

ANTI-MICROBIAL ACTIVITY OF TAMARIND (*Tamarindus indica*) EXTRACTS FROM SEMI-ARID EASTERN PARTS OF KENYA

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 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

Introduction

Tamarind (*Tamarindus indica*) 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 bitartrate 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].

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 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 [11]. The extract was dried using a rotary evaporator at 30-40 °C and 0.2 g of the extract was dissolved in 1ml of the extracting solvent as described by [12] and stored at 4 °C.

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 [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.

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.

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 $p < 0.05$.

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 |

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.

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 |

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*, *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).

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

| Samples | KT007 | KT011 | MW002 | MW005 | MW006 | MW01 | KB002 | E020 |
|-------------------------|------------|------------|-----------|------------|------------|-----------|------------|------------|
| Inhibition zone in 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 in 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 in (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
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: Inhibition of *Bacillus subtilis* with methanol fruit extracts from semi-arid Eastern Kenya

| Samples | KB001 | KB002 | KB003 | KB004 | KB005 | KB006 | KB007 | KB008 |
|-----------------------|------------|------------|-----------|------------|------------|-----------|------------|------------|
| Inhibition zone in 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 in 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 in 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

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).

Table 2: 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 in 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 in 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 in 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

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).

Table 3: Inhibition of *Bacillus subtilis* with tamarind water extracts from semi-arid Eastern Kenya

| Samples | KT015 | E003 | E004 | E007 | E008 | E013 | E014 | KB007 |
|-----------------------|------------|------------|-----------|------------|------------|-----------|------------|------------|
| Inhibition zone in 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 in 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

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).

Table 4: Inhibition of *Pseudomonas aeruginosa* by methanol leaf extracts from semi-arid Eastern Kenya

| Samples | KT012 | E001 | E020 | KB008 | KB010 | KB011 | KT022 |
|-----------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| Inhibition zone in 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 in 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

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).

Table 5: 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 in 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 in 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

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).

Table 6: Inhibition of *Pseudomonas aeruginosa* by tamarind fruits extracted using water from semi-arid Eastern Kenya

| Samples | KT011 | KT012 | E004 | E005 | E012 | E013 |
|-----------------------|------------------|------------------|------------------|-----------|-----------|------------------|
| Inhibition zone in 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 in 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

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. 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).

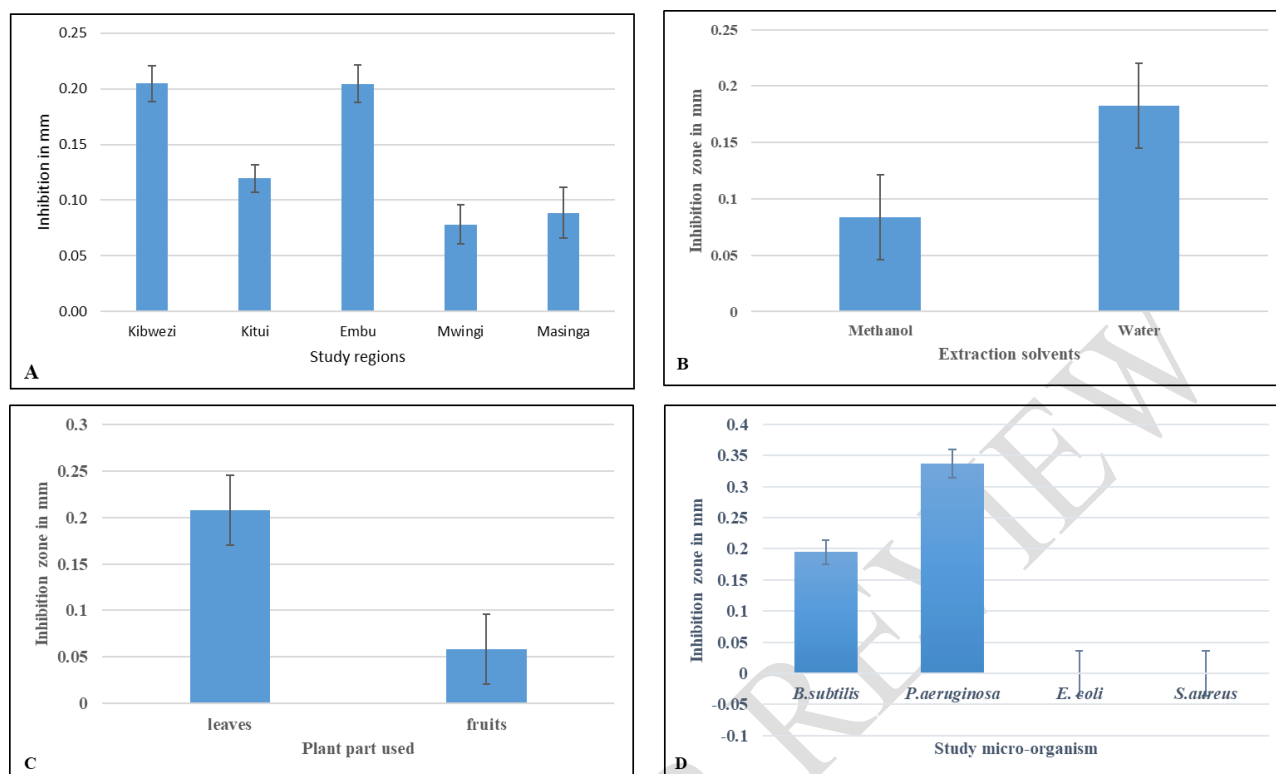


Figure 8: Tamarind extracts from semi-arid Eastern Kenya: study regions (A), extraction solvents (B), plant parts (C), inhibition by micro-organisms (D)

Discussion

Plant extracts are considered active against micro-organisms 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 [15].

The leaf extracts of tamarind had higher zones of inhibition compared to fruit extracts which was contrary to the reports by [16] that tamarind fruits had a higher zones of inhibition than the leaves. Similarly, reports by [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 [17] indicated high inhibition in fruits extracted using methanol against *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa*. [16] also indicated that tamarind fruits extracted using methanol were effective against *E. coli*. [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 [17] revealed there was no inhibition. [18] and [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 [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 [15].

Water extracts had a significant inhibition compared to methanol. This is supported by findings of [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 [21]. [14] also showed that water extracts inhibited 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 [22] who reported that water extracts of *A. discondis* had a high inhibition against *P. aeruginosa*. Conversely reports by [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 [24].

Methanol extracts had the least inhibition against the pathogens. This finding was contrary to the reports by [25] who revealed that most antimicrobial compounds of Japapeno were extracted using methanol had a high inhibition against the pathogens. Additionally [26] reported that *Ocinum gratissimum* and *Vernonia amydalina* extracted using methanol highly inhibited *E. coli*. Experiments by [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

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 not meet the required threshold to be used as alternative medicine. Tamarind extracts were not effective against *E. coli* and *S. aureus*

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

References

1. Gupta, C., Prakash, D., & Gupta, S. (2014). Studies on the antimicrobial activity of Tamarind (*Tamarindus indica*) and its potential as food bio-preservative. *International food research journal*. 21(6), 2437–2441
2. Biswas, A., Melmaiee, K., Elavarthi, S., Jones, J., & Reddy, U. (2019). Characterization of strawberry (*Fragaria* spp.) accessions by genotyping with SSR markers and phenotyping by leaf antioxidant and trichome analysis. *Scientia Horticulturae*. 256, 108561.
3. Kalinganire, A.; Weber, J.C.; Uwamariya, A. and Kone, B. Improving Rural Livelihoods through Domestication of Indigenous Fruit Trees in the Parklands of the Sahel. World Agroforestry Centre. fruit trees Ch 10 4/9/07 13:55 Page 186 www.worldagroforestrycentre.org
4. Escalona-Arranz JC, Péres-Roses R, Urdaneta-Laffita I, Camacho-Pozo MI, Rodríguez-Amado J, Licea-Jiménez I. Antimicrobial activity of extracts from *Tamarindus indica* L. leaves. *Pharmacognosy Magazine*. 2010 Jul;6(23):242.
5. Meléndez PA, Capriles VA. Antibacterial properties of tropical plants from Puerto Rico. *Phytomedicine*. 2006 Mar 13;13(4):272-6.
6. Du Preez, R. J. (2003). Fruits of tropical climates Lesser-known Fruits of Africa. In B. Caballero (Ed.), *Encyclopedia of Food Sciences and Nutrition* (Second Edition) (pp. 2800–2810). Academic Press.
7. Nazir, A., Asghar, A., & Aslam Maan, A. (2017). Chapter 13—Food Gels: Gelling Process and New Applications. In J. Ahmed, P. Ptaszek, S. Basu (Eds.), *Advances in Food Rheology and Its Applications* (pp. 335–353). Woodhead Publishing.
8. Nwodo UU, Obiyeke GE, Chigor VN, Okoh AI. Assessment of *Tamarindus indica* extracts for antibacterial activity. *International Journal of Molecular Sciences*. 2011 Sep 26;12(10):6385-96
9. Abukakar MG, Ukwuani AN, Shehu RA. Phytochemical screening and antibacterial activity of *Tamarindus indica* pulp extract. *Asian Journal of Biochemistry*. 2008;3(2):134-8.
10. Banerjee S.D, Majumder S, Paul Chowdhury B, Goswami A, Halder K, Chakraborty U, Pal NK, Majumdar S. Immune subversion by *Mycobacterium tuberculosis* through CCR5 mediated signaling: involvement of IL-10. *PLoS One*. 2014 Apr 2;9(4): e92477.
11. Uthayarasa K, Pathmanathan K, Jeyadevan JP, Jeeyaseelan EC. Antibacterial activity and qualitative phytochemical analysis of medicinal plant extracts obtained by sequential extraction method. *IJIB*. 2010;10(2):76-81.
12. Predrag, L., Song, H., Cogan, U., Azaizeh, H., & Bomzom, A. (2005). The effects of aqueous extracts prepared from the leaves of *Pistacia lentiscus* in experimental liver disease. *Journal of Ethnopharmacology*. 100(1–2), 198–204.
13. Sandle, T. (2016). 14—Antibiotics and preservatives. In T. Sandle (Ed.), *Pharmaceutical Microbiology* (pp. 171–183). Woodhead Publishing. Oxford
14. Saadabi, A. M. A., & Ayoub, S. M. H. (2009). Comparative bioactivity of *Hydnora abyssinica* A. Braun against different groups of fungi and bacteria. *Journal of medicinal plants research*. 3(4), pp 262–265
15. Yahia Y, Benabderrahim MA, Tlili N, Bagues M, Nagaz K. Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two *Ziziphus* Mill. species. *PloS one*. 2020 May 19;15(5): e0232599.

16. Abdallah MS, Muhammad A. Antibacterial activity of leaves and fruits extract of *Tamarindus indica* against clinical isolates of *Escherichia coli* and *Shigella* at potiskum yobe state, Nigeria. *J Anal Pharm Res.* 2018;7(5):606-9.
17. Ali, B. A., Almagboul, Z. A., & Mohammed, M. O. (2015). Antimicrobial activity of fruits, leaves, seeds and stems extracts of *Ziziphus spina-christi*. *Arabian journal of medicinal and aromatic plants.* 1(2): pp 94–107.
18. Aliyu, A. M., Musa, F. M., & Abeku, R. (2017). Antibacterial activity of fruit and bark extracts of *Tamarindus indica* against *Escherichia coli* and *Staphylococcus aureus*. *Bayero Journal of pure and applied science.* 10(1), 184–187.
19. Compean, K. L., & Ynalves, R. A. (2014). A Review on Antimicrobial Activity of Plant Secondary Metabolites. *Research journal of medicinal plants.* 8(5), 204–213.
20. Hijazi, A., Bandar, H., Rammal, H., Hachem A, A., Saad, Z., & Badran, B. (2013). Techniques for extraction of bioactive compounds from *Lananese urtica dioica*. *American journal of phytomedicine and clinical therapeutics.* 1(6), 507–513.
21. Esimone, C. O., Attama, A. A., Mundi, S. K., Ibekw, N. N., & Chah, F. K. (2012). Antimicrobial activity of *Psidium guajava* Linn. Stem extracts against methicillin-resistant *Staphylococcus aureus*. *African journal of biotechnology.* 11(89), 15556-15559
22. Obeidat, M., Shatnawi, M., Al-alawi, M., Al-Zu`bi, E., Haneer Al-Dmoor, H., Al-Qudah, M., El-Qudah, J., & Otri, I. (2012). Antimicrobial Activity of Crude Extracts of Some Plant Leaves. *Research journal of microbiology.* 7(1), 59–67
23. Mudzengi, C. P., Murwira, A., Tivapasi, M., Murungweni, C., Burumu, J. V., & Halimani, T. (2017). Antibacterial activity of aqueous and methanol extracts of selected species used in livestock health management. *Pharmaceutical Biology.* 55(1), 1054–1060
24. Thouri, A., Chahdoura, H., Arem, A. E., Hichri, A. O., Hassin, R. B., & Achour, L. (2017). Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (*var. Korkobbi and Arechti*). *BMC complementary medicine and therapies.* 17, 248-278
25. Bacon, K., Boyer, R., Denbow, C., O'Keefe, S., Neilson, A., & Williams, R. (2016). Evaluation of different solvents to extract antibacterial compounds from jalapeño peppers. *Food Science and Nutrition.* 5(3), 497–503. PubMed.
25. Alo, M. M., Anyim, C., Igwe, J. C., Elom, M., & Uchenna, D. S. (2012). Antimicrobial activity of water, Ethanol and methanol extracts of *Ocimum gratissimum*, *Vernonia amydalina* and *Afromomum mulagueta*. *Advances in applied science research.* 3(2), 844–848.
26. Mariita, R. M., Ogot, C. K. P. O., Oguge, N. O., & Okemo, P. O. (2011). Methanol Extract of Three Medicinal Plants from Samburu in Northern Kenya Show Significant Antimycobacterial, Antibacterial, and Antifungal Properties. *Research journal of medicinal plants.* 5(1), 54–64.