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Usha MS
Department of Microbiology, JAIN (Deemed-to-be University), School of Sciences, Bengaluru, Karnataka, India

Diya Jangada
Department of Microbiology, JAIN (Deemed-to-be University), School of Sciences, Bengaluru, Karnataka, India

D Sanjana
Department of Microbiology, JAIN (Deemed-to-be University), School of Sciences, Bengaluru, Karnataka, India

N Harini
Department of Microbiology, JAIN (Deemed-to-be University), School of Sciences, Bengaluru, Karnataka, India

Harshitha Dandina
Department of Microbiology, JAIN (Deemed-to-be University), School of Sciences, Bengaluru, Karnataka, India

B Pragathi
Department of Microbiology, JAIN (Deemed-to-be University), School of Sciences, Bengaluru, Karnataka, India

Dona Suresh
Department of Microbiology, JAIN (Deemed-to-be University), School of Sciences, Bengaluru, Karnataka, India

Corresponding Author:
Usha MS
Department of Microbiology, JAIN (Deemed-to-be University), School of Sciences, Bengaluru, Karnataka, India

Biofilm inhibition and Biofilm eradication against clinical isolates using *Artocarpus heterophyllus* and *Mangifera indica*

Usha MS, Diya Jangada, D Sanjana, N Harini, Harshitha Dandina, B Pragathi and Dona Suresh

Abstract
The study focuses on the biofilm inhibition and eradication capabilities of *Artocarpus heterophyllus* (jackfruit) and *Mangifera indica* (mango) extracts against clinical bacterial isolates namely *Escherichia coli*, *Klebsiella spp*., and *Staphylococcus aureus*. Biofilm formation, a key factor in bacterial resistance and chronic infections, was targeted using methanolic, ethanolic, and water extracts derived from seeds, pulp, and peels of these plants. The results demonstrated significant biofilm inhibition and eradication. Ethanolic extracts were found to be more effective, methanolic extracts showing better inhibition compared to water extracts. *Mangifera indica* was found to be more effective when compared to *Artocarpus heterophyllus*. When biofilm inhibition studies were carried out ethanolic extract of *Mangifera indica* seed and pulp both at 25 mg/ml concentration were found to be showing good inhibition against *E. coli*. Ethanolic extract of *Artocarpus heterophyllus* seed at 10 mg/ml concentration, ethanolic extract of *Artocarpus heterophyllus* pulp at 25 mg/ml concentration and ethanolic extract of *Mangifera indica* seed at 150 mg/ml were found to be effective against *Klebsiella* spp. Similarly, Methanolic extract of *Mangifera indica* seed and ethanolic extract of *Mangifera indica* peel both at 25 mg/ml were found to be effective against *S. aureus*. When biofilm eradication studies were carried out methanolic extract of *Mangifera indica* peel at 25 mg/ml concentration was found to be effective against *E. coli*. Whereas ethanolic extract of *Mangifera indica* seed at 150 mg/ml concentration, methanolic extract of *Mangifera indica* pulp at 200 mg/ml concentration and methanolic extract of *Mangifera indica* peel at 150 mg/ml concentration were found to show good inhibition against *Klebsiella* spp. Similarly, ethanolic extract of *Mangifera indica* seed and ethanolic extract of *Mangifera indica* peel both at 25 mg/ml concentractions were found to be effective against *S. aureus*.

Keywords: *Artocarpus heterophyllus*, *Mangifera indica*, biofilm inhibition, biofilm eradication

1. Introduction
The search for new antimicrobial agents is critical in the face of increasing antibiotic resistance. Plants, with their rich array of secondary metabolites like flavonoids, alkaloids, and terpenoids, have long been a source of bioactive compounds with antimicrobial properties. These compounds offer promising alternatives to synthetic antibiotics, particularly against biofilm-associated infections, which are notoriously difficult to treat. Biofilms—communities of bacteria encased in a protective matrix—are responsible for chronic infections and contribute to antibiotic resistance. Plant extracts have shown potential not only in inhibiting bacterial growth but also in preventing or disrupting biofilm formation, thus providing a multi-target approach that may reduce the development of resistance.

Artocarpus heterophyllus (jackfruit) and *Mangifera indica* (mango) are two plants with significant antimicrobial and antibiofilm potential. Jackfruit, particularly its seeds and peel, contains flavonoids and phenolic compounds that exhibit broad-spectrum antibacterial activity against pathogens like *Escherichia coli* and *Staphylococcus aureus.* While its antibiofilm effects are less explored, there is growing interest in evaluating jackfruit extracts for their ability to disrupt biofilm formation. Similarly, mango, especially its peel and seeds, is rich in phenolic acids, flavonoids, and terpenoids, which exhibit strong antibacterial effects. Mango extracts have also demonstrated the ability to inhibit biofilm formation and disrupt existing biofilms, making them valuable candidates for treating infections caused by biofilm-forming bacteria. Both plants offer promising leads for the development of natural antimicrobial and antibiofilm therapies.

The review of literature in this study emphasizes the ongoing global challenge posed by antibiotic resistance, which has spurred the search for alternative therapeutics, particularly from plant-based sources. Historically, plant materials were the cornerstone of treatments for infectious diseases. Research has increasingly shown that plant extracts, rich in bioactive compounds such as flavonoids, tannins, and alkaloids, exhibit significant antibacterial and antibiofilm properties. Ahmed conducted studies on the antimicrobial activity of mango seeds extract against Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus* spp.), Gram-negative bacteria (*Pseudomonas aeruginosa* and *E. coli*) and yeast (*Candida albicans*) by well diffusion method [1]. Similarly, Olawuwo *et al.* explored the antimicrobial properties of various plant extracts, reinforcing the potential of natural compounds in combating resistant pathogens [2]. These studies highlight the critical role that phytochemicals from plants such as *Artocarpus heterophyllus* and *Mangifera indica* could play in the development of new antibacterial therapies, particularly for biofilm-related infections. Kalbassi *et al.* determined the antibiofilm, antimicrobial efficacy and cytotoxicity of Iran's endemic plants against pathogenic SRB Sulfate-Reducing Bacteria (SRB [3]). Mehrishi *et al.* determined the antibacterial and antibiofilm properties of 4 plant extracts i.e. clove (*Syzygium aromaticum*), tea (*Camellia sinensis*), garlic (*Allium sativum*) and coriander (*Coriandrum sativum*) [4]. Blando *et al.* investigated the potential of polyphenolic extracts from cladodes of *Opuntia ficus-indica* (L.) Mill. to inhibit the growth of some enterobacteria and the biofilm formation by *Staphylococcus aureus* [5]. Sánchez *et al.* conducted studies on the antimicrobial and antibiofilm activities of 8 methanolic plant extracts against clinically isolated microorganisms [6]. Slobodníková *et al.* conducted studies on the effects of polyphenols against bacterial biofilms [7]. Geethashri *et al.* estimated the efficacy of aqueous extract of *Azadirachta indica*, *Mangifera indica*, *Piper betel* and *Pepper nigrum* for antibiofilm activity against *E. faecalis* and *S. aureus* [8]. Despite the known antimicrobial properties of *Artocarpus heterophyllus* (jackfruit) and *Mangifera indica* (mango), limited research specifically addresses their ability to inhibit or disrupt biofilm formation, a critical factor in chronic infections. While both plants have demonstrated antibacterial activity, their effects on biofilm-associated pathogens like *E. coli*, *Klebsiella* spp, and *S. aureus* remain underexplored. Furthermore, the mechanisms through which these plants' bioactive compounds disrupt biofilm formation are not well understood, particularly in terms of molecular interactions and pathways. Most existing studies focus on single-species biofilms, leaving a gap in knowledge regarding their impact on multi-species biofilm formation. To date, comprehensive studies evaluating both the antimicrobial and antibiofilm properties of jackfruit and mango extracts are scarce, especially concerning clinical isolates and biofilm-associated infections.

Materials and Methods

Clinical isolates

The three clinical isolates i.e., *Escherichia coli, Klebsiella* spp. and *Staphylococcus aureus* were collected from Care Diagnostics, Bengaluru, Karnataka. They were maintained on Eosin Methylene Blue agar (EMB), Mac Conkey Agar (MCA) and Mannitol Salt Agar (MSA) respectively (Fig. 1).

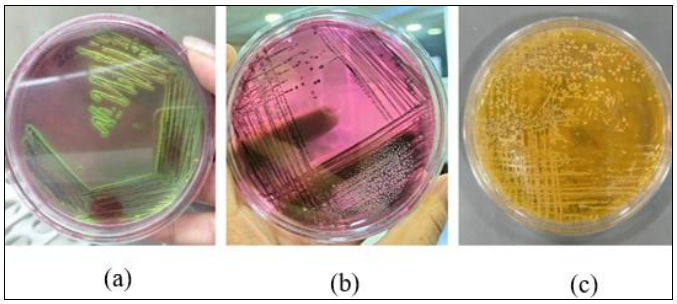

Fig 1: Growth of Clinical isolates (a) *E. coli*, (b) *Klebsiella* spp. and (c) *S. aureus* on specific media

Collection of plant materials

Mangifera indica (Mango) and *Artocarpus heterophyllus* (Jackfruit) were collected from the local markets in Bengaluru (Fig. 2).

Fig 2: Image of (a) *Mangifera indica* and (b) *Artocarpus heterophyllus* fruits

Sample preparation

Peel, pulp and seeds of mango were separated. Similarly pulp and seeds of jackfruit were also separated. The samples were then spread on tray for drying in hot air oven to avoid fungal contamination at 50 ˚C. Samples were then macerated to form a coarse powder (Fig. 3, 4, 5 and 6).

Fig 3: *M. indica* pulp

Fig 4: *M. indica* peel

Fig 5: *A. heterophyllus* pulp

Fig 6: *A. heterophyllus* seed

Fig 7: Solvent extracts of *M. indica* peel

Fig 8: Solvent extracts of *A. heterophyllus* pulp

Preparation of plant extracts

Mangifera indica peel, pulp and seeds were powdered and extracted with different solvents. Similarly, *Artocarpus heterophyllus* pulp and seeds were also powdered and extracted with different solvents. Methanol, ethanol and water were used as solvents for extraction (Fig. 7 and 8).

Estimation of Minimum Inhibitory Concentration (MIC)

The agar well diffusion method was used to check the antibacterial effects of plant extracts on nutrient agar plates. Three clinical isolates namely *E. coli*, *Klebsiella* spp. and *S. aureus* were used to check antibacterial activity of plant extracts. In order to adjust the turbidity to 0.5 McFarland standards, the test organisms were inoculated into Nutrient broths separately and cultured overnight at 37°C, yielding a final inoculum of $1.5x10^8$ CFU/ml. The culture broths were spread plated onto nutrient agar plates. Ethanol, methanol and water were used as solvents for extraction of plant materials. Concentrations of plant materials ranged from 25 mg/ml to 100 mg/ml concentrations. Using a sterile cork-borer, 6 mm wells were made, and the plant extracts were loaded separately into the wells. The solvents were used as negative control. Samples were allowed to diffuse at room temperature for 15 mins and later they were incubated for 18-24 hrs at

37°C. After incubation, plates were observed for the formation of a clear zone around the well. The zones of inhibition (ZOI) were observed and measured in mm. For plant extracts where zones of inhibition were not shown for the concentration ranging from 25 to 100 mg/ml concentration, further studies were carried out with the concentrations ranging from 100 to 300 mg/ml. Whereas with the plant extracts where zones of inhibition were shown even at 25 mg/ml concentration, further studies were carried out with the concentrations ranging from 5 to 20 mg/ml. MIC values were recorded for all the plant extracts with all the three solvents.

Microtiter plate assay for biofilm inhibition

Using the microtiter plate method, the effect of extracts of *Mangifera indica* and *Artocarpus heterophyllus* on biofilm formation by *E. coli*, *Klebsiella* spp. and *S. aureus* were investigated. For biofilm inhibition, all the extracts were used at concentrations. The extracts and overnight culture were added into the wells. At 37 °C, the microtiter plate was incubated overnight. Following the incubation, the medium was then removed from the wells, and each well received 1-2 drops of 0.1% crystal violet before the plate was incubated for 20 minutes. Three ethanol washes were performed on the stain after 20 minutes of incubation. At 590 nm, readings were taken with an ELISA reader. As negative controls, methanol, ethanol, and sterilized distilled water were used [9].

Microtiter plate assay for biofilm eradication

The wells received the addition of the overnight-grown cultures, followed by the addition of nutrient broth while maintaining the concentration of 1:3. The plate was incubated for 24 hours at 37°C. Without disturbing the biofilm that had adhered to the wells during incubation, the medium was removed from the wells. All of the extracts of both the plants were used at various concentrations. The microtiter plate was incubated for 24 hours at 37°C. After the wells had been incubated, the contents were decanted, and the wells were stained with crystal violet for 20 minutes. Three ethanol washes were performed on the wells, and readings were taken using an ELISA reader at 590 nm [9].

Results

When estimations of MIC were carried out with different solvent extracts of plant materials ethanolic extract of *Artocarpus heterophyllus* seed showed 150 mg/ml as MIC against *E. coli* and 10 mg/ml as MIC against *Klebsiella* spp. Whereas on *S. aureus* ethanolic extract of *Artocarpus heterophyllus* seed did not show any inhibition with the range of concentrations experimented. Mthanolic extract of *Artocarpus heterophyllus* seed showed 15 mg/ml as MIC against *Klebsiella* spp. But on both *E. coli* and *S. aureus* it did not show any inhibition even upto 300 mg/ml concentration. However water extract of *Artocarpus heterophyllus* seed did not have any effect against all the three bacterial cultures.

With ethanolic and methanolic extract of *Artocarpus heterophyllus* pulp 25 mg/ml concentration was found to be MIC value against both *E. coli* and *Klebisella* spp. But against *S. aureus* no zone of inhibition was observed with both the extracts. Water extract of *Artocarpus heterophyllus* pulp did not show any zone of inhibition on all the three bacterial cultures tested.

Ethanolic extract of *Mangifera indica* seed showed 25 mg/ml as MIC against both *E. coli* and *S. aureus*. But against *Klebsiella* spp. it failed to show zone of inhibition. With methanolic extract of *Mangifera indica* seed 150 mg/ ml was found to be MIC value against both *E. coli* and *Klebisella* spp. But against *S. aureus* at 25 mg/ml concentration it showed zone of inhibition. Water extract of *Mangifera indica* seed was able show zone of inhibition at 25 mg/ml concentration against only *S. aureus*, but against both *E. coli* and *Klebsiella* spp. it did not show any inhibition.

Ethanolic extract of *Mangifera indica* pulp showed 25 mg/ml as MIC against both *E. coli* and *s. aureus*. But against *Klebisella* spp. 300 mg/ml concentration was recorded as MIC value. With methanolic extract of *Mangifera indica* pulp against both *E. coli* and *S. aureus* 25 mg/ml concentration showed zone of inhibition, whereas against *Klebsiella* spp. 200 mg/ ml was found to be MIC value. Water extract showed inhibition against both *E. coli* and *S. aureus* at 25 mg/ml concentration, but against *Klbsiella* spp. there was no zone of inhibition even up to 300 mg/ml concentration.

When *Mangifera indica* peel extracts were subjected to estimation of MIC, ethanolic extract showed inhibition at 25 mg/ml concentration of *E. coli* and *S. aureus*. Methanolic extract showed different MIC values against all three isolates, i.e. 25 mg/ml against *E. coli*, 25 mg/ml against *S. aureus* and 150 mg/ml against *Klebsiella* spp. Water extract of *Mangifera indica* peel showed inhibition against *S. aureus* at 25 mg/ml concentration, whereas against *E. coli* and *Klebsiella* spp. there was no inhibition even up to 300 mg/ml concentration

Biofilm inhibition assay was done by using a 96-well microtiter plate. Negative controls were taken for each test. Each plant extracts were tested against all three bacterial isolates. The ethanolic extracts of *M. indica* seed and pulp both at a concentration of 25 mg/ml showed to be effective in inhibiting the biofilm created by *E. coli*. Whereas methanolic extract of *M. indica* peel at a concentration of 25 mg/ml showed to be least effective against the biofilm formed by *E. coli*. Against *Klebsiella* spp. ethanolic extract of *M. indica* seed at a concentration of 150 mg/ml, ethanolic extract of *Artocarpus heterophyllus* seed at 10 mg/ml concentration and ethanolic extract of *Artocarpus heterophyllus* pulp at 25 mg/ml concentration showed to be effective in inhibiting the biofilm. Whereas ethanolic extract of *M. indica* pulp at a concentration of 300 mg/ml and methanolic extract of *Artocarpus heterophyllus* seed were found to be least effective against the biofilm formed by *Klebsiella* spp. Against *S. aureus* methanolic extract of *M. indica* seed, ethanolic extract of *M. indica* peel and water extract of *M. indica* peel all three at 25 mg/ml concentractions showed to be effective in inhibiting the biofilm. Whereas ethanolic and water extracts of *M. indica* pulp both at a concentration of 25 mg/ml showed to be least effective against the biofilm formed by *S. aureus* (Fig. 10, Table 2).

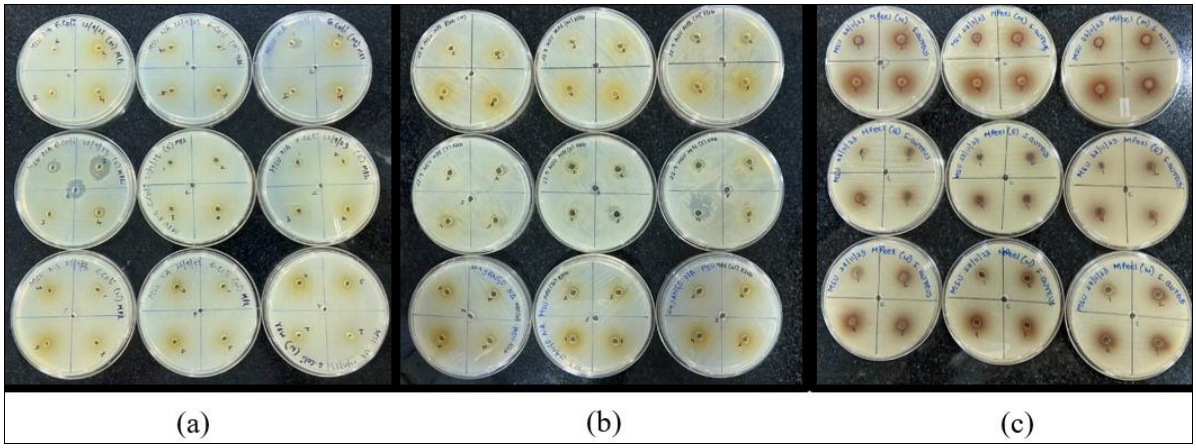

Fig 9: Plates showing zones of inhibition by different solvent extracts of *A. heterophyllus* and *M. indica* against clinical isolates

Table 1: MIC of methanolic, ethanolic and water extracts of *A. heterophyllus* seed, *A. heterophyllus* pulp, *M. indica* seed, *M. indica* pulp *M. indica* peel against *E. coli*, *Klebsiella* spp and *S. aureus*

Organism		***E. coli***	***Klebsiella* spp.**	***S. aureus***
Plant Extract	**Sol.**	**Conc. (mg/ml)**	**Conc. (mg/ml)**	**Conc. (mg/ml)**
A. heterophyllus seed	E	150	10	-
	M	-	150	-
	W	-	-	-
A. heterophyllus pulp	E	25	25	-
	M	25	25	-
	W	-	-	-
M. indica seed	E	25	-	25
	M	150	150	25
	W	-	-	25
M. indica pulp	E	25	300	25
	M	25	200	25
	W	25	-	25
M. indica peel	E	25	-	25
	M	25	150	25
	W	-	-	25

Table 2: Biofilm inhibition by *Artocarpus heterophyllus* and *Mangifera indica* against bacterial isolates

E. coli		***Klebsiella* spp.**		***S. aureus***	
+ve control: 0.611 -ve control: 0.0498		+ve control: 0.498 -ve control: 0.0585		+ve control: 0.1886 -ve control: 0.0856	
Extract	**Absorbance at 590 nm**	**Extract**	**Absorbance at 590 nm**	**Extract**	**Absorbance at 590 nm**
JS(E) (150)	0.043	JS(E) (10)	0.038	MPl(M) (25)	0.095
JP(M) (25)	0.048	JP(M) (25)	0.046	MPl(E) (25)	0.069
MS(E) (25)	0.036	JP(E) (25)	0.038	MPl(W) (25)	0.070
MP(E) (25)	0.038	MS(E) (150)	0.034	MS(M) (25)	0.067
MP(M) (25)	0.051	MP(M) (200)	0.043	MS(E) (25)	0.087
MPl(E) (25)	0.044	MPl(M) (150)	0.048	MS(W) (25)	0.084
MPl(M) (25)	0.056	JS(M) (150)	0.051	MP(M) (25)	0.080
		MP(E) (300)	0.052	MP(E) (25)	0.113
				MP(W) (25)	0.113

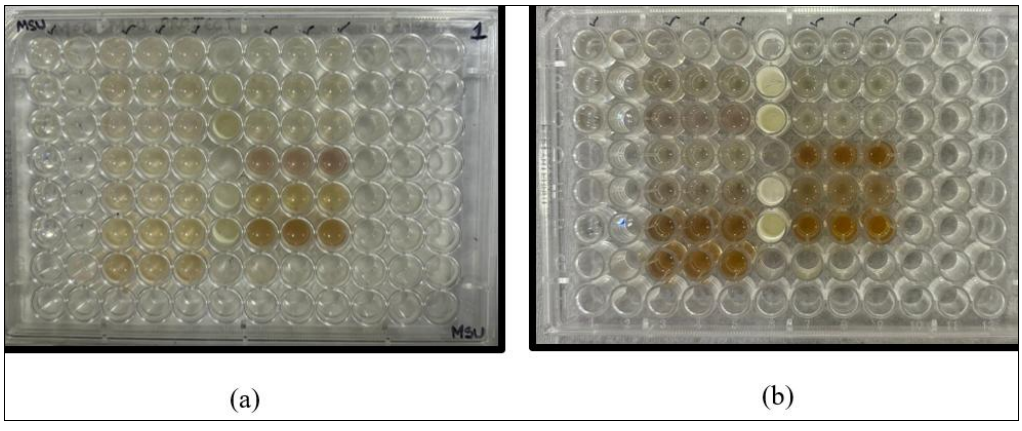

Fig 10: Biofilm inhibition by *Artocarpus heterophyllus* and *Mangifera indica* against bacterial isolates (a) Day 1 (b) Day 2

The methanolic extract of *M. indica* peel at the concentration of 25 mg/ml had the highest activity in eradicating the biofilm formed by *E. coli*. Whereas the ethanolic extract of *M. indica* peel at the concentration of 25 mg/ml showed to be least effective in eradicating the biofilm formed by *E. coli*. The methanolic extract of *M. indica* pulp at the concentration of 200 mg/ml, ethanolic extract of *M. indica* seed at 150 mg/ml concentration and methanolic extract of *M. indica* peel at 150 mg/ml concentration had the highest activity in eradicating the biofilm formed by *Klebsiella* spp. The least eradicating power was shown by ethanolic extract of *M. indica* pulp at the concentration of 300 mg/ml against biofilm formed by *Klebsiella* spp. The ethanolic extract of *M. indica* seed at the concentration of 25 mg/ml and ethanolic extract of *M. indica* peel at 25 mg/ml concentration have shown to be more effective in eradicating biofilm formed by S. aureus. The least eradicating power was shown by methanolic extract of *M. indica* peel at the concentration of 25 mg/ml against in eradicating biofilm formed by S. aureus (Fig. 11, Table 3).

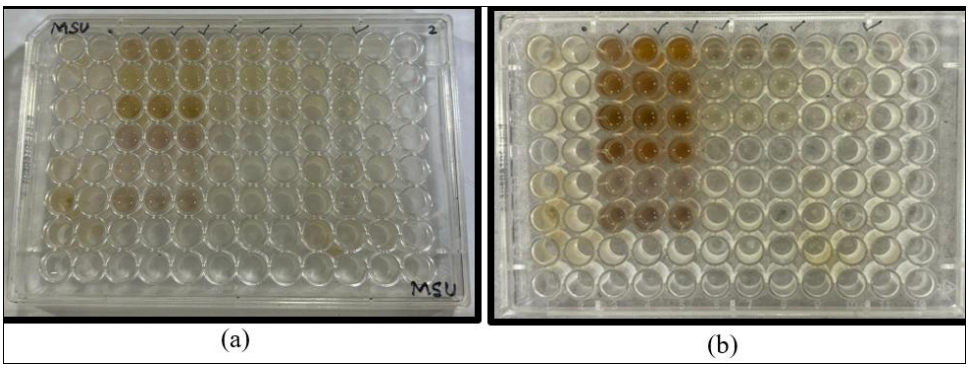

Fig 11: Biofilm eradication by *Artocarpus heterophyllus* and *Mangifera indica against* bacterial isolates (a) Day 1 (b) Day 2

Table 3: Biofilm eradication by *Artocarpus heterophyllus* and *Mangifera indica against* bacterial isolates

E. coli		***Klebsiella* spp.**		***S. aureus***	
+ve control: 0.39 -ve control: 0.050		**+ve control: 0.50 -ve control: 0.048**		**+ve control: 0.087 -ve control: 0.048**	
Plant material with solvent and concentration	**Absorbance at 590 nm**	**Plant material with solvent and concentration**	**Absorbance at 590nm**	**Plant material with solvent and concentration**	**Absorbance at 590 nm**
JS(E) (150)	0.209	JS(E) (10)	0.122	MPl(M) (25)	0.188
JP(M) (25)	0.209	JP(M) (25)	0.077	MPl(E) (25)	0.050
MS(E) (25)	0.131	JP(E) (25)	0.072	MPl(W) (25)	0.148
MP(E) (25)	0.256	MS(E) (150)	0.046	MS(M) (25)	0.084
MP(M) (25)	0.133	MP(M) (200)	0.043	MS(E) (25)	0.046
MPl(E) (25)	0.215	MPl(M) (150)	0.044	MS(W) (25)	0.076
MPl(M) (25)	0.038	JS(M) (150)	0.132	MP(M) (25)	0.070
		MP(E) (300)	0.265	MP(E) (25)	0.078
				MP(W) (25)	0.072

Discussion

In the current investigation, estimation of MIC values with seed and pulp of *A. heterophyllus* and seed, pulp and peel of *M. indica* were carried out using ethanol, methanol and water as solvents. Three clinical isolates i.e. *E. coli*, *Klebsiella* spp and *S. aureus* were used in the study. Against *E. coli* methanolic extract of *M. indica* peel was found to be effective at 25 mg/ml concentration being MIC value. Against *Klebsiella* spp. ethanolic extract of *A. heterophyllus* seed was found to be effective with MIC value of 10 mg/ml. Against *S. aureus* both seed and pulp of *A. heterophyllus* had no effect with either ethanol or methanol or water as solvent for extraction. Whereas with *M. indica* all the three solvent extracts of seed, pulp and peel had equal effects, MIC value being 25 mg/ml. Ethanolic extracts of plant extracts were found to be comparatively more effective than methanolic extracts, water extracts being least effective.

In the study conducted by Kalbassi *et al.* on the antibacterial activity of the four herbal extracts (*Aloe vera, E. caryophyllata, T. chebula, P. ovata*) against SRB strains, the MIC values for the extracts ranged between 0.11-96.7 µg/mL against strain 1, 57.2-219.22 µg/mL for strain 2, and 40.2-75.5 µg/mL for strain 3 [3]. These results indicated that the extracts were active against all SRB strains. In the experiments conducted by Mehrishi *et al.* extracts of the plants (*Syzygium aromaticum, Camellia sinensis, Coriandrum sativum* and *Allium sativum*) were tested for their antimicrobial properties against *S. aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* [4]. According to their studies plant extracts have shown maximum zone of inhibition at either 50 mg/ml or 25 mg/ml concentrations. Sánchez *et al.* tested a total of 8 methanolic plant extracts against 5 clinical bacterial isolates [6]. The highest zone of inhibition was obtained with *P. laevigata* extract (2.8±0.5 cm), followed by *G. microcephala* (2.3±0.2 cm) and *S. secundiflora* (2.1±0.3 cm) against *S. aureus* strain. *S. maltophilia* was the only microorganism that was not inhibited by any of the extracts. The antibacterial activity of plant extracts in various solvents was investigated in the study done by Ahmed [1]. According to the study ethanol extract was slightly more effective than acetone extract. A zone of inhibition with 20 mm diameter was shown by *P. aeruginosa* and *S. aureus* against ethanolic extract. on the other hand 17 mm and 18 mm of zone of inhibitions were shown by *P. aeruginosa* and *S. aureus* respectively against acetone extract.

In the current investigation when biofilm inhibition assay was carried out the ethanolic extracts of *M. indica* seed and pulp both at a concentration of 25 mg/ml showed to be effective in inhibiting the biofilm created by *E. coli*. Whereas methanolic

extract of *M. indica* peel at a concentration of 25 mg/ml showed to be least effective against the biofilm formed by *E. coli*. Against *Klebsiella* spp. ethanolic extract of *M. indica* seed at a concentration of 150 mg/ml, ethanolic extract of *Artocarpus heterophyllus* seed at 10 mg/ml concentration and ethanolic extract of *Artocarpus heterophyllus* pulp at 25 mg/ml concentration showed to be effective in inhibiting the biofilm. Whereas ethanolic extract of *M. indica* pulp at a concentration of 300 mg/ml and methanolic extract of *Artocarpus heterophyllus* seed were found to be least effective against the biofilm formed by *Klebsiella* spp. Against *S. aureus* methanolic extract of *M. indica* seed, ethanolic extract of *M. indica* peel and water extract of *M. indica* peel all three at 25 mg/ml concentractions showed to be effective in inhibiting the biofilm. Whereas ethanolic and water extracts of *M. indica* pulp both at a concentration of 25 mg/ml showed to be least effective against the biofilm formed by *S. aureus*.

Kalbassi *et al.* carried out antibiofilm activities of hydroalcoholic plant extracts of *A. vera* (leaf), *T. chebula* (fruit), and *E. caryophyllata* (flower bud) on two strains isolated from dentistry clinic and orthodontic appliances producing strong biofilm [3]. Biofilm formation screening showed that three isolated strains were capable of producing strong biofilms. Examination of the effect of subMIC extracts on these strains showed that *A. vera*, *T. chebula* and *E. caryophyllata* had an anti-biofilm effect on two strains producing strong biofilm. In contrast, *P. ovata* had an anti-biofilm impact on one of the strains. Strain 1 showed the highest sensitivity and strain 3 had the highest resistance to extracts. Mehrishi *et al.* carried out antibiofilm properties of methanolic extracts of flower buds (*Syzygium aromaticum*), dried leaves (*Camellia sinensis*, *Coriandrum sativum*) and bulb part (*Allium sativum*) of plants were tested against clinically isolated *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* from various specimens [4]. According to their studies biofilm inhibition was more than 50% with *Syzygium aromaticum* for all the concentrations. Biofilm reduction was more than 50% for each isolate at all concentrations of *Camellia sinensis*. With extract of *A. sativum* and *Coriandrum sativum* reduction of biofilm formation was less than 50% (range between 20-43%) for all concentrations. The effects of *Opuntia ficus-indica* (L.) Mill. Cladode polyphenolic extracts were investigated by Blando *et al.* on biofilm formation by *Staphylococcus aureus* ATCC 35556, a strong biofilm producer. Both cladode extracts (mature and immature) at concentration of 500 µg/mL did not inhibit the biofilm formation [5]. At 1000 µg/mL, only the immature cladode extract inhibited significantly the biofilm formation with an inhibition rate of 80%. At 1500 µg/mL concentration, both extracts significantly inhibited the biofilm formation, with an inhibition rate of 71% for the mature cladode and 85% for the immature cladode. Geethashri *et al.*, (2014) [8] carried out antibiofilm activities of aqueous leaf extracts of four medicinal plants i.e., *M. indica*, *A. indica*, *P. betel* and *P. nigrum* [8]. Neem leaf extract suppressed the *E. faecalis* biofilm significantly at 7.5 mg/ml. Betel and pepper leaf extracts at 30 mg/ml suppressed the *E. faecalis* biofilm. The Mango leaf extract did not suppress the *E. faecalis* biofilm significantly. Neem leaf at 7.5 mg/ml and pepper leaf extract at 15 mg/ml were highly significant against *S. aureus* biofilm. The betel leaf extract was significant in suppressing *S. aureus* at 15 mg/ml. The aqueous extract of mango leaves significantly effective only against *S. aureus* biofilm at 30 mg/ml.

In the current investigation when biofilm eradication study was carried out the methanolic extract of *M. indica* peel at the concentration of 25 mg/ml had the highest activity in eradicating the biofilm formed by *E. coli.* Whereas the ethanolic extract of *M. indica* peel at the concentration of 25 mg/ml showed to be least effective in eradicating the biofilm formed by *E. coli.* The methanolic extract of *M. indica* pulp at the concentration of 200 mg/ml, ethanolic extract of *M. indica* seed at 150 mg/ml concentration and methanolic extract of *M. indica* peel at 150 mg/ml concentration had the highest activity in eradicating the biofilm formed by *Klebsiella* spp. The least eradicating power was shown by ethanolic extract of *M. indica* pulp at the concentration of 300 mg/ml against biofilm formed by *Klebsiella* spp. The ethanolic extract of *M. indica* seed at the concentration of 25 mg/ml and ethanolic extract of *M. indica* peel at 25 mg/ml concentration have shown to be more effective in eradicating biofilm formed by *S. aureus*. The least eradicating power was shown by methanolic extract of *M. indica* peel at the concentration of 25 mg/ml against in eradicating biofilm formed by *S. aureus*.

Adeyemo *et al.* carried out biofilm eradication studies using extracts of *Brachylaena transvaalensis*, *Searsia batophylla*, *S. pendulina*, *S. leptodictya*, *S. lancea*, *S. gueinzii*, *Bauhinia galpinii*, *B. bowkeri* and *B. variegate* [10]. Strains of *Escherichia coli* O157: H7 (ATCC 43888), *E. coli* (ATCC 25922), *E. coli* (ATCC 35218), *Bacillus cereus* (ATCC 21366), *Staphylococcus aureus* (ATCC 29213), *Salmonella enterica* subsp. enterica serovar Typhimurium (*S. typhimurium* ATCC 39183), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* subsp. enterica serovar Enteritidis (*S. enteritidis* ATCC 13076) were used in their studies. In the biofilm eradication test (treatment after 24 h), negative percentage inhibition of plant extracts was noted for five out of six tested bacteria. However, all the extracts had good biofilm eradication activity against *B. cereus* strains. Also, *S. leptodictya* had good biofilm eradication activity against *P. aeruginosa* with a percentage of biofilm eradication of 79.85%. However, *S. pendulina*, *S. lancea*, *B. galpinii* and *B. bowkeri* had weak biofilm eradication activity against *S. typhimurium* with percentages lower than 50. Similar results were obtained with *S. batophylla* against *E. faecalis* and *B. galpinii* against *E. coli* ATCC 35218. Teanpaisan *et al.* carried out evaluation of antibacterial activity of 12 ethanol extracts of Thai traditional herb against oral pathogens [11]. The concentrations of *P. betle* extract to eradicate for 50% biofilm formation (MBEC50) of *S. mutans* ATCC 25175 and *A. actinomycetemcomitans* ATCC 33384 were 0.78±0.74 and 0.78±0.11 mg/mL, respectively. And for 90% biofilm eradication (MBEC90), concentrations of *P. betle* extract were, 6.25±0.58 and 3.13±0.28 mg/mL respectively. CHX could completely (100%) inhibit or remove biofilm formation of both tested strains. In an investigation carried out by Perumal and Mahmud, the methanolic extract of *Euphorbia hirta* effectively eradicated the established biofilm of *P. aeruginosa*, with minimum biofilm eradication concentration (MBEC) value of 0.5 mg/ml [12]. The methanol extract also showed weak antibiofilm activities against *E. faecalis* and *P. aeruginosa* (ATCC 27853) exhibiting MBEC value of 1.0 mg/ml.

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References

1. Ahmed EF. Antimicrobial and antibiofilm activity of mango seeds extract. Iraqi J Sci. 2015;56(4B):3121-9.
2. Olawuwo OS, Famuyide IM, McGaw LJ. Antibacterial and antibiofilm activity of selected medicinal plant leaf extracts against pathogens implicated in poultry diseases. Front Vet Sci. 2022;9:820304. DOI: 10.3389/fvets.2022.820304.
3. Kalbassi S, Yarahmadi M, Mohammadifard H, Ahmadi F. The antibiofilm and antibacterial effects of medicinal plant extracts on isolated sulfate-reducing bacteria from orthodontic appliances. Food Sci Technol. 2022;42:e38322.
4. Mehrishi P, Agarwal P, Broor S, Sharma A. Antibacterial and antibiofilm properties of medicinal plant extracts against multidrug-resistant Staphylococcus species and non-fermenter bacteria. J Pure Appl Microbiol. 2020;14:403-13.
5. Blando F, Russo R, Negro C, De Bellis L, Frassinetti S. Antimicrobial and antibiofilm activity against Staphylococcus aureus of *Opuntia ficus-indica* (L.) Mill. Cladode polyphenolic extracts. Antioxidants. 2019;8(5):117.
6. Sánchez E, Morales CR, Castillo S, Leos-Rivas C, García-Becerra L, Martínez DMO. Antibacterial and antibiofilm activity of methanolic plant extracts against nosocomial microorganisms. Evid Based Complement Alternat Med. 2016;2016:1572697.
7. Slobodníková L, Fialová S, Rendeková K, Kováč J, Mučaji P. Antibiofilm activity of plant polyphenols. Molecules. 2016;21(12):1717.
8. Geethashri A, Manikandan R, Ravishankar B, Shetty AV. Comparative evaluation of biofilm suppression by plant extracts on oral pathogenic bacteria. J Appl Pharm Sci. 2014;4(3):020-3.
9. Teanpaisan R, Senapong S, Puripattanavong J. *In vitro* antimicrobial and antibiofilm activity of *Artocarpus lakoocha* (Moraceae) extract against some oral pathogens. Trop J Pharm Res. 2014;13(7):1149-55.
10. Adeyemo RO, Famuyide MI, Dzoyem JP, McGaw LJ. Anti-biofilm, antibacterial, and anti-quorum sensing activities of selected South African plants traditionally used to treat diarrhoea. Evid Based Complement Alternat Med. 2022;2022:1307801.
11. Teanpaisan R, Kawsud P, Pahumunto N, Puripattanavong J. Screening for antibacterial and antibiofilm activity in Thai medicinal plant extracts against oral microorganisms. J Tradit Complement Med. 2017;7(2):172-7.
12. Perumal S, Mahmud R. Chemical analysis, inhibition of biofilm formation and biofilm eradication potential of *Euphorbia hirta* L. against clinical isolates and standard strains. BMC Complement Altern Med. 2013;13:346.