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Antibacterial potentials of *Tamarindus indica* L. leaf extracts against multidrug resistant pathogens

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Abstract

Multidrug resistance (MDR), which has a major effect on patient's morbidity and mortality, is the bacterial resistance to antimicrobial agents not with standing previous sensitivity to them. Due to a number of reasons like irrational use of antibiotics, it has been predicted that, by 2050, the antimicrobial resistance (AMR) will reach to epidemic proportions, becoming the major cause of death. So, the scientific world is searching for potential antibacterial agents from the nature and various plant materials have proven to be one of the promising sources. *Tamarindus indica* L. (Tamarind) which is widely seen in tropical and subtropical zones has shown to possess a number of potential biochemical compounds, many of them yet to be explored, with a wide range of activities including against microorganisms. So, the present study focuses on evaluating the anti-MDR bacterial activity of the methanolic leaf extracts of this plant against *Escherichia coli* and *Staphylococcus aureus*. The minimum inhibitory concentrations (MICs) and the antioxidant activities were also examined. 500 mg/ml and 1000 mg/ml extracts concentrations showed higher zones of inhibition against both the test organisms. The MICs of *E. coli* and *S. aureus* were found to be 7.8 mg and 31.5 mg. The crude leaf extract showed better antioxidant values, 78.73 ± 0.46% of DPPH scavenging and 62.8 ± 0.42% of reducing power was observed on 1000 µg of extract. From this analysis, it has been concluded that, further studies will help in exploring the chances of using potential phytocompounds from *T. indica* in the treatment of MDR infections.

1. Introduction

Antimicrobial resistance (AMR) arises when the pathogens from bacteria, viruses, fungi or parasites develop adapting capabilities in the presence of pharmaceutical agents which were once used to suppress or kill them. AMR has been realized as a serious danger to the public health systems throughout the world as a number of the commercially available antibiotics are not effective any more, in treating infections, and thus posing an unprecedented challenge and hence, an uncertain future in healthcare (Porooshat *et al.*, 2019). AMR infections cause significant diseases, lengthy hospitalizations, increased healthcare expenses, higher second-line medication prices, treatment failures and higher mortality rates. It has been estimated that, currently, the complications and secondary diseases arising from drug-resistant infections claim the lives of around 700,000 individuals every year across the world and by 2050, the AMR mortality rates and primary causes of death will have reached epidemic proportions over the world (Bin *et al.*, 2017). Despite the difficulties of establishing specific death statistics, official researches

propose that around 10 million people would die globally by 2050 if robust and effective antimicrobial resistance tackling measures are not adopted (Chaw *et al.*, 2018). Also, the antimicrobial resistance has been associated to a total cost of above nine billion Euros per annum in Europe alone. According to the CDC (Centers for Disease Control and Prevention), the antibiotic resistance adds a \$20 billion excess in direct healthcare expenditures in the United States every year, not including the estimated \$35 billion in lost productivity (Founou *et al.*, 2017).

Indiscriminate use of antibiotics, lack of adequate diagnostic tools, unprescribed use of antibiotic medications (Lekagul *et al.*, 2019), inferior quality antibiotics, self-treating behaviors of the common public, *etc.* (Robertson *et al.*, 2019) are responsible for the acceleration in the antimicrobial resistance development. Also, the antibiotic usage has been established to be positively connected with increases in GDP and living standards in low and middle-income countries (LMICs) (Chokshi *et al.*, 2019). Microorganisms have established a variety of strategies to overcome the efficacy of medications, allowing them to survive drug exposure (Chatterjee *et al.*, 2018). As a result, the multidrug-resistant Gram-negative bacteria (MDR-GNB) have complicated the treatment of diseases such as urinary tract infections, pneumonia (Shrestha *et al.*, 2018), TB, gonorrhea typhoid fever, *etc.* (Bassetti *et al.*, 2019).

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The scientific world is now searching back in the nature for novel antibiotics that may overcome the resistance development by pathogens including the multidrug resistant bacteria. As they possess a number of potential compounds, plants and several herbal formulations have been widely used in conventional medicinal practices since time unknown (Raghavan *et al.*, 2020). *Tamarindus indica* L. a single species genus belonging to the Fabaceae, is a medium-sized tropical tree with a crown height of 12 to 18 meters (Shaymaafouad Rasheed, 2014; Mbaye *et al.*, 2017), cultivated in various countries including India and has been widely used in treating colds, dysentery, cough, sore throat, rheumatism, furuncles, malaria, stomach disorders, diarrhea, diabetes, jaundice, *etc.*, and has been used as a skin cleanser in the traditional Indian system (Lakhe *et al.*, 2017; Meena *et al.*, 2018; Bhawana Sharma *et al.*, 2021). Therefore, the present study focuses on evaluating the anti-MDR bacterial activity of the *T. indica* methanolic leaf extracts. As there are several reports on antibacterial potential of *T. indica* extracts, this is the first study to investigate the antibacterial potential against multidrug resistant (MDR) bacterial pathogens. The leaves of some species were characterized by higher content of bioactive and nutritional compounds than other other parts (Escalona-Arranz *et al.*, 2010; Bhadoriya *et al.*, 2011; James Ronald Bayoï *et al.*, 2021). Therefore, the leaf extracts were evaluated for antioxidant activity and minimum inhibitory concentration against multidrug resistant *E. coli* and *S. aureus*.

2. Materials and Methods

2.1 Collection and identification of pathogens

Clinical pathogens were collected from throats of infected patients at the male ICU of a tertiary care hospital and the swabs were streaked on nutrient media (Himedia) to cultivate the pathogens. For the cultural identification, the specimens were grown on Eosin Methylene Blue (EMB-for green metallic sheen colonies of *E. coli*) agar and on Mannitol Salt Agar (MSA-for the mannitol fermenting yellow colonies of *S. aureus*) (Mohanty *et al.*, 2013). The identified strains were utilised for further analyses.

2.2 Antibiotic susceptibility test (ABST)

To test the antibiotic susceptibility of the isolated pathogens, disc diffusion (Kirby-Bauer method) protocol was used. The isolated *S. aureus* and *E. coli* were distinctly tested against five antibiotics (ampicillin –25 mcg, methicillin –5 mcg, amoxicillin –30 mcg, tetracycline –30 mcg and streptomycin –30 mcg) on Muller-Hinton Agar (MHA). By measuring the diameters of zones of inhibition (in mm), the antibacterial potentials of the extracts were quantified and interpreted by comparing with the standard chart (as per CLSI). To analyse the results, disk diffusion method susceptibility/resistance interpretations as defined in the National Committee for Clinical Laboratory Standards (NCCLS 2000, Microbiology Systems, Becton Dickinson, USA) was used.

2.3 Collection and processing of plant

The collected *T. indica* leaves were rinsed in distilled water and dried in shade under room temperature. The dried leaves were grounded to powders and kept in sterile container for extraction (Figure 1).



Figure 1: Collection of *T. indica* leaves.

2.4 Extraction of bioactive compounds using Soxhlet apparatus

Powders of *T. indica* leaves were loaded in a porous bag that is made from a cellulose paper in thimble chamber of the Soxhlet apparatus. The solvent-methanol was filled in the extractor and a temperature of 60°C was set and left for 6 h. The extracts were obtained and the solvents were vaporised. The dried extracts were acquired and reserved in sterile containers.

2.5 Antioxidant activity of the plant extracts

2.5.1 DPPH radical scavenging assay

Using the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH), the antioxidant activity of the methanolic *T. indica* leaf extracts were evaluated in terms of its ability to donate hydrogen or scavenging radicals. Several concentrations (100 µg to 1000 µg) of the extract were taken and the total volume was made to 100 µl with methanol. About 3 ml of methanol solution of DPPH (2.4 mg in 100 ml) was mixed and permitted to rest for 30 min at 27°C. The absorbance (OD values) was calculated at 517 nm. Ascorbic acid was used as standard for comparison. The percentage radical scavenging activity of the sample was calculated as follows:

$$\% \text{ DPPH radical scavenging activity} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100$$

The assay was implemented in triplicates and the results were determined in mean $\pm$ SD.

2.5.2 Ferric reducing antioxidant power (FRAP) assay

The ferric reducing antioxidant power assay was used conferring to the technique defined by Benzie and Strain (1996) to estimate the reducing ability of the plant extract. The FRAP reagent comprising 2.5 ml of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 2.5 ml of a 10 mM TPTZ (2,4,6-tri(2-pyridyl)-1,3,5-triazine) solution in 40 mM HCl, and 25 ml of 300 mM acetate buffer (pH 3.6) were prepared and kept at 37°C.

3 ml FRAP reagent was added with 60 µl of the sample. The reaction mixture was set to incubation for 30 min at 37°C and the absorbance (OD values) was measured at 610 nm. FeSO₄ at diverse concentrations (100 µg, 250 µg, 500 µg, 750 µg and 1000 µg) were used for standardization.

2.6 Antibacterial activity using well diffusion method

Antibacterial potentials of the plant extract were estimated against the isolated clinical pathogens *S. aureus* and *E. coli*. Sterile nutrient agar (composition for 100 ml: peptone: 0.5 g; yeast extract: 0.2 g; agar 1.5 g; 0.5 g, sodium chloride: 0.5 g, beef extract: 0.3 g, total pH: 7.0 ± 0.2) plates were inoculated with the clinical specimen by swabbing. To bore wells on the agar surface, 6 mm well borer was used. To each well, 100 µl of the samples were introduced and the plates were incubated in an incubator at 37°C for 48 h. Based on zone of inhibition around the wells in all the nutrient agar plates having test pathogens, the antibacterial activity was recognized. The clear zones were detected and measured in millimetre (mm).

2.7 Evaluation of the minimum inhibitory concentrations (MICs) of the plant extracts

A slight modification of the dilution technique was used to evaluate the minimum inhibitory concentration (MICs). 1 ml of plant extracts was diluted into many concentrations (1.95 mg/ml, 3.9 mg/ml, 7.8 mg/ml, 15.6 mg/ml, 31.5 mg/ml, 62.5 mg/ml, 125 mg/ml and 250 mg/ml, in 1 ml of sterile nutrient broth in test tubes. A 100 µl of *E. coli* culture at 0.5 McFarland standard, was inoculated to the tubes. Correspondingly, this was repeated for *S. aureus*. The tubes were incubated at 37°C for 24 h and observed for growth or turbidity by using unaided eye.

3. Results

3.1 Antibiotic sensitivity test (ABST)

The antibiotic sensitivity test (ABST) of the isolated pathogens was carried out to determine the drug resistance pattern against several commercial drugs. The isolated *E. coli* and *S. aureus* showed resistance to all the antibiotics except streptomycin (Figures 2 and 3). Intermediate resistance was observed for streptomycin (Table1). This confirms that both the isolated pathogens were found to be multidrug resistant.

Table 1: Antibiotic sensitivity test

Sl. No.	Pathogens	Antibiotic	Inhibitory	Interpretation
1	<i>Escherichia coli</i>	Methicillin	-	R
		Ampicillin	-	R
		Tetracycline	-	R
		Amoxicillin	-	R
		Streptomycin	11	I
2	<i>Staphylococcus aureus</i>	Methicillin	-	R
		Ampicillin	-	R
		Tetracycline	-	R
		Amoxicillin	-	R
		Streptomycin	14	I

*S-sensitive; I-intermediate; R-resistant

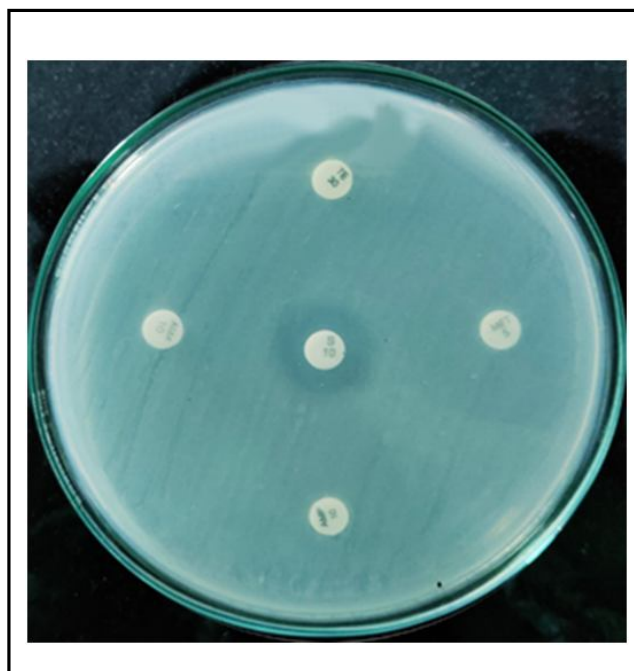


Figure 2: Antibiotic susceptibility of the *E. coli*.

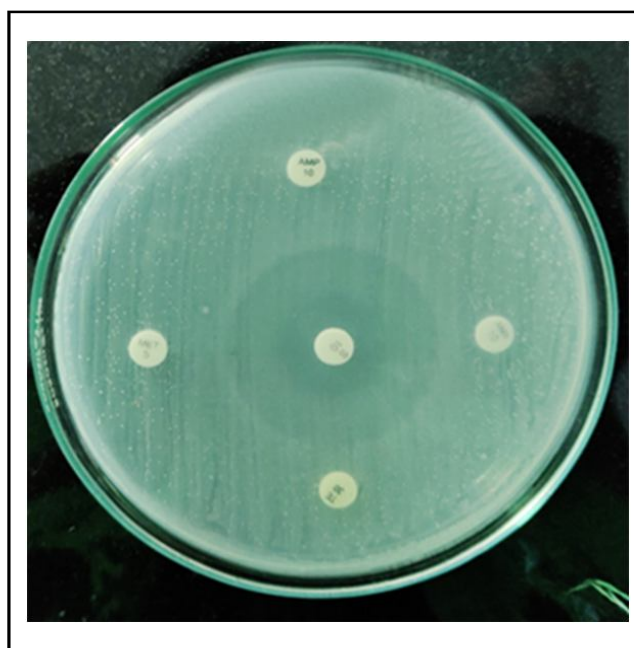


Figure 3: Antibiotic susceptibility of the *S. aureus*.

3.2 DPPH radical scavenging activity

The DPPH radical scavenging activity of the *T. indica* leaf extracts was carried out using five different concentrations (100 µg, 250 µg, 500 µg, 750 µg and 1000 µg). 100 µg, 250 µg, 500 µg, 750 µg and 1000 µg *T. indica* leaf extracts showed 26.08 ± 0.52%, 34.7 ± 1.04%, 50.23 ± 0.68%, 63.23 ± 0.81% and 78.73 ± 0.46% inhibition, whereas ascorbic acid showed 42.63 ± 0.76%, 66.25 ± 0.45%, 75.14 ± 0.37%, 75.14 ± 0.37%, 81.46 ± 0.23% and 94.26 ± 0.18% inhibition (Figure 4).

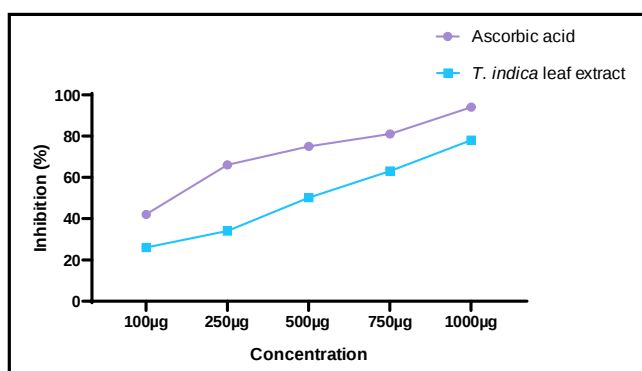


Figure 4: DPPH radical scavenging activity of *T. indica* leaf extracts.

3.3 Ferric reducing antioxidant power

Five different concentrations of *T. indica* leaf extracts were used for evaluating the ferric reducing power. 100 µg, 250 µg, 500 µg, 750 µg and 1000 µg *T. indica* leaf extracts showed 33.66 ± 0.66%, 41.72 ± 1.23%, 54.27 ± 1.14%, 59.29 ± 0.58% and 62.8 ± 0.42% inhibition, whereas ascorbic acid showed 45.26 ± 0.54%, 65.42 ± 0.22%, 71.35 ± 0.35%, 87.77 ± 0.69%, 81.46 ± 0.23% and 96.28 ± 0.47% inhibition (Figure 5).

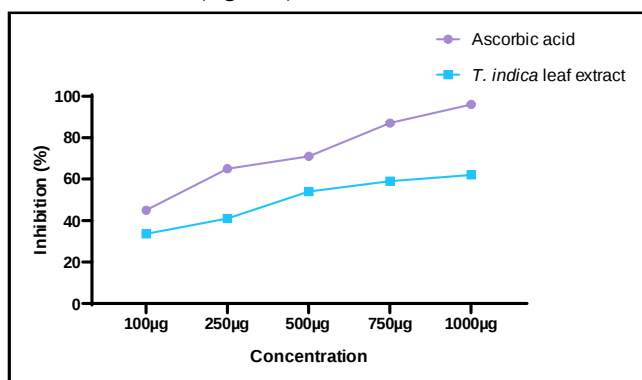


Figure 5: Ferric reducing antioxidant power of *T. indica* leaf extracts.



Figure 6: Antibacterial activity of *T. indica* leaf extracts against *E. coli* using well diffusion.

3.4 Antibacterial analysis

The antibacterial activity of the *T. indica* leaf extracts was evaluated using well diffusion method. Four different concentration (125 mg/ml, 250 mg/ml, 500 mg/ml and 1000 mg/ml) were used for analysis. *T. indica* leaf extracts showed 18.66 ± 0.57 mm and 25.66 ± 1 mm against MDR *E. coli* at 500 mg/ml and 1000 mg/ml concentration (Figure 6).

No inhibitory zones were observed at 125 mg/ml and 250 mg/ml. Similarly, 250 mg/ml, 500 mg/ml, 750 mg/ml and 1000 mg/ml showed 7.33 ± 0.57 mm, 12.66 ± 1.52 mm and 15.33 ± 0.57 mm of inhibitory zones against MDR *S. aureus*. No inhibitory zones were observed at 125 mg/ml (Figures 7 and 8).

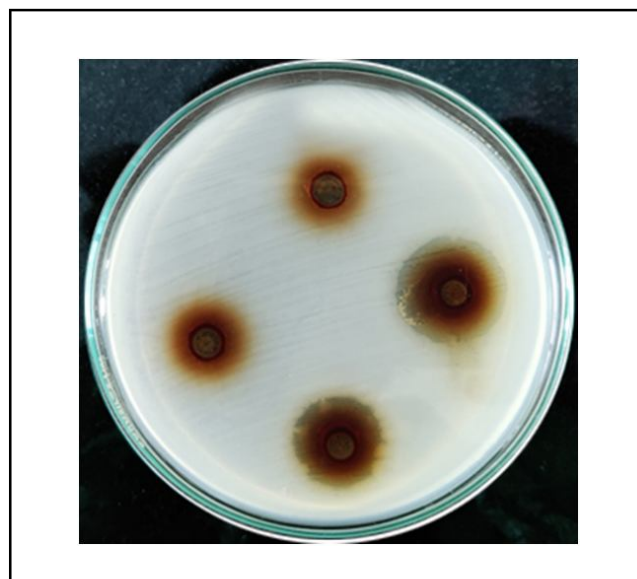


Figure 7: Antibacterial activity of *T. indica* leaf extracts against *S. aureus* using well diffusion.

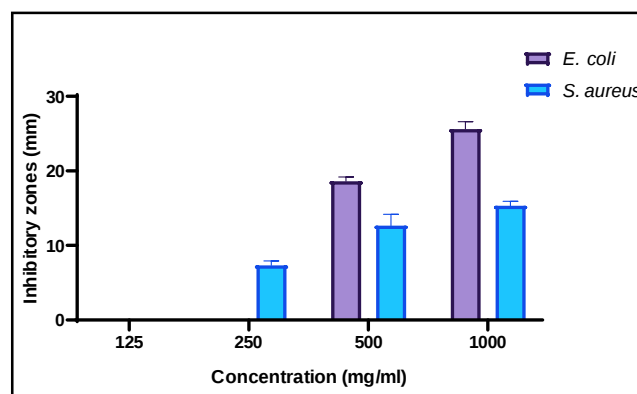


Figure 8: Graphical representation of inhibitory zones of *T. indica* leaf extracts against MDR pathogens.

3.5 Minimum inhibitory concentration analysis

The MIC values of the *T. indica* leaf extracts against *E. coli* and *S. aureus* were found to be 7.8 mg and 31.5 mg (Figures 9 and 10).

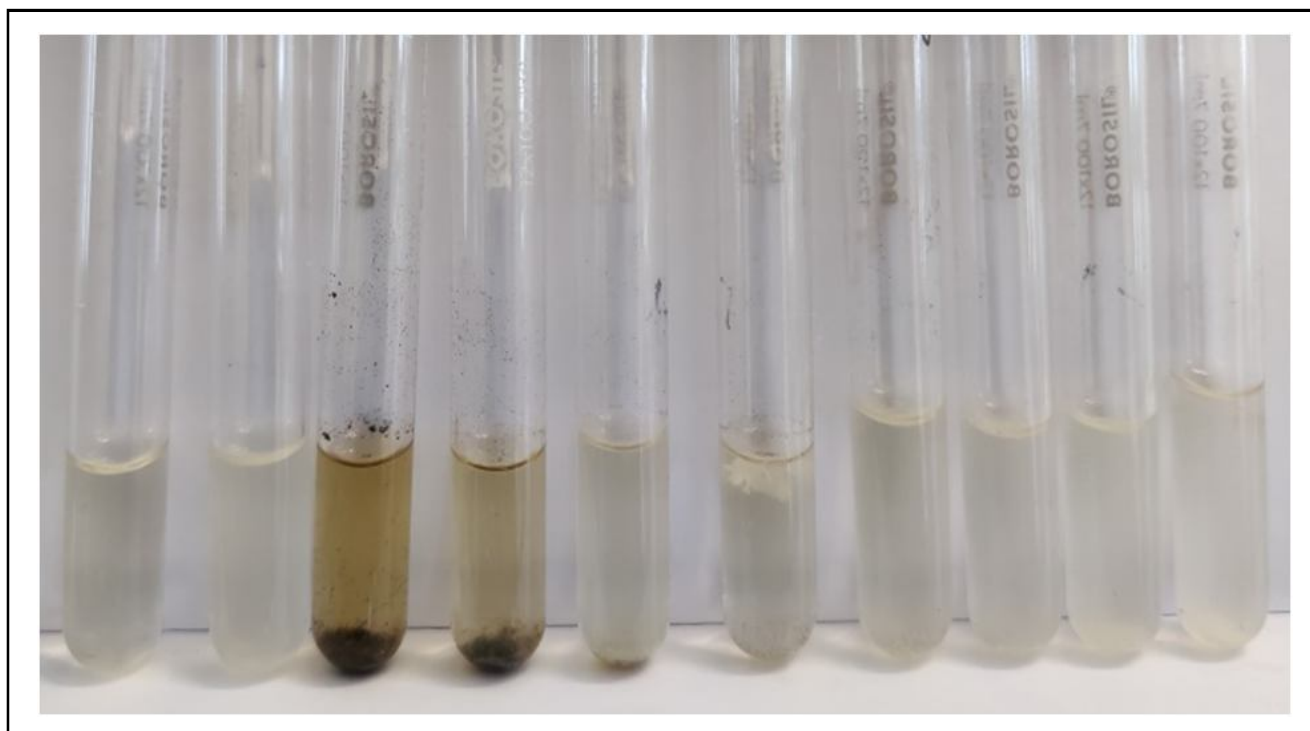


Figure 9: MIC evaluation *T. indica* leaf extracts against *E. coli* (tubes from left to right: 1: positive control, 2: negative control, 3: 250 mg, 4: 125 mg, 5: 62.5 mg, 6: 31.5 mg, 7: 15.6 mg, 8: 7.8 mg, 9: 3.9 mg and 10: 1.95 mg).

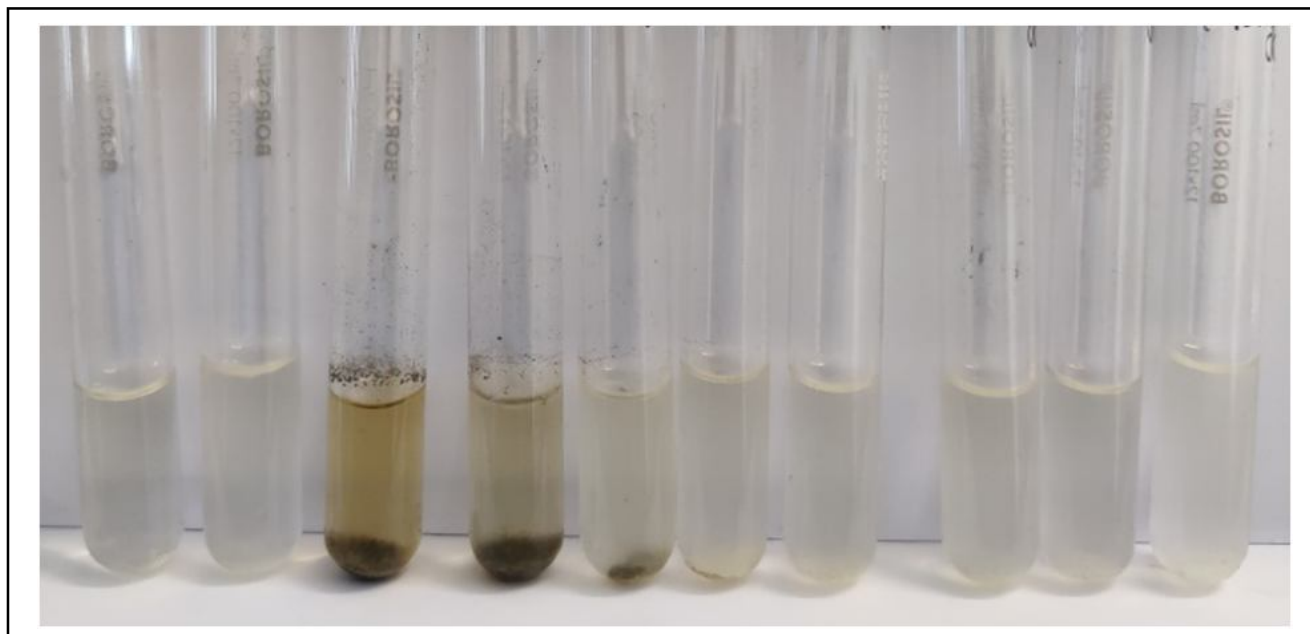


Figure 10: MIC evaluation *T. indica* leaf extracts against *S. aureus* (tubes from left to right: 1: positive control, 2: negative control, 3: 250 mg, 4: 125 mg, 5: 62.5 mg, 6: 31.5 mg, 7: 15.6 mg, 8: 7.8 mg, 9: 3.9 mg and 10: 1.95 mg).

4. Discussion

E. coli, a gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium (Bhadoriya *et al.*, 2018), can cause serious complications like pneumonia, urinary tract infections and blood stream infections in hospitalized patients. One of the most prevalent

causes of HAIs is due to multidrug-resistant *E. coli* that have developed resistance to numerous, if not all, antibiotics (Adeniyi *et al.*, 2017; Mohammed, 2019; Ahmed *et al.*, 2020). *S. aureus* is also known for wide resistance against many of the drugs and various strains and sub-strains were being isolated from clinical specimens (Katsayal *et al.*, 2019).

The tamarind tree, *T. indica* has been used in medicinal practices since time unknown and its various parts have displayed potential antibacterial activities. According to Chakraborty *et al.* (2016), the alcoholic extract of *T. indica* seeds had improved antibacterial activity against gram-negative *Shigella dysenteriae*, *E. coli*, *Salmonella typhi*, and the gram-positive *Bacillus subtilis* and *S. aureus*. This is in correlation with our present results. Another investigation report by Narina *et al.* (2019) stated that, when compared to other bacterial isolates, *Salmonella typhi* and *E. coli* were more sensitive, with the largest inhibition zone of 35 mm, when screened against *T. indica* extracts. This also supports the findings in our analyses.

5. Conclusion

The bioactivity of the *T. indica* leaf extracts against multidrug resistant *E. coli* and *S. aureus* was investigated in the present study. Higher inhibitory zones were observed against both the test organisms by well diffusion method. The minimum inhibitory concentration showed the complete inhibition of pathogens. In addition, another biological property of the methanolic extract, i.e., antioxidant assays were also performed. The *T. indica* leaf extracts showed significant DPPH radical scavenging activity and ferric reducing antioxidant power due to the presence of potential bioactive compounds. Further, studies are required to identify the phytochemical compounds responsible for the responsible for the anti-MDR bacterial activity and can be utilized for development of novel drugs.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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