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Antibacterial Activity of Different Parts of *Tamarindus indica*

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Abstract

The present work assessed the antibacterial activity of *Tamarindus indica* against *B. subtilis*, *S. aureus*, *S. typhi*, *L. acidophilus*, and *E. coli*. All *Tamarindus* methanolic extracts exhibited the strongest antibacterial activity against *L. acidophilus*. Out of the four plant parts that were analyzed, the *Tamarindus* stem extracts (ethanol, methanol, and aqueous) had the strongest antibacterial activity, followed by the fruit, bark, and leaves. *Tamarindus indica* has shown high antibacterial action against *B. subtilis*, *S. aureus*, *S. typhi*, and *E. coli*, in contrast to numerous common drugs listed in Hexa g+7.

Keyword: Tamarind, antimicrobial, Drugs, Laxative

Introduction

Traditional plant-based medicines, which are the primary source of antibacterial chemicals, are used by almost 80% of people in Asia, Latin-America, and Africa. It is well known that these treatments have little adverse effects (Bibitha *et, al*., 2002; Maghrani *et, al*., 2005) [1, 11]. In an effort to provide more economical and efficient treatments, pharmaceutical corporations have recently dedicated a significant amount of time and money to the development of natural medications derived from plants. The hunt for new sources of antibiotics has accelerated due to the growing issue of multidrug resistance in pathogenic microorganisms. Tropical Africa is the native home of the tamarind (*Tamarindus indica*), a leguminous tree in the Fabaceae family. It yields fruits that resemble pods and are used in many different cuisines around the world. In the past, tamarind seeds have been used as a laxative, to treat diarrhea, and to treat intestinal infections, fevers, and diabetes. Poly-hydroxylated chemicals, many of which are flavonoids, are responsible for the tamarind tree's leaves' well-known hepatoprotective qualities Meléndez and Carriles (2006) [12]; El-Siddig *et al*. (2004) [4]. The bark and seeds also have therapeutic properties. Tamarind leaves offer a wide range of ethnobotanical uses due to their antibacterial, antifungal, and antiseptic qualities (Escalona-Arranz *et al*., 2010; Lans, 2007) [5, 9]. Phytochemicals from various plant species, including tamarind, are recognized for their biological activity, including antimicrobial, allelopathic, and antioxidant effects. Tamarind has a long history of use as a medicinal plant, with its fruits frequently noted for their therapeutic value in various pharmacopoeias. Recent studies have investigated the antimicrobial properties of tamarind extracts against six bacterial strains known for causing food spoilage.

Materials and Methods

Bacterial and fungal strains

Five organisms were used for the study: *Salmonella typhimurium* (MTCC no 3224), *Lactobacillus acidophilus* (MTCC no 10307), *Staphylococcus aureus* (MTCC no 3160), *Escherichia coli* (MTCC no 1610), and *Bacillus subtilis*

(MTCC no 441). The standard bacterial stock cultures were supplied by the Institute of Microbial-Technology (IMTECH) culture collection in Chandigarh, India. To assess vitality, each isolate was resurrected using nutritive agar-medium and Sabouraud's dextrose-agar (SDA) media. The stock cultures were maintained on nutritious agar medium (Hi Media, Mumbai, India) and potato-dextrose agar medium. To screen for bacteria, they were grown for 24 hours at 37 °C. After that, they were stored in a refrigerator at 4 °C until sensitivity testing was necessary.

Making a Tamarind-Extract

After being bought from the neighborhood market, the tamarind fruits were cleaned and, if required, descaled before being rinsed in sterile distilled water. One gram of tamarind pulp was dissolved in five milliliters of a 50% aqueous ethanol solvent in a 250 mL Erlenmeyer flask to create the herbal extract. The best solvent for removing the physiologically active phytochemicals from the tamarind pulp was found to be this 50% aqueous ethanol solution. Water, ethanol, isopropanol, petroleum ether, and chloroform were among the other solvents that were tested. After sealing the flasks with aluminum foil and cotton plugs, the tamarind pulp was left to soak in the 50% aqueous ethanol for 48 hours at room temperature, shaking them occasionally. The mixture was soaked, centrifuged for 20 minutes at 3500 g, and then filtered through Whatman filter paper No. 1. The pellet was disposed of, and a rotating vacuum evaporator was used to collect and concentrate the supernatant at a lower pressure until a semisolid residue was obtained. To create a solid powder, this residue was subsequently dried in a crucible at a regulated temperature of 45ºC. Until 500 mg of powder were collected, the extraction procedure was repeated. Prior to being sterilized using a 0.45-micron membrane filter, the powder was weighed, reconstituted in dimethyl sulfoxide (DMSO), and sieved through fine mesh cloth. For antimicrobial sensitivity testing, this extract, which represented a 100% concentration, was kept in a refrigerator at 4 ºC.

Anti-bacterial activity testing using agar well-method (cup-plate method)

The positive control was sodium propionate, a common food preservative, and the negative control was sterile DMSO. After allowing the extract to diffuse for at least an hour, the plates were incubated for 24 hours at 37ºC. To the closest millimeter, the zones of inhibition were measured (NCCLS, 1999). Every experiment was carried out three times. Sterile DMSO served as the negative control, and sodium propionate, a common food preservative, served as the positive control. The plates were incubated for 24 hours at 37 ºC after the extract had been allowed to diffuse for at least an hour. The zones of inhibition were measured to the nearest millimeter (NCCLS, 1999). All experiments were conducted three times.

Results

Antimicrobial Activity of *Tamarindus indica* Plant Parts against *Lactobacillus acidophilus*

The antimicrobial properties of *Tamarindus indica* extracts were tested against Lactobacillus acidophilus. The experimental data indicate that ethanolic extracts from the stem, bark, and fruit exhibited antimicrobial activity, while the leaf extract showed no effect against *L. acidophilus*. The sensitivity of *L. acidophilus* increased with higher extract concentrations, ranging from low (+) to very high (++++) sensitivity. At a concentration of 200 µg, the inhibition zone (IZ) for stem, bark, and fruit extracts was between 11.4 mm and 11.6 mm, compared to 5.4 mm at 50 µg. At 100 µg, the inhibition zone ranged from 8.4 mm to 10.2 mm (Table 1).

Methanolic extracts of *Tamarindus indica* displayed the highest antimicrobial activity against *L. acidophilus*. Inhibition zones increased with extract concentration. At 50 µg, leaf, bark, and fruit extracts had a 5.4 mm inhibition zone. For fruit, leaf, stem, and bark extracts, the corresponding inhibition zones were 8.4 ± 0.24 mm, 9.4 ± 0.40 mm, 10.6 ± 0.75 mm, and 11.6 ± 0.68 mm at 100 µg. For fruit, leaf, stem, and bark methanolic extracts, the inhibition zones grew to 10.4 ± 0.68 mm, 11.8 ± 0.37 mm, 12.8 ± 0.20 mm, and 14.6 ± 0.51 mm at 200 µg. In aqueous extracts, the stem exhibited antimicrobial activity at all concentrations, with inhibition zones ranging from 6.2 mm to 13.6 mm. The fruit showed antibacterial activity only at 200 µg, with a 13.0 mm IZ . Other plant parts (leaf and bark) did not show any inhibitory effects against *L. acidophilus.*

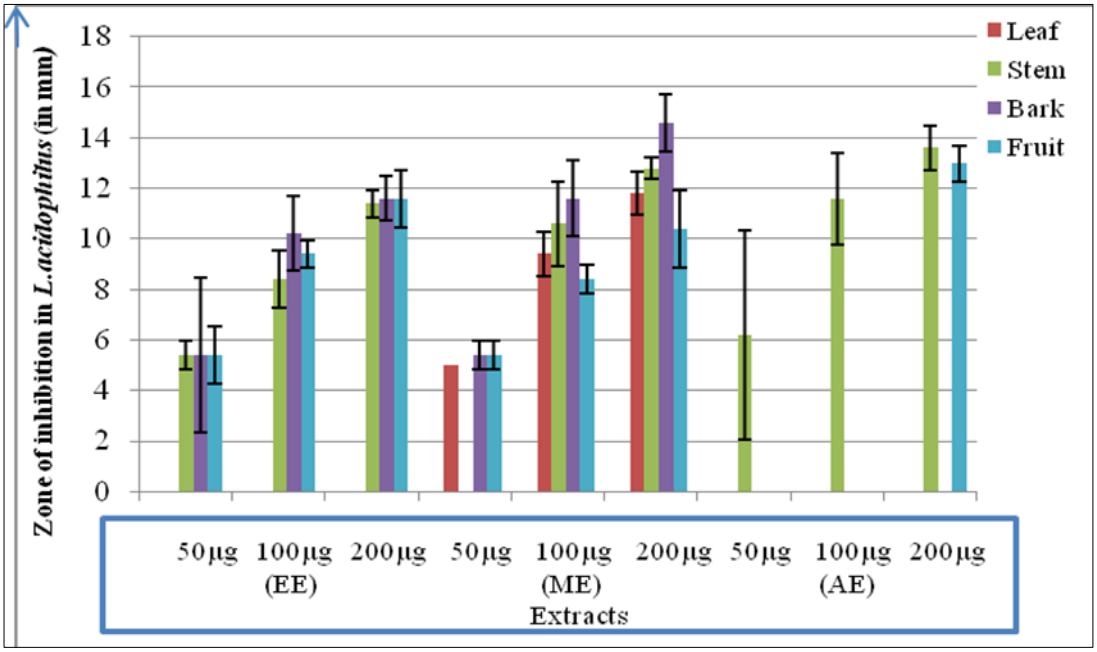


Table 1: Inhibition zone size (Diameter in mm ± SD) against *L. acidophilus* /level of senstivity

Plant parts	Ethanol			Methanol			Aqueous		
	10 µl	20 µl	40 µl	10 µl	20 µl	40 µl	10 µl	20 µl	40 µl
Leaf	0±0.0	0±0.0	0±0.0	5±0.0	9.4±0.40	11.8±0.37	0±0.0	0±0.0	0±0.0
	-	-	-	+	+++	++++	-	-	-
Stem	5.4±0.24	8.4±0.51	11.4±0.24	0±0.0	10.6±0.75	12.8±0.20	6.2±1.85	11.6±0.81	13.6±0.40
	+	++	++++	-	+++	++++	+	++++	++++
Bark	5.4±1.36	10.2±0.66	11.6±0.40	5.4±0.24	11.6±0.68	14.6±0.51	0±0.0	0±0.0	0±0.0
	+	+++	++++	+	++++	++++	-	-	-
Fruit	5.4±0.51	9.4±0.24	11.6±0.51	5.4±0.24	8.4±0.24	10.4±0.68	0±0.0	0±0.0	13±0.32
	+	+++	++++	+	++	+++	-	-	++++

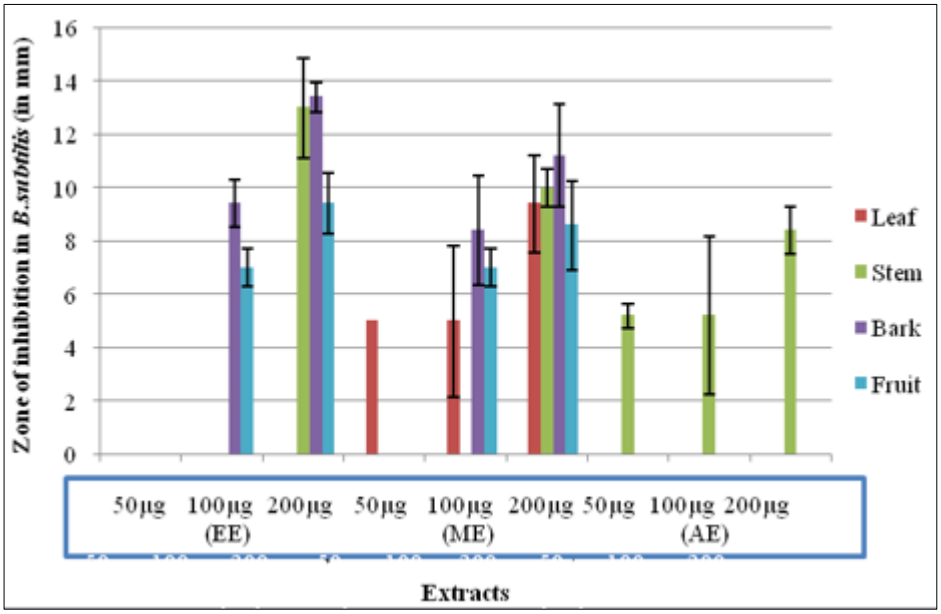
Antibacterial activity of *T. indica* plant parts leaf, stem, bark and fruit against *B. subtilis* strain

At a concentration of 200 µg, ethanolic stem extracts reduced the development of *B. subtilis*, with a zone of inhibition (IZ) of 13.0±0.84 mm. At 100 and 200 µg doses, bark ethanolic extracts displayed IZs of 9.4±0.4 mm and 13.4±0.24 mm, respectively. The activity of the fruit ethanolic extract ranged from 7.0±0.32 mm to 9.5±0.51 mm.

Methanolic leaf extracts inhibited *B. subtilis* growth by up to 5.0 mm at 50 and 100 µg, increasing to 9.4 mm at 200 µg. Methanolic stem extracts showed activity only at 200 µg (10.0±0.32 mm). Bark and fruit methanolic extracts inhibited *B. subtilis* at 100 and 200 µg, with IZs of 8.4±0.93 mm and 11.2±0.86 mm for bark, and 7.0±0.32 mm and 8.6±0.75 mm for fruit. Aqueous stem extracts inhibited *B. subtilis* growth with IZs ranging from 5.2±0.20 mm to 8.4±0.40 mm at different concentrations. Other aqueous extracts showed no inhibition. Among the plant parts, the ethanolic extract of bark exhibited the highest inhibition (13.4 mm), followed by the stem extract (13.0 mm).

Table 2: Inhibition zone size (Diameter in mm ± SD) against B. subtilis /level of sensitivity

Plant parts	Ethanol			Methanol			Aqueous		
	10µl	20µl	40µl	10µl	20µl	40µl	10µl	20µl	40µl
Leaf	0±0.0	0±0.0	0±0.0	5±0.0	5±1.26	9.4±0.81	0±0.0	0±0.0	0±0.0
	-	-	-	+	+	+++	-	-	-
Stem	0±0.0	0±0.0	13±0.84	0±0.0	0±0.0	10±0.32	5.2±0.20	5.2±1.32	8.4±0.40
	-	-	++++	-		+++	+	+	++
Bark	0±0.0	9.4±0.40	13.4±0.24	0±0.0	8.4±0.93	11.2±0.86	0±0.0	0±0.0	0±0.0
	-	+++	++++	-	++	++++	-	-	-
Fruit	0±0.0	7±0.32	9.4±0.51	0±0.0	7±0.32	8.6±0.75	0±0.0	0±0.0	0±0.0
	-	+	+++	-	+	+++	-	-	-



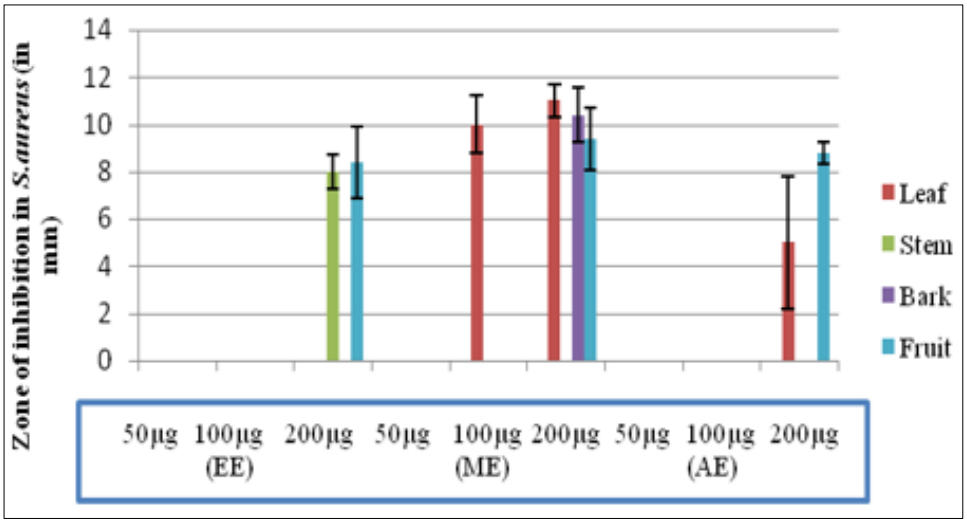
Anti-bacterial Activity of *Tamarindus-indica* Plant Parts Against Staphylococ*cus aureus*:

In the analysis of *Tamarindus indica* extracts against *Staphylococcus aureus*, only the ethanolic extracts of stem and fruit showed anti-microbial activity against *S. aureus*. The stem extract exhibited an inhibition zone of 8.0±0.71 mm, while, the fruit extract showed an IZ of 8.4±1.52 mm at a concentration of 200 µg. Inhibition zones for the leaf extracts were 10±1.22 mm and 11±0.71 mm at doses of 100 µg and 200 µg, respectively. At 200 µg, the bark extract's inhibitory zone measured 10.4±1.14 mm. At 200 µg, the fruit extract showed an inhibitory zone of 9.4±1.34 mm.

The leaf extract at 200 µg concentration showed a low to medium growth inhibition with an inhibition zone of 5.0±2.83 mm. The fruit extract at 200 µg concentration demonstrated a medium level of inhibition with an inhibition zone of 8.8±0.45 mm. The results indicate varying levels of antimicrobial activity against *S. aureus* based on the type of extract and concentration (Table 3).

Table 3: Inhibition zone size (Diameter in mm ± SD) against Saureus/level of sensitivity

Plant parts	Ethanol			Methanol			Aqueous		
	10µl	20µl	40µl	10µl	20µl	40µl	10µl	20µl	40µl
Leaf	0±0.0	0±0.0	0±0.0	0±0.0	10±1.22	11±0.71	0±0.0	0±0.0	5±2.83
	-	-	-	-	+++	+++	-	-	+
Stem	0±0.0	0±0.0	8±0.71	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
	-	-	++	-	-	-	-	-	-
Bark	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	10.4±1.14	0±0.0	0±0.0	0±0.0
	-	-	-	-	-	+++	-	-	-
Fruit	0±0.0	0±0.0	8.4±1.52	0±0.0	0±0.0	9.4±1.34	0±0.0	0±0.0	8.8±0.45
	-	-	++	-	-	+++	-	-	++

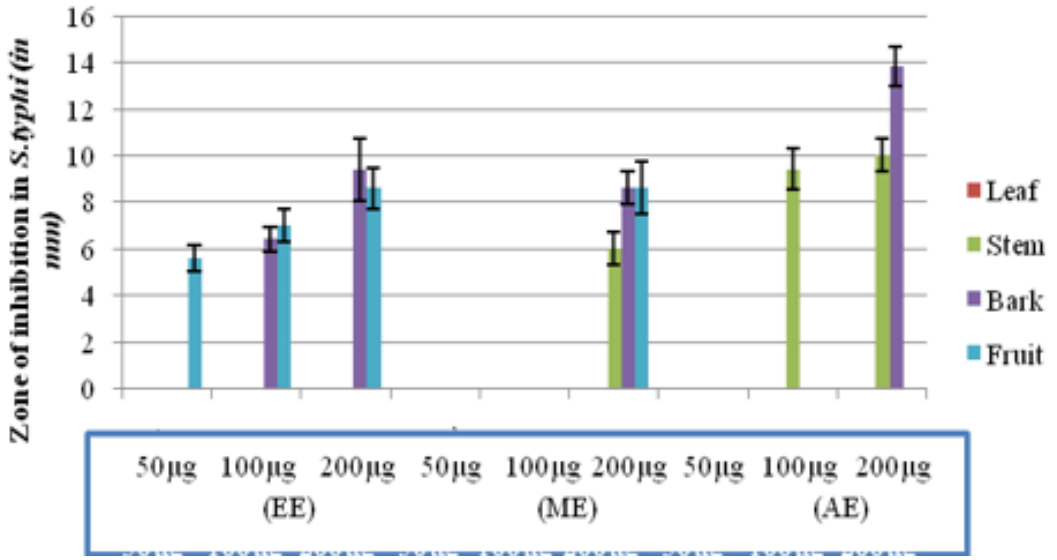


Anti-bacterial Activity of *Tamarindus-indica* Plant Parts Against *Salmonella typhi*

Leaf does not show any activity with either of the extracts and fruit also does not exhibit activity in aqueous extract. No effect was shown by Stem in ethanolic extract and fruit in aqueous extract against *Salmonella typhi*. The stem extract however had inhibition zones ranging from 6.0±0.71 mm to 10 ±0.71mm in methanolic and aqueous extracts (Table 4).

Table 4: Inhibition zone size (Diameter in mm ± SD) against *S. typhi* /level of sensitivity

Plant parts	Ethanol			Methanol			Aqueous		
	10µl	20µl	40µl	10µl	20µl	40µl	10µl	20µl	40µl
Leaf	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0
	-	-	-	-	-	-	-	-	-
Stem	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	6.0±0.71	0±0.0	9.4±0.89	10±0.71
	-	-	-	-	-	+	-	+++	+++
Bark	0±0.0	6.4±0.55	9.4±1.34	0±0.0	0±0.0	8.6±0.71	0±0.0	0±0.0	13.8±0.84
	-		+++	-	-	++	-	-	++++
Fruit	5.6±0.55	7.0±0.71	8.6±0.89	0±0.0	0±0.0	8.6±1.14	0±0.0	0±0.0	0±0.0
	+	+	++	-	-	++	-	-	-

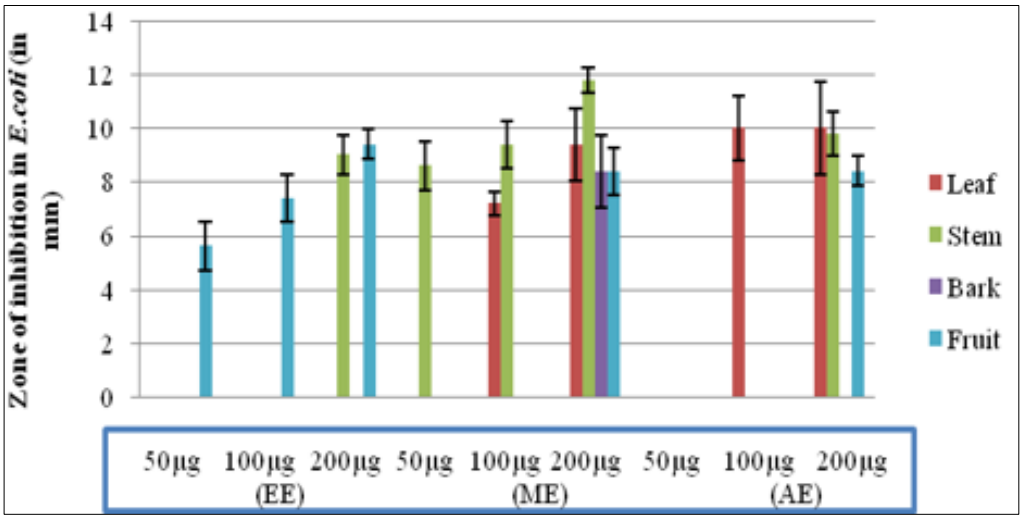


Anti-bacterial Activity of *Tamarindus indica* Plant Parts Against *E. coli*

The leaf and bark extracts showed no antimicrobial activity. The stem extract inhibited *E. coli* growth only at a 200 µg concentration, with a zone of inhibition of 9.0±0.71 mm. The fruit extract demonstrated notable activity, with inhibition zones of 5.6±0.89 mm, 7.4±0.89 mm, and 9.4±0.55mm at 50 µg, 100 µg, and 200 µg concentrations, respectively. Overall, the methanolic extracts of *Tamarindus indica* were more effective against *E. coli* compared to the ethanolic extracts. According to the results in Table 5, the aqueous extracts of *Tamarindus indica* showed antimicrobial activity against *E. coli* at various concentrations: 10.0mm for leaf (100, 200 µg), 9.8mm for stem (200 µg), and 8.4mm for bark (200 µg). *E. coli* displayed relatively high sensitivity to these extracts, with growth inhibited by 9 out of the 12 extracts tested. The most effective extracts were methanolic extracts of leaf, stem, bark, and fruit; aqueous extracts of leaf, stem, and fruit; and ethanolic extracts of stem and fruit. Among the plant parts, the stem and fruit extracts were the most effective, followed by the leaf. The bark extract had the least effect. The maximum inhibition was recorded with the stem methanolic extract, showing a zone of inhibition ranging from 8.4 mm to 11.8 mm, while the leaf aqueous extract had a zone of 10.0 mm (Table 5).

Table 5: Inhibition zone size (Diameter in mm ± SD) against *E. coli* /level of senstivity

Plant parts	Ethanol			Methanol			Aqueous		
	10 µl	**20 µl**	**40 µl**	**10 µl**	**20 µl**	**40 µl**	**10 µl**	**20 µl**	**40 µl**
Leaf	0±0.0	0±0.0	0±0.0	0±0.0	7.2±0.45	9.4±1.34	0±0.0	10±1.22	10±1.73
	-	-	-	-	++	+++	-	+++	+++
Stem	0±0.0	0±0.0	9±0.71	8.6±0.89	9.4±0.89	11.8±0.45	0±0.0	0±0.0	9.8±0.84
	-	-	++	++	+++	++++	-	-	+++
Bark	0±0.0	0±0.0	0±0.0	0±0.0	0±0.0	8.4±1.34	0±0.0	0±0.0	0±0.0
	-	-	-	-	-	++	-	-	-
Fruit	5.6±0.89	7.4±0.89	9.4±0.55	0±0.0	0±0.0	8.4±0.89	0±0.0	0±0.0	8.4±0.55
	+	++	+++	-	-	++	-	-	+++



Discussions

Alkaloids, tannins, saponins, glycosides, flavonoids, anthraquinones, reducing sugars, terpenoids, and phenols were among the substances found in *Tamarindus indica* leaves and fruit extracts after phytochemical screening. The antibacterial qualities of the plant are facilitated by these compounds. In particular, saponins are recognized for their antibacterial qualities (Gonzalez–Lamothe *et al*., 2009; Cowan, 1999) [6, 2], whereas flavonoids have shown anti-inflammatory, anti-hepatotoxic, and antimicrobial activities (Madubunyi, 1995) [10]. Additionally, tannins have antibacterial properties and promote wound healing. Alkaloids are a broad class of nitrogenous compounds that are present in both leaves and fruits. They are employed in a variety of medical applications, including as anesthetics, central nervous system stimulants, and anticancer therapies. Alkaloids have an impact on cell division and metabolism, which may account for their antibacterial properties. Sravanthi *et al*. (2017) [15] found that extracts of *Tamarindus indica* that contained tannins, saponins, alkaloids, and triterpenoidal saponins were effective against both Gram-positive and Gram-negative bacteria. This investigation confirms their findings.

Extracts from *Tamarindus indica* leaves and fruits shown antimicrobial activity in the antibacterial tests; the methanol extract was more effective than the aqueous extract. In particular, the aqueous extract demonstrated a zone of inhibition of 13.8 mm against *S. typhi*, while the methanol extract exhibited a zone of inhibition of 14.6 mm against *L. acidophilus*. The higher solubility of active chemicals in methanol as opposed to water is the reason for the methanol extract's increased activity. This implies that methanol is a more soluble solvent for the active ingredients than other solvents. According to Doughari *et al*. (2006) [3], the plant's bioactive components are also responsible for its antibacterial properties. Different test isolates had differing degrees of susceptibility, according to the study. *L. acidophilus* was the most sensitive to the extracts, with a 12.2 mm zone of inhibition as opposed to 8.5 mm for other bacteria. Our results are consistent with those of Nwodo *et al*. (2011) [13], who also evaluated the antibacterial activity of *Tamarindus indica* fruit pulp, stem bark, and leaf extracts against specific bacterial

strains. They found that the fruit pulp extracts exhibited a wide range of activity, with the ethanolic and hot water extracts demonstrating activity against 90.9% and 86.4% of the test bacterial strains, respectively, and the cold-water extract demonstrating activity against 95.5% of the pathogens.

Conclusion

All methanolic extracts of Tamarindus demonstrated the strongest antimicrobial activity against *L. acidophilus*. Among the four plant parts tested, extracts from the Tamarindus stem (ethanol, methanol, and aqueous) exhibited the greatest antimicrobial properties, followed by the fruit, bark, and leaves. In comparison to several common medications included in Hexa g+7, *Tamarindus indica* has demonstrated strong antibacterial activity against *B. subtilis*, *S. aureus*, *S. typhi*, and *E. coli*. Limited quantities of 50, 100, and 200 µg of the extracts were used in the study; raising these concentrations could improve their efficacy. Future research should focus on quantitatively analyzing the phytochemicals in each extract and their individual effects on microorganisms to potentially identify superior biological molecules for use in antimicrobial drugs.

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