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Anti-microbial Activity of Tamarind (*Tamarindus indica* L.) Extracts from Semi-arid Eastern Parts of Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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. Tamarind (*Tamarindus indica*) belongs to the Leguminosae family and subfamily Fabaceae. Its native to Africa, Asia and S, America. Tamarind tree produces pod like edible fruits that are widely used in cuisines globally. The fruits have been reported to be therapeutic in several pharmacopoeias. The seeds have been explored in the treatment of diabetes, fevers, intestinal infections and diarrhea. In Kenya, tamarind is present in the arid and semi-arid areas and there is limited information on its antimicrobial activity. This study aimed at evaluating the antimicrobial activity of leaf and fruit extracts from tamarind trees growing in semi-arid Eastern Kenya. 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

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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 24 hrs. 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. The results 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 in extraction solvents against *B. subtilis and P. aeruginosa*. 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 than *B. subtilis*; also, water fruit extracts from accessions E008 and E014 had a higher inhibition than *B. subtilis*. Methanol leaf extracts from accessions KT012, E001, KB008 and KB011 had higher inhibition against *P. aeruginosa* compared to Streptomycin, Kanamycin, and Co-trimoxazole. Water fruit extracts from the accession of KT012 had a higher inhibition of *P. aeruginosa* compared to Streptomycin, Kanamycin, and Co-trimoxazole. Water leaf extracts from accessions of KT001, KB004, KB005, KB011, KB012, KB014 and KB016 had a higher inhibition of *P. aeruginosa* compared to kanamycin, gentamycin, streptomycin, ampicillin, and co-trimoxazole. Tamarind extracts did not inhibit *S. aureus* and *E. coli*. In conclusion, Eastern Kenyan tamarind had limited potential against *B. subtilis* and *P. aeruginosa*.

Keywords: Anti-microbial; Eastern Kenya; tamarind; methanol; water.

1. INTRODUCTION

Tamarind (*Tamarindus indica* L.) is an evergreen fruit tree that serves a both as a fruit and forest tree. It belongs to leguminosae family and has it origin in Africa, Asia and S. America [1,2]. In Africa its widely distributed in Bukina Faso, Mali, Cameroon, Ghana, Cameroon Senegal, Ethiopia, Kenya, Uganda, Tanzania, Sudan. It is cultivated in parklands, arid and semi-arid areas, along roads. In Kenya it's in abundance in semi-arid areas of Eastern, North Eastern and Nyanza [3]. The tree has been used for many years to control bacterial pathogens as different parts of tamarind contain different medicinal properties [4]. The antimicrobial properties of tamarind increased its ethnobotanical use in Latin America, Asia and Africa [5]. Tamarind fruit extracts are used as refrigerant in fever and as laxatives and carminatives alone or as a combination. In South East Asia the pulp has been used to cure sore throats [6]. Tamarind pulp is composed of tartaric acid, malic acid, citric acid, pectin gum, potassium bitartarate and paranchymatous fibre [7].

In West Africa, tamarind has been used as food and in herbal therapies [8]. In Nigeria, the pulp is used in production of local drink, preservation of food and general traditional medicine as a drug carrier. A combination of tamarind with other herbs was reported to be effective against constipation, fever and sore throats [9].

Most rural communities worldwide depended on traditional medicines for health solutions [9], which were more effective compared to the predominant synthetic drugs popularly found in urban areas where resistance to conventional medicine was a challenge. Plant extracts with biologically active compounds were reported to offer new sources of anti-bacterial compounds [9,10].

2. MATERIALS AND METHODS

2.1 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 removed. The leaves and the fruits were pulverized separately. Twenty grams of the pulverized leaves and fruits from each accession were weighed and each dissolved in 120 ml of each solvent. This was extracted sequentially using methanol and water as described by Uthayarasa et al. [11]. The extract was dried using a rotary evaporator at 30-40°C and 0.2g of the extract was dissolved in 1ml of the extracting solvent as described by Predrag et al. [12] and stored at 4°C.

2.2 Pathogen Inoculation

Two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus)* and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas 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 [13]. 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.

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

3. 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 solvents at $p<0.05$.

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* and *S. aureus*.

The methanol leaf extracts that were active against *B. subtilis* included accessions of KT007, KT011, E004, E008, E009, E010, E012, E015, E017, E020, E021, MW002, MW005, MW006, MW010. 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, and KB016). KB 003, KB004, KB014 and KB016 had inhibition equivalent to ampicillin while the other extracts had inhibition lower than the common antibiotics (Table 4).

Table 1. Analysis on Inhibition of *Bacillus 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

Table 2. Analysis on inhibition of *Pseudomonas 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

Table 3. Inhibition of *Bacillus subtilis* with tamarind methanol leaf extracts from semi-arid Eastern Kenya

Samples	**KT007**	**KT011**	**MW002**	**MW005**	**MW006**	**MW01**	**KB002**	**E020**
Inhibition zone (mm)	**1.47±0.29**	1.07±0.58	1.17±0.44	1.33±0.88	1.00±0.58	1.67±0.88	1.17±0.17	**2.67±0.67**
Samples	**E008**	E009	E010	E012	E015	E017	E021	E004
Inhibition zone (mm)	**2.33±0.33**	1.33±0.33	1.33±0.33	1.93±0.07	1.33±0.67	1.67±0.33	1.17±0.44	1.33±0.67
Antibiotics	**Gen**	**T**	**Amp**	**COT**	**C**	**SX**	**S**	**K**
Inhibition zone (mm)	22.67±0.67	22.33±0.33	**1.33±0.33**	23.67±0.88	19.33±1.33	2.67±0.44	21.67±1.20	20.67±0.67

Key: KB-Kibwezi, E-Embu, KT-Kitui, MW-Mwingi, Gen-Gentamycin, T- Tetracyclin, COT- Co-trimoxazole, C- Chloromphenical, SX-Sulfamethoxazole, S- Streptomycin, K- Kanamycin

Table 4. Inhibition of *Bacillus subtilis* with tamarind methanol fruit extracts from semi-arid Eastern Kenya

Samples	**KB001**	**KB002**	**KB003**	**KB004**	**KB005**	**KB006**	**KB007**	**KB008**
Inhibition zone (mm)	1.33±0.49	1.00±0.21	1.33±0.27	1.33±0.24	1.17±0.33	1.00±0.12	1.00±0.12	1.00±0.20
Samples	**KB009**	KB010	KB011	KB012	KB013	KB014	KB016	
Inhibition zone (mm)	1.00±0.15	1.00±0.06	1.00±0.15	1.00±0.12	1.00±0.25	1.33±0.24	1.33±0.27	
Antibiotics	**Gen**	**T**	**Amp**	**COT**	**C**	**SX**	**S**	**K**
Inhibition zone (mm)	22.67±0.67	22.33±0.33	1.33±0.33	23.67±0.88	19.33±1.33	2.67±0.44	21.67±1.20	20.67±0.67

Key: KB-Kibwezi, E-Embu, KT-Kitui, MW-Mwingi, Gen-Gentamycin, T- Tetracyclin, COT- Co-trimoxazole, C- Chloromphenical, SX-Sulfamethoxazole, S- Streptomycin, K- Kanamycin

Table 5. Inhibition of *Bacillus subtilis* with tamarind water leaf extracts from semi-arid Eastern Kenya

Samples	**KB001**	**KB002**	**KB003**	**KB004**	**KB005**	**KB007**	**KB009**	**E005**
Inhibition zone (mm)	1.33±0.33	1.07±.58	1.33±0.33	1.33±0.33	1.17±0.44	1.17±0.17	1.00±0.5	0.53 ± 0.03
Samples	**KB011**	KB012	KB013	KB014	KB016	KB006	KB008	KB015
Inhibition zone (mm)	1.00±0.5	1.00±0.42	1.00±0.29	1.33±0.33	1.33±0.60	0.77±0.15	0.83±0.34	0.67±0.17
Antibiotics	**Gen**	**T**	**Amp**	**COT**	**C**	**SX**	**S**	**K**
Inhibition zone (mm)	22.67±0.67	22.33±0.33	1.33±0.33	23.67±0.88	19.33±1.33	2.67±0.44	21.67±1.20	20.67±0.67

Key: KB-Kibwezi, E-Embu, Gen-Gentamycin, T- Tetracyclin, COT- Co-trimoxazole, C- Chloromphenical, SX-Sulfamethoxazole, S- Streptomycin, K- Kanamycin

Table 6. Inhibition of *Bacillus subtilis* with tamarind water extracts from semi-arid Eastern Kenya

Samples	**KT015**	**E003**	**E004**	**E007**	**E008**	**E013**	**E014**	**KB007**
Inhibition zone (mm)	1.33±0.17	1.33±0.33	1.33±0.44	1.33±0.33	**4.00±0.58**	1.67±0.33	**2.67±0.33**	1.13±0.24
Antibiotics	**Gen**	**T**	**Amp**	**COT**	**C**	**SX**	**S**	**K**
Inhibition zone (mm)	22.67±0.67	22.33±0.33	1.33±0.33	23.67±0.88	19.33±1.33	2.67±0.44	21.67±1.20	20.67±0.67

Key: KB-Kibwezi, E-Embu, KT-Kitui, Gen-Gentamycin, T- Tetracyclin, COT- Co-trimoxazole, C- Chloromphenical, SX-Sulfamethoxazole, S- Streptomycin, K- Kanamycin

Table 7. Inhibition of *Pseudomonas aeruginosa* by tamarind methanol leaf extracts from semi-arid Eastern Kenya

Samples	**KT012**	**E001**	**E020**	**KB008**	**KB010**	**KB011**	**KT022**
Inhibition zone (mm)	**1.67±0.44**	**1.67±0.44**	0.67±0.13	1.17±0.33	1.00±0.29	1.33±0.17	0.67±0.27
Antibiotics	**Gen**	**K**	**Amp**	**COT**	**C**	**SX**	**S**
Inhibition zone (mm)	1.67±0.33	**1.00±0.23**	1.87±0.23	**1.00±0.10**	6.00±2.00	4.67±1.33	**1.00±0.12**

Key: KB-Kibwezi, E-Embu, KT-Kitui, Gen-Gentamycin, T- Tetracyclin, COT- Co-trimoxazole, C- Chloromphenical, SX-Sulfamethoxazole, S- Streptomycin, K- Kanamycin

Table 8. Inhibition of *Pseudomonas aeruginosa* by water leaf extract of tamarind from semi-arid Eastern Kenya

Samples	**KT001**	**KB004**	**KB005**	**KB011**	**KB012**	**KB014**	**KB016**
Inhibition zone (mm)	3.33±0.88	3.67±0.23	3.00±0.40	3.33±0.57	3.33±0.33	3.00±0.25	4.00±0.29
Antibiotics	**Gen**	**K**	**Amp**	**COT**	**C**	**SX**	**S**
Inhibition zone (mm)	**1.67±0.33**	**1.00±0.23**	1.87±0.23	**1.00±0.10**	6.00±2.00	4.67±1.33	**1.00±0.12**

Key: KB-Kibwezi, KT-Kitui, Gen-Gentamycin, T- Tetracyclin, COT- Co-trimoxazole, C- Chloromphenical, SX-Sulfamethoxazole, S- Streptomycin, K- Kanamycin

Table 9. Inhibition of *Pseudomonas aeruginosa* by tamarind fruits extracted using water from semi-arid Eastern Kenya

Samples	**KT011**	**KT012**	**E004**	**E005**	**E012**	**E013**
Inhibition zone (mm)	1.00±0.29	**1.67±0.17**	1.27±0.18	1.00±0.31	1.00±0.29	1.00±0.31
Antibiotics	K	**Amp**	COT	C	SX	S
Inhibition zone (mm)	**1.00±0.23**	1.87±0.23	**1.00±0.10**	6.00±2.00	4.67±1.33	**1.00±0.12**

Key: E-Embu, KT-Kitui, Gen-Gentamycin, T- Tetracyclin, COT- Co-trimoxazole, C- Chloromphenical, SX-Sulfamethoxazole, S- Streptomycin, K- Kanamycin

Tamarind water leaf extracts that were active against *B. subtilis* were from Embu (E005) and Kibwezi (KB001, KB002, KB003, KB004, KB005, KB006, KB007, KB008, KB009, KB011, KB012, KB013, KB014, KB015, KB016. 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 (KT015,), Embu (E003, E005, E006, E007, E008, E013, E014,) and Kibwezi (KB007). 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 (KT0012, KT022) Embu (E001, and E020) and Kibwezi (KB008, KB010, KB011) were active against *P. aeruginosa* accessions. 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-trimoxazole (Table 7).

Leaves extracted using water that were active against *P. aeruginosa* included extracts of accessions; KT001, KB004, KB005, KB011, KB012, KB015, KB014, and KB016). Leaf Extracts of accessions KT001, KB004, KB005, KB011, KB012, KB014 and KB016 had inhibition zones greater than kanamycin, gentamycin, streptomycin, ampicillin, and co-trimoxazole (Table 8).

Tamarind fruits extracted using water that were active against *P. aeruginosa* were from Kitui, Embu and Kibwezi. Fruits extracted using water had less inhibition zones compared to the common antibiotics except for extract of accession KT012 which performed better than streptomycin, kanamycin and co-trimoxazole (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* and *S. aureus* were not inhibited by tamarind extracts.

Tamarind extracts from Embu had high inhibition while the least inhibition was from Mwingi (Fig. 1A). Water extracts had higher inhibition than methanol (Fig. 1B). The leaves had higher inhibition than the fruits (Fig. 1C). Tamarind extracts inhibited *P. aeruginosa* highly followed by *B. subtilis* while there was no inhibition in *E. coli* and *S. aureus* (Fig. 1D).

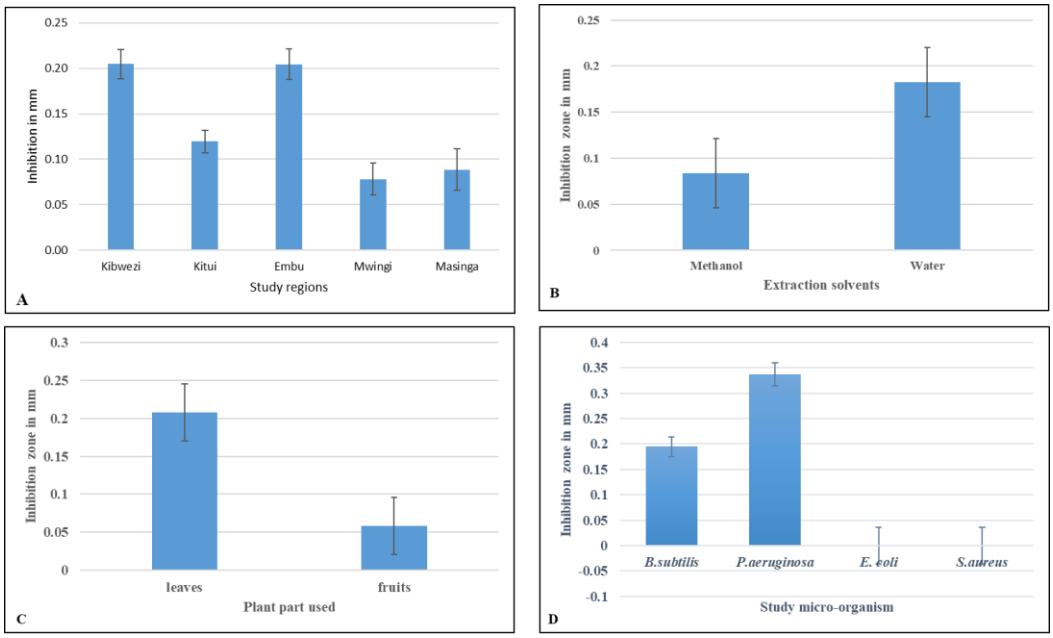

Fig. 1. Tamarind extracts from semi-arid Eastern Kenya: study regions (A), extraction solvents (B), plant parts (C), inhibition by micro-organisms (D)

4. DISCUSSION

Plant extracts are considered active against microorganisms when they have inhibition zones of more than 6 mm [14]. 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, Kitui, Masinga and least inhibition in Mwingi. This was attributed to the differences in soil types, rainfall, temperatures and humidity as these factors contribute greatly to the availability and different antimicrobial compounds in different plants Yahia et al. [15].

The leaf extracts of tamarind had higher zones of inhibition compared to fruit extracts which was contrary to the reports by Abdallah and Muhammad [16] that tamarind fruits had a higher zones of inhibition than the leaves. Similarly, reports by Nwodo et al. [8] 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) [17] indicated high inhibition in fruits extracted using methanol against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa*. Abdallah and Muhammad [16] also indicated that tamarind fruits extracted using methanol were effective against *E. coli*. Banerjee et al. [10] reported that tamarind fruits extracted using methanol were effective against *B. subtilis* with inhibition zone of 15.6 mm which was higher compared to 1.66 mm from this study.

Aqueous fruit extracts exhibited insignificant zones of inhibition but report by Ali et al. [17] revealed there was no inhibition. Aliyu et al. [18] and Compean & Ynalves [19] reported that aqueous fruit extracts of tamarind were active against *S. aureus* and *E. coli* which was contrary to this finding 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. [17] 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. [15].

Water extracts had a significant inhibition compared to methanol. This is supported by findings of Hijaz et al. [20] 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. [21]. Saadabi et al. [14] also showed that water extracts inhibited seven strains of *S. aureus.*

Water and methanol solvents were able to extracts 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. [22] who reported that water extracts of *A. discondis* had a high inhibition against *P. aeruginosa*. Conversely reports by Mudzengi et al. [23] showed that aqueous extracts of *Dichrostachys cinera*, and *Salvadora persica* 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. Bacon et al. [24].

Methanol extracts had the least inhibition against the pathogens. This finding was contrary to the reports by Thouri et al. [25] who revealed that most antimicrobial compounds of Japapeno were extracted using methanol had a high inhibition against the pathogens. Additionally Alo et al. [26] reported that *Ocinum gratissimum* and *Vernonia amydalina* extracted using methanol highly inhibited *E. coli.* Experiments by Mariita et al. [27] showed that methanol extracts of *Thilachium africanum, Bacharis angustifolia, Scadoxus multiflorus* and *Acacia nilotica* 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 kanamycin, gentamycin, streptomycin, ampicillin and Co-trimoxazole.

5. 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*

6. RECOMMENDATION

Activity of tamarind extracts against *B. subtilis* and *P. aeruginosa* is important in ethnobotany. However, further study is necessary to identify antimicrobial compounds in tamarind parts such as roots and bark using other extraction solvents.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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