was no inhibition in *E. coli* and *S. aureus* (Fig. 8D).

Table 1: Inhibition of *B. subtilis* by tamarind extracts from semi-arid Eastern Kenya

Source	Wald Chi-Square	Sig.
Study regions	65.484	.000
Plant parts (leaves and fruits)	.001	.973
Extraction solvent (-water and methanol)	22.456	.000

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There was a significant inhibition of *P. aeruginosa* by tamarind extracts from study regions; Kitui, Mwingi, Masinga, Embu and Kibwezi extracts (Table 2). Tamarind leaf and fruit extracts showed significant inhibition. The extraction solvents; water and methanol revealed significant inhibition.

Table 2: Inhibition of *P. aeruginosa* by tamarind extracts from semi-arid Eastern Kenya

Source	Wald Chi-Square	Sig.
Study sites	16.460	.002
Plant parts (leaves and fruits)	242.176	.000
Extraction solvents (methanol and water)	207.033	.000

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Methanol leaf and fruits, water leaf and fruit extracts showed activity against *B. subtilis*. Methanol leaf extracts, water leaf and fruit extracts showed activity against *P. aeruginosa*. *E. coli*, *S. aureus*, *Penicillium digitatum*, *Alternaria solani* and *Colletotrichum gloeosporioides* were not inhibited by tamarind extracts.

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Inhibition of *Bacillus subtilis* by tamarind extracts from semi-arid Eastern Kenya

Methanol Leaf extracts that were active against *B. subtilis* included accessions of KT001, KT002, KT004, KT007, KT011, KT012, KT015, KT018, KT020, E001, E003, E004, E005, E008, E009, E010, E012, E014, E015, E016, E017, E018, E020, E021, MW002, MW005, MW006, MW010, MS004, KB002, KB004, KB006, KB009, KB010 and KB022 (Fig 1).

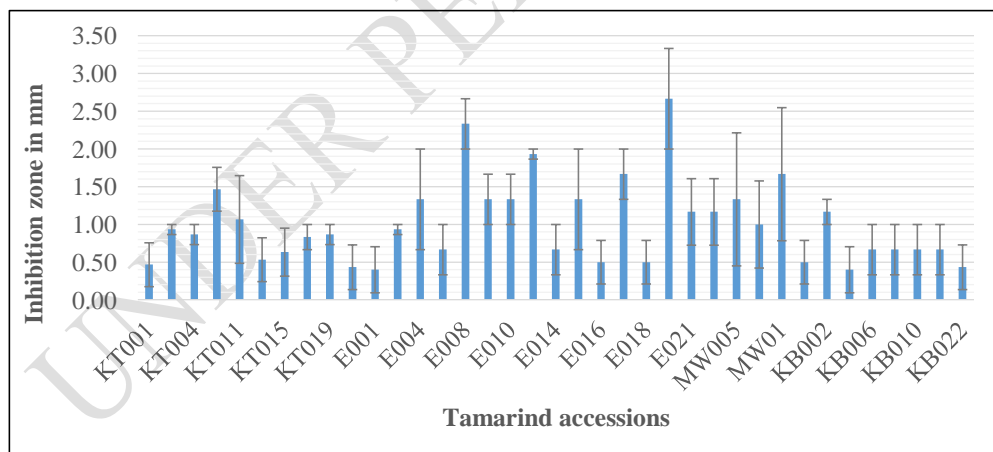


Figure 1: Inhibition of *Bacillus subtilis* by methanol leaf extract of tamarind from semi-arid Eastern Kenya

Inhibition of *B. subtilis* by tamarind leaves extracted using methanol

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Tamarind methanol leaf extracts of accessions of KT007, E017 and E020 inhibited *B. subtilis* better than ampicillin (Table 3).

Table 3: Inhibition of *B. subtilis* with methanol leaf extracts from semi-arid Eastern Kenya compared to common antibiotics

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Leaf samples	Mean (mm)	Leaf samples	Mean (mm)	Common antibiotics	Mean (mm)
KT007	1.47±0.29	E004	1.33±0.67	Gentamycin	22.67±0.67
KT011	1.07±0.58	E008	2.33±0.33	Tetracycline	22.33±0.33
MW002	1.17±0.44	E009	1.33±0.33	Ampicillin	1.33±0.33
MW005	1.33±0.88	E010	1.33±0.33	Co-trimoxale	23.67±0.88
MW006	1.00±0.58	E012	1.93±0.07	Chloramphenicol	19.33±1.33
MW01	1.67±0.88	E015	1.33±0.67	Sulfamethoxazole	2.67±0.44
KB002	1.17±0.17	E017	1.67±0.33	Streptomycin	21.67±1.20
E020	2.67±0.67	E021	1.17±0.44	Kanamycin	20.67±0.67

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Tamarind methanol fruit extracts that were active against *B. subtilis* were from Kibwezi (KB001, KB002, KB003, KB004, KB005, KB006, KB007, KB008, KB009, KB011, KB012, KB013, KB014, KB015, KB016 and KB017) and Embu (E003 and E005) (Fig 2).

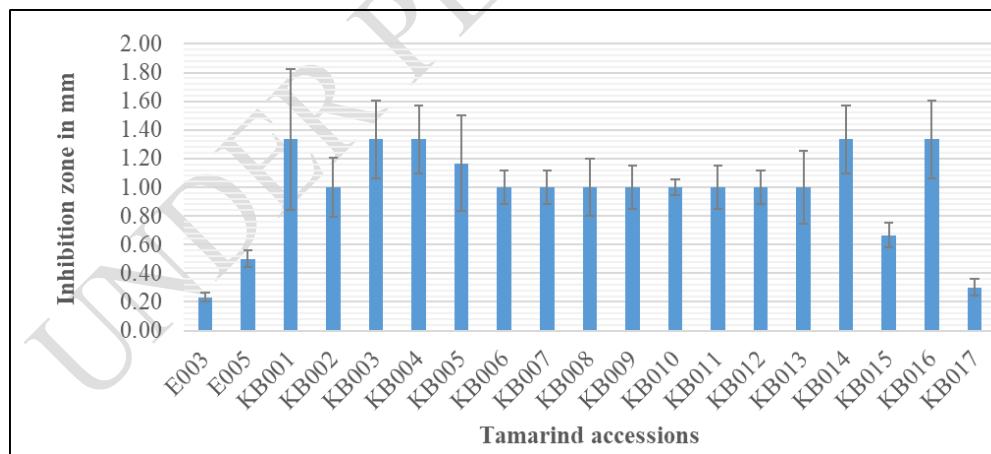


Figure 2: Inhibition of *Bacillus subtilis* by methanol fruit extract of tamarind from semi-arid Eastern Kenya

Inhibition of *B. subtilis* by tamarind fruits extracted using methanol

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All fruit extracts from methanol as a solvent had inhibition zones less than the common antibiotics. Leaf extracts from methanol showed some inhibition against *B. subtilis* (Table 4).

Table 14: Inhibition of *B. subtilis* with methanol fruit extracts from semi-arid Eastern Kenya compared to standard antibiotics.

Fruit samples	Mean (mm)	Fruit samples	Mean (mm)	Common antibiotics	Mean (mm)
E003	0.23±0.03	KB010	1.00±0.06	Gentamycin	22.67±0.67
E005	0.50±0.06	KB011	1.00±0.15	Tetracyclin	22.33±0.33
KB001	1.33±0.49	KB012	1.00±0.12	Ampicillin	1.33±0.33
KB002	1.00±0.21	KB013	1.00±0.25	Co-trimoxale	23.67±0.88
KB003	1.33±0.27	KB014	1.33±0.24	Chloromphenical	19.33±1.33
KB004	1.33±0.24	KB015	0.67±0.09	Sulfamethoxazole	2.67±0.44
KB005	1.17±0.33	KB016	1.33±0.27	Streptomycin	21.67±1.20
KB006	1.00±0.12	KB017	0.30±0.06	Kanamycin	20.67±0.67
KB007	1.00±0.12	KB009	1.00±0.15		
KB008	1.00±0.20				

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Tamarind water leaf extracts that were active against *B. subtilis* were from Embu (E003, E005) and Kibwezi (KB001, KB002, KB003, KB004, KB005, KB006, KB007, KB008, KB009, KB011, KB012, KB013, KB014, KB015, KB016 and KB017) (Fig 3).

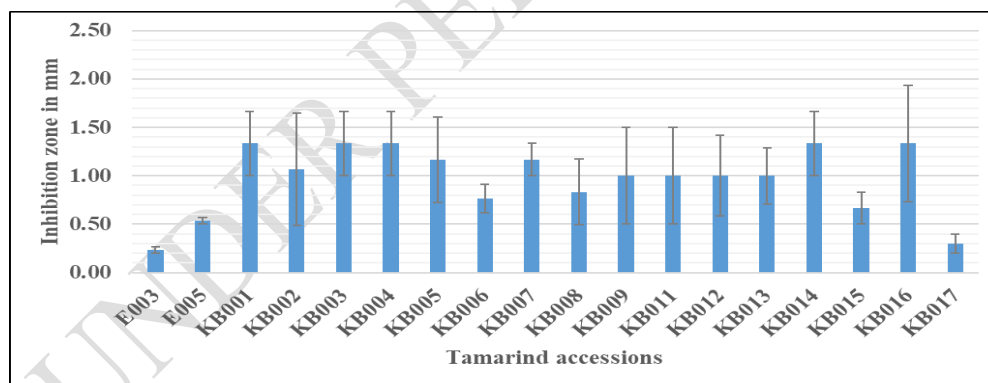


Figure 3: Inhibition of *Bacillus subtilis* by water leaf extract of tamarind from semi-arid Eastern Kenya

Inhibition of *B. subtilis* by tamarind leaves extracted using water

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Almost all tamarind leaves extracted using water had less inhibition zones compared to common antibiotics (Table 5).

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Table 25: Inhibition of *B. subtilis* with tamarind-water leaf extracts from semi-arid Eastern Kenya compared with common antibiotics.

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Inhibition zones					
Leaf samples	Mean (mm)	leaf samples	Mean (mm)	Common antibiotics	Mean (mm)
E003	0.23 ± 0.03	KB008	0.83±0.34	Kanamycin	20.67±0.67
E005	0.53 ± 0.03	KB009	1.00±0.5	Gentamycin	22.67±0.67
KB001	1.33±0.33	KB011	1.00±0.5	Tetracycline	22.33±0.33
KB002	1.07±.58	KB012	1.00±0.42	Ampicillin	1.33±0.33
KB003	1.33±0.33	KB013	1.00±0.29	Co-trimoxale	23.67±0.88
KB004	1.33±0.33	KB014	1.33±0.33	Chloramphenicol	19.33±1.33
KB005	1.17±0.44	KB015	0.67±0.17	Sulfamethoxazole	2.67±0.44
KB006	0.77±0.15	KB016	1.33±0.60	Streptomycin	21.67±1.20
KB007	1.17±0.17	KB017	0.30±0.10		

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Tamarind fruit extracted using water that were active against *B. subtilis* were from Kitui (KT004, KT009, KT011, KT015, KT025), Embu (E003, E005, E006, E007, E008, E010, E013, E014, E017, E018, E019, E021) and Kibwezi (KB007 and KB022) (Fig 4).

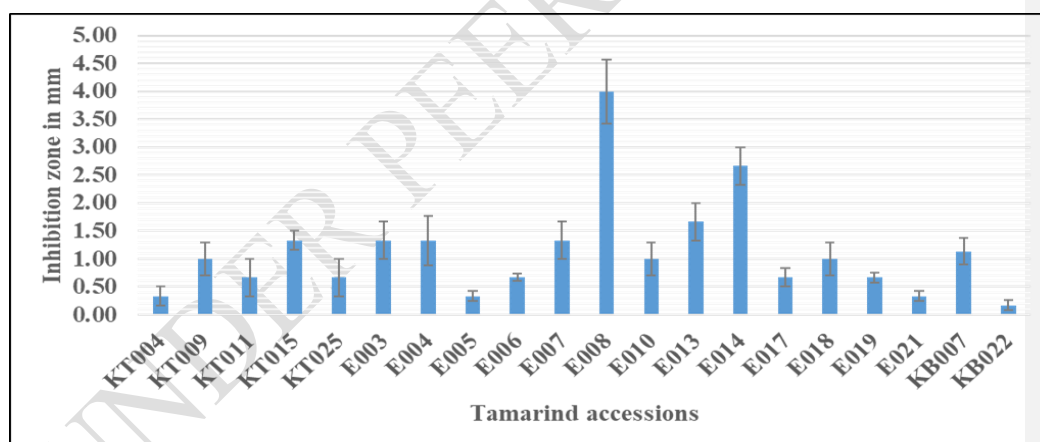


Figure 4: Inhibition of *Bacillus subtilis* by water fruit extract of tamarind from semi-arid Eastern Kenya

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Inhibition of *B. subtilis* by tamarind fruits from semi-arid Eastern Kenya extracted using water

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Fruits extracted using water had less inhibition compared to common antibiotics except for extracts from accessions E008, E014 that performed better than ampicillin (Table 6)

Table 36: Inhibition of *B. subtilis* with tamarind water extracts from semi-arid Eastern Kenya compared to common antibiotics

Fruit samples	Mean (mm)	Common antibiotics	Mean (mm)
KT015	1.33±0.17	Kanamycin	20.67±0.67
E003	1.33±0.33	Gentamycin	22.67±0.67
E004	1.33±0.44	Tetracycline	22.33±0.33
E007	1.33±0.33	Ampicillin	1.33±0.33
E008	4.00±0.58	Co-trimoxale	23.67±0.88
E013	1.67±0.33	Chloramphenicol	19.33±1.33
E014	2.67±0.33	-Sulfamethoxazole	2.67±0.44
KB007	1.13±0.24	Streptomycin	21.67±1.20

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Inhibition of *P. aeruginosa* by tamarind extracts from semi-arid Eastern Kenya

Tamarind methanol leaf extracts from Kitui (KT005, KT007, KT0012, KT013, KT016, KT022, KT023), Embu (E001, E002, E005, E006, E009, E010, E015, E016 and E020) and Kibwezi (KB008, KB011, KB016 and KB017) were active against *P. aeruginosa* accessions (Fig 6). Tamarind fruits extracted using methanol were not active against *P. aeruginosa*

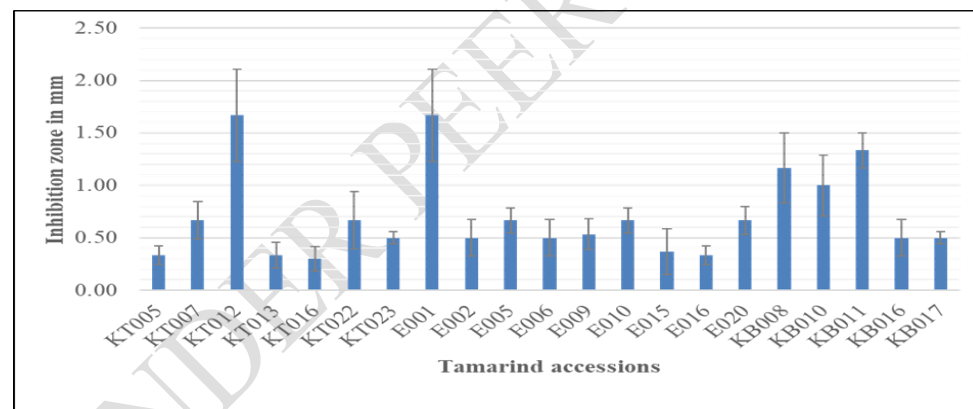


Figure 5: Inhibition of *Pseudomonas aeruginosa* by methanol leaf extract of tamarind from semi-arid Eastern Kenya

Inhibition of *P. aeruginosa* by tamarind leaves extracted using methanol from semi-arid Eastern Kenya

Methanol leaf extracts of accessions KT012, E001, KB008 and KB011 had inhibitions greater than streptomycin, kanamycin and co trimoxale (Table 7)

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Table 47: Inhibition of *P. aeruginosa* by methanol leaf extracts from semi-Eastern Kenya compared to common antibiotics

Leaf samples	Mean (mm)	Leaf samples	Mean (mm)	Common antibiotics	Mean (mm)
KT007	0.67±0.18	E009	0.53±0.15	Co-trimoxale	1.00±0.10
KT012	1.67±0.44	E010	0.67±0.12	Chloramphenicol	6.00±2.00
KT022	0.67±0.27	E020	0.67±0.13	Sulfamethoxazole	4.67±1.33
KT023	0.50±0.06	KB008	1.17±0.33	Streptomycin	1.00±0.12
E001	1.67±0.44	KB010	1.00±0.29	Kanamycin	1.00±0.23
E002	0.50±0.17	KB011	1.33±0.17	Ampicillin	1.87±0.23
E005	0.67±0.12	KB016	0.50±0.17	Gentamycin	1.67±0.33

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Leaves extracted using water that were active against *P. aeruginosa* included extracts of accessions; KT001, KT002, KT003, KT004,KT005, KT008, KT009, KT011, KT012, KT013, KT014, KT015, KT016, KT017, KT018, KT019,KT020, KT022, KT023, KT024, KT025, E001, E002, E003, E004, E005, E006, E007, E008,E009, E010, E011, E012, E013, E015, E016, E019, E020, E021, MW001, MW003, MW004, MW006, MW007, MW008, MS001, MS002, MS003, MS004, MS005, MS006, KB001, KB002, KB003, KB004, KB005, KB006, KB008, KB009, KB010, KB011,KB012, KB013, KB015, KB014, KB016, KB017, KB018, KB019, KB020, KB021, KB022, KB023,KB024, KB025, KB026 and KB0027 (Fig 6).

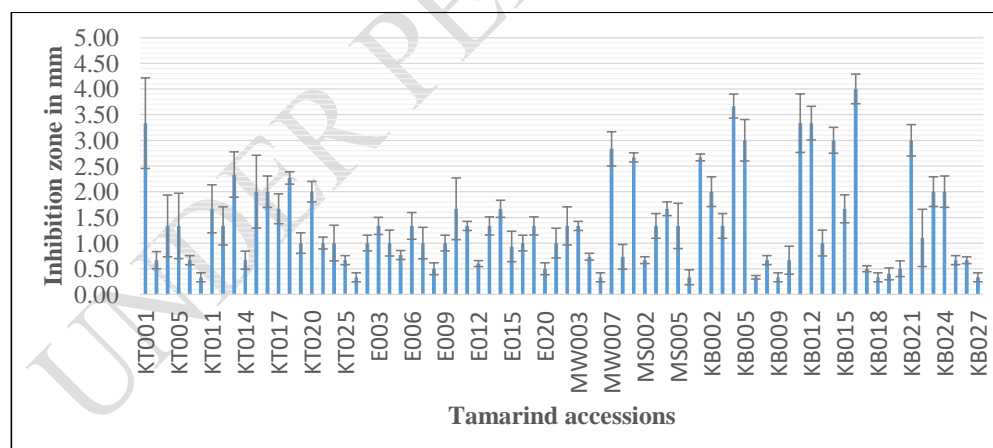


Figure 6: Inhibition of *Pseudomonas aeruginosa* by water leaf extract of tamarind from semi-arid Eastern Kenya

Inhibition of *P. aeruginosa* by tamarind leaves extracted using water from semi-arid Eastern Kenya

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Leaf Extracts of accessions KT001, KB004, KB005, KB011, KB012, KB014 and KB016 had inhibition zones greater than kanamycin, gentamycin, streptomycin, ampicillin, and co-trimoxale (Table 8).

Table 58: Inhibition of *P. aeruginosa* by water leaf extract of tamarind leaves extracted using water from semi-arid Eastern Kenya compared to common antibiotics.

Samples	Mean (mm)	Samples	Mean (mm)
KT001	3.33±0.88	Chloramphenicol	6.00±2.00
KB004	3.67±0.23	Kanamycin	1.00±0.23
KB005	3.00±0.40	Sulfamethoxazole	4.67±1.33
KB011	3.33±0.57	Gentamycin	1.67±0.33
KB012	3.33±0.33	Streptomycin	1.00±0.12
KB014	3.00±0.25	Tetracycline	20.67±0.67
KB016	4.00±0.29	Ampicillin	1.87±0.23
		Co-trimoxale	1.00±0.10

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Tamarind fruits extracted using water that were active against *P. aeruginosa* were from Kitui, Embu and Kibwezi (Fig7).

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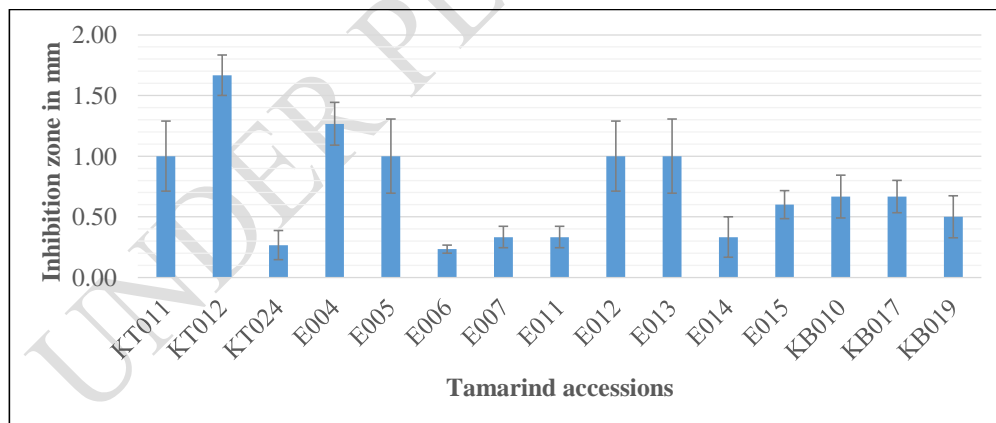


Figure 7: Inhibition of *Pseudomonas aeruginosa* by fruit leaf extract of tamarind from semi-arid Eastern Kenya

Inhibition of *P. aeruginosa* by tamarind fruits extracted using water from semi arid Eastern Kenya

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Fruits extracted using water had less inhibition zones compared to the common antibiotics except for extract of accession KT012 that performed better than streptomycin, kanamycin and co-trimoxale (Table 9).

Table 69: Inhibition of *P. aeruginosa* by tamarind fruits extracted using water from semi-arid Eastern Kenya compared to common antibiotics

Fruit samples	Mean(mm)	Fruit samples	Mean(mm)	Common antibiotics	Mean(mm)
KT011	1.00±0.29	E015	0.60±0.12	Co-trimoxale	1.00±0.10
KT012	1.67±0.17	KB010	0.67±0.18	Chloramphenicol	6.00±2.00
KT024	0.27±0.12	KB017	0.67±0.13	Sulfamethoxazole	4.67±1.33
E004	1.27±0.18	KB019	0.50±0.17	Streptomycin	1.00±0.12
E005	1.00±0.31	E012	1.00±0.29	kanamycin	1.00±0.23
E006	0.23±0.03	E013	1.00±0.31	Ampicillin	1.87±0.23
E007	0.33±0.09	E014	0.33±0.17		
E011	0.33±0.09				

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Inhibition of *E. coli* by tamarind extracts

Inhibition zones by all extracts from the different regions (Kitui, Mwingi, Embu, Kibwezi and Masinga) were not significantly different. The inhibition zones for the leaves and fruits were not significantly different. In addition, extracts from the different extraction solvents were not significantly different. *E. coli* was not inhibited by tamarind extracts.

Inhibition of *S. aureus* by tamarind extracts

Inhibition zones by all extracts from different for the regions (Kitui, Mwingi, Embu, Kibwezi and Masinga) were not significantly different. The inhibition zones for the leaves and fruits were not significantly different. In addition, the extracts from different solvents were not significantly different. Tamarind extracts were not active against *S. aureus*.

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Inhibition activity of tamarind extracts from Kitui, Mwingi, Embu, Kibwezi and Masinga), solvents (Water and methanol) and plant parts (leaves and fruits)

Tamarind extracts from Embu had high inhibition while the least inhibition was from Mwingi (Fig 8A). Water extracts had higher inhibition than methanol (Fig 8B). The leaves as plant part had higher inhibition than fruits (Fig 8 C). Tamarind extracts inhibited *P. aeruginosa* highly followed by *B. subtilis* and there was no inhibition of *E. coli* and *S. aureus* (Fig 8D).

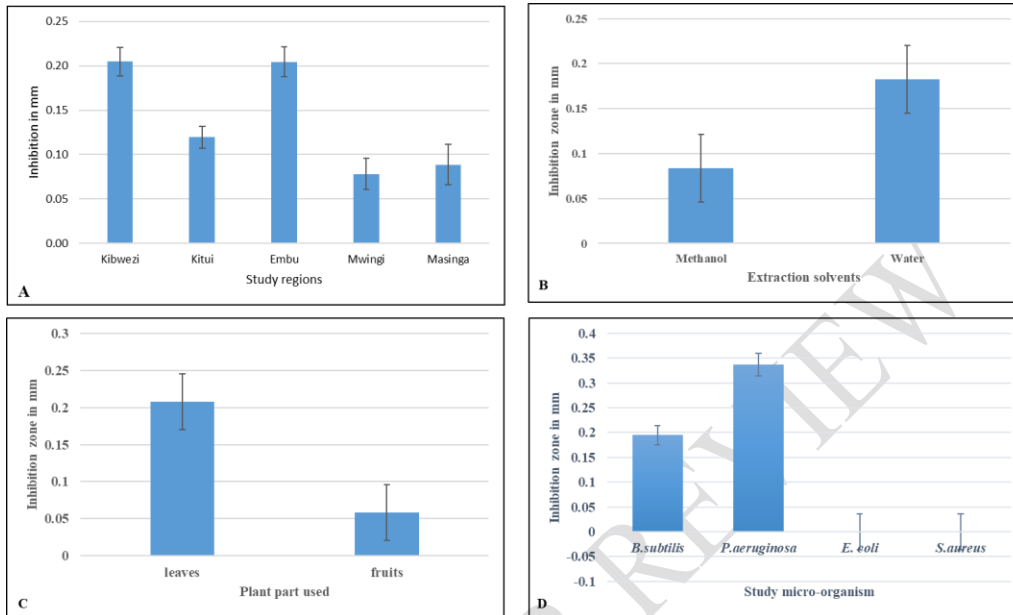


Figure 8: Inhibition zones of Tamarind extracts from semi-arid Eastern Kenya: Study regions (A), extraction solvents (B), plant parts (C), inhibition by micro-organisms (D) parts (C) and study micro-organism (D)

Discussion

Inhibition of bacteria (*B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*) by tamarind extracts

Plant extracts are considered active against micro-organisms when they have inhibition zones more than 6 mm (Saadabi and Ayoub, 2009). In this study, both gram-positive and negative were inhibited by the extracts but all the inhibitions were less than 6 mm.

The five regions of the study had significant inhibition against *B. subtilis* and *P. aeruginosa* with Embu having the highest inhibition, followed by Kibwezi, then Kitui, then Masinga and least inhibition in Mwingi. This ~~which~~ was attributed to the differences in soil types, rainfall availability, temperatures and humidity as ~~this~~ ~~these~~ factors contribute greatly to the availability and different antimicrobial compounds in different plants (Yahia *et al.*, 2020).

The ~~l~~ leaf extracts of tamarind had higher zones of inhibition compared to fruit extracts which was contrary to the reports by Abdallah and Muhammad, (2018) who reported that tamarind fruits had a higher zones of inhibition than the leaves. Similarly, reports by Nwodo *et al.* (2011) showed that fruit and bark of tamarind which are storage organs had higher inhibition zones.

It was observed that fruits extracted using methanol had no inhibition while reports by Ali *et al.* (2015) indicated high inhibition in fruits extracted using methanol against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. Abdallah and Muhammad, (2018) also indicated that tamarind fruits extracted using methanol were effective against *E. coli*. Paul das and Banerjee, (2014) reported

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that tamarind fruits extracted using methanol were effective against *B. subtilis* with ~~and~~ inhibition zone of 15.6 mm which was higher compared to 1.66 mm from ~~my-this~~ study.

Aqueous fruit extracts exhibited insignificant zones of inhibition but report by Ali *et al.* (2015) revealed there was no inhibition. Aliyu *et al.* (2017) and Compean and Ynalves, (2014) reported that aqueous fruit extracts of tamarind were active against *S. aureus* and *E. coli* which was contrary to ~~my-this~~ findings where *S. aureus* and *E. coli* were not inhibited at all. Aqueous leaf extracts were active against *B. subtilis* and *P. aeruginosa* but the findings of Ali *et al.* (2015) revealed that the extracts were inactive against all micro-organisms. Different inhibition ability could be associated with different composition of antimicrobial compounds in different regions (Yahia *et al.*, 2020).

Water extracts had a significant inhibition compared to methanol. This is supported by ~~findings of the study by~~ Hijazi *et al.* (2013) who reported that polar solvents had a higher ability to extract more compounds though this would have a combination of high numbers of impurities. This was in agreement with the findings by Esimone *et al.*, (2012). Saadabi and Ayoub, (2009) also showed that water extracts inhibited ~~of~~ seven strains of *S. aureus*.

Water and methanol solvents were able to extract compounds that were active against the microbes. In this study, water had significant inhibition compared to methanol. This finding was in agreement with Obeidat *et al.* (2012) who reported that water extracts of *A. discondis* had a high inhibition against *P. aeruginosa*. Conversely reports by Mudzengi *et al.* (2017) showed that aqueous extracts of *D. cinera*, *S. persica* and *C. mponne* inhibited *E. coli* higher than *S. aureus*. This could be associated with the polarity of water to extract and dissolve more antimicrobial compounds than methanol (Thouri *et al.*, 2017).

Methanol extracts had the least inhibition against the pathogens. This finding was contrary to the reports by Bacon *et al.* (2016) who revealed that most antimicrobial compounds of Japapeno were extracted using methanol had a high inhibition against the pathogens. Additionally Alo *et al.* (2012) reported that *Ocinum gratissimum* and *Vernonia amydalina* extracted using methanol highly inhibited *E. coli*. Experiments by Mariita *et al.* (2011) showed that methanol extracts of *T. africanum*, *B. angustifolia*, *S. multiflorus*, *A. nilotica* and *G. simi* had high inhibition against *S. aureus*, *E. coli* and *P. aeruginosa*.

Commercial antibiotics had higher inhibition than most of the extracts. These results were similar to Abdallah -and Muhammad, (2018) report. Tamarind extracts hardly inhibited *E. coli* and *S. aureus* which indicates that these extracts could not be used in treating diseases caused by the two micro-organisms. Extracts of KT001, KB004, KB005, KB011, KB014, KB016, E008 and E014 could be exploited more as they were effective against *P. aeruginosa* than K, Gen, S, Amp and COT.

Conclusion

Tamarind extracts of KB004, KB005, KB011, KB012, KB014, KB015 E008 and E014 showed antimicrobial activity against *B. subtilis* and *P. aeruginosa*. However, they did meet the required threshold to be used as alternative medicine. Tamarind extracts were not effective against *E. coli* and *S. aureus* and horticultural fungal pathogens of *A. solani*, *P. digitatum* and *C. gloeosporioides*

Recommendation

Activity of tamarind extracts against *B. Subtilis-subtilis* and *P. aeruginosa* is important in ethnobotany. However, further study is necessary to identify antimicrobial compounds in

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tamarind parts such as roots and bark using other extraction solvents. Additionally, testing tamarind extracts against plant bacterial pathogens is recommended.

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References

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