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Medicinal Plants Exhibited Promising Potential to Inhibit Biofilm Formation by Catheter-Associated Bacteria in UTI Patients from Lahore, Pakistan

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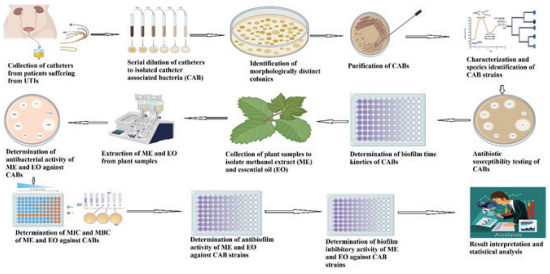

Abstract: The current study was designed to evaluate the antibacterial, antibiofilm, and biofilm inhibitory potential of six medicinal plants, including *Trachyspermum ammi*, *Trigonella foenum-graecum*, *Nigella sativa*, *Thymus vulgaris*, *Terminalia arjuna*, and *Ipomoea carneaid* against catheter-associated bacteria (CAB). Eighteen CAB were identified up to species level using 16S rRNA gene sequencing, *viz*., *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. *T. ammi* essential oil and *T. foenum-graecum* methanolic extract combination exhibited the highest antibacterial activity (ZOI; 32.0) against *S. aureus*. *N. sativa* essential oil (EO) showed highest ZOI (31.0; $p \leq 0.05$) against *Proteus mirabilis* at 100 µgmL^{-1}. Among 18 CAB isolated, 13 showed mature biofilm formation on 5th day. All plant extracts demonstrated more than 80% antibiofilm and biofilm inhibition activity. A concentration-dependent increase was observed with plant extracts against CAB during antibacterial, antibiofilm, and biofilm inhibition activities. The study suggests that EO and methanolic extract (ME) of tested plants possess promising antibiofilm and biofilm inhibitory potential against CABs. To our knowledge, this is the first study to report antibacterial, antibiofilm, and biofilm inhibitory potential of *T. ammi* and *N. sativa* seed EO, as well as *T. foenum-graecum*, *N. sativa*, *T. vulgaris*, *T. arjuna*, and *I. carnea* ME against CAB from medical setting.

Key words: catheter-associated bacteria, plant extracts, essential oils, antibacterial activity, biofilm inhibition, medicinal plants

Abbreviations: **CAB**; catheter associated bacteria, **CAUTIs**; catheter-associated urinary tract infections, **EO**; essential oil, **ICME**; *I. carnea* methanolic extract, **MBC**; minimum inhibitory concentration, **MDR**; multi drug resistant bacteria, **ME**; methanolic extract, **MIC**; minimum inhibitory concentration, **NSEO**; *N. sativa* essential oil, **OD**; optical density, **TAEO**; *T. arjuna* essential oil, **TAME**; *T. arjuna* methanolic extract, **TFME**; *T. foenum-graecum* methanolic extract, **TVME**; *T. vulgaris* methanolic extract, **UTIs**; urinary tract infections, **ZOI**; zone of inhibition

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1 Introduction

Catheter-associated urinary tract infections (CAUTIs) are a major healthcare concern, accounting for nearly half of all hospital-acquired infections. Indwelling urinary catheters, tubes placed in the bladder, are the primary cause of catheter-associated UTIs[1]. Urinary catheters are used daily for treating patients with urological infections[2]. Each day of catheterization increases the risk of infection by 3–7%[3]. The duration of catheterization is directly linked to the chances of CAUTI[4].

Most microbes colonizing urinary catheters are *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus epidermidis*, *K. pneumoniae*, *Candida* species, and other Gram-negative organisms[5].

Pathogenic microbial biofilms are a primary factor causing catheter-associated infections[6]. Microbes in biofilm mode can colonize the whole catheter and even move along the lumen and then into the bladder, kidneys, and sometimes into bloodstreams[7]. Biofilm formation occurs along the catheter surface and begins immediately after catheter insertion[8].

Predominantly the pathogens causing the infection are multi-drug-resistant (MDR)[9]. The rise of antibiotic resistance and side effects is making them less effective against various infections, especially those involving biofilm[10].

Novel biofilm management measures are needed since biofilm-related infections have become harder to control due to the inappropriate use of antibiotics[11]. Due to the increasing prevalence of catheter-associated infection, recent methods that are used to prevent microbial contamination often involve coating catheters with antibiotics[12]. Lately, traditional herbs have become the source of novel antibiotics[13].

Medicinal plants have a long history of use and contain natural compounds (phytochemicals) that can offer benefits with potentially fewer side effects[14]. Unlike antibiotics, plant extracts often target different bacterial mechanisms, offering an alternative approach. Due to their potential to complement antibiotics, particularly against biofilm infections that are becoming increasingly resistant to traditional treatments[15].

A study reported by[16], identified promising antibiofilm properties in extracts from *T. arjuna* and *I. carnea*, suggesting their potential as future UTI therapies, especially against resistant strains. Likewise, studies have shown that *T. ammi* has antioxidant and antibacterial benefits, suggesting potential as a natural supplement[14, 17]. In addition, *T. foenum-graecum* seeds and green leaves have a variety of chemical components, including amino acids, steroids, alkaloids, and flavonoids[18].

To date, no study has reported the use of *T. ammi* and *N. sativa* seed EO, as well as *T. foenum-graecum*, *N. sativa*, *T. vulgaris*, *T. arjuna*, and *I. carnea* ME against CAB isolated from patients suffering from UTIs from hospitals in Lahore Pakistan. Therefore, this study was designed to evaluate the antibacterial, antibiofilm, biofilm inhibition activity of *T. ammi* and *N. sativa* seed EO, as well as *T. foenum-graecum*, *N. sativa*, *T. vulgaris*, *T. arjuna*, and *I. carnea* ME.

2 Materials and Methods

2.1 Collection of samples

Catheter samples from suspected UTI patients from the intensive care unit of Ghurki Trust Teaching Hospital and the labor ward of Shalamar Hospital, Lahore were collected and immediately transferred to Microbiology Lab Department of Zoology, Government College University, Lahore.

2.2 Isolation of the bacteria

Catheter samples were rinsed with saline solution. The solution was serially diluted and 100 μL was spread on nutrient agar petri dishes. After 24 h incubation at 37℃, morphologically distinct colonies were selected and purified by consecutive rounds of quadrant streaking.

2.3 Characterization and identification of the CAB

The isolated bacterial strains were characterized morphologically and biochemically. Then molecular characterization was performed to identify the catheter associated bacteria (CAB) up to species level using 16S rRNA gene sequencing as already reported by Liaqat et al.[19]. Phylogeny was determined using MEGA X software.

2.4 Determination of antibiotic susceptibility

Antibiotic susceptibility test was performed to check sensitivity pattern of CAB against commercially available antibiotics (lincomycin, 100 $\mu g mL^{-1}$; ciproflaxin, 40 $\mu g mL^{-1}$; erythromycin, 20 $\mu g mL^{-1}$; rifampicin, 100 $\mu g mL^{-1}$) using Kerby-Bauer disc diffusion method as reported by Liaqat et al.[20]. The zone of inhibition (ZOI) was measured in mm.

2.5 Plant collection and extract preparation

Seeds of *T. ammi*, *N. sativa*, bark of *T. arjuna*, and leaves of *T. foenum-graecum*, *T. vulgaris*, and *I. carnea* were collected and identified by the Department of Botany, Government College University, Lahore. Extracts of selected plants were prepared using different methods.

2.6 Hydro-distillation of seeds of *T. ammi* and *N. sativa*

The principle of hydro-distillation was employed for the extraction of essential oil using a Clevenger apparatus following Noori et al.[21]. *T. ammi* and *N. sativa* seeds were grounded and soaked in distilled water (1:10) for essential oil (EO) extraction. After 4 h consecutive extraction, the EO was dried over anhydrous sodium sulphate and stored

(4℃).

2.7 Soxhlet extraction

Soxhlet extraction method was used to extract compounds from *T. vulgaris*, *T. arjuna*, *T. foenum-graecum*, and *I. carnea* leaves following Saxena et al.[22]. 15 g of powder underwent 16 h extraction with 70% methanol (65-70℃). Later, the solvent was evaporated and the extract was concentrated and stored at 4℃.

2.8 Preparation of the working concentrations of plant extracts

Four different concentrations (40, 60, 80, and 100 μgmL^{-1}) of methanolic extracts (ME) of *T. arjuna* (TAME), *I. carnea* (ICME), *T. foenum-graecum* (TFME) and *T. vulgaris* (TVME) and essential oil (EO) of *T. arjuna* (TAEO) and *N. sativa* (NSEO) were prepared by dissolving each concentration in 1 mL of 3% DMSO and methanol respectively. Mixed concentrations were prepared by combining the extracts of *T. arjuna* and *I. carnea*, *T. ammi* and *T. foenum-graecum*, and *N. sativa* and *T. vulgaris* in a 1:1 ratio.

2.9 Determination of the antibacterial activity

The antibacterial activity of plant extracts against CABs was tested using the agar well diffusion method as reported by Liaqat et al.[20]. In brief, fresh inoculums of each isolated CAB, adjusted to the 0.5 McFarland standard, were spread on Mueller-Hinton agar plates. Wells were made with the help of a sterile cork borer and labeled for the respective concentrations and controls. Rifampicin (100 μgmL^{-1}) and distilled water were used as positive and negative controls, respectively. 100 µL of the plant extracts and their combinations at the concentrations discussed above were poured into the wells and incubated at 37℃ for 24 h. Zone of inhibition were measured in mm.

2.10 Determination of minimum inhibitory concentration (MIC)

MIC and MBC of plant extracts (alone and combined) were determined using the methodology of Liaqat et al.[20]. MIC was evaluated in the range of 1 μgmL^{-1} to 80 μgmL^{-1} for TAEO, 0.5 μgmL^{-1} to 64 μgmL^{-1} for NSEO, 1 μgmL^{-1} to 60 μgmL^{-1} for TFME and TVME, 10 μgmL^{-1} to 160 μgmL^{-1} for TAME, and 20 μgmL^{-1} to 160 μgmL^{-1} for ICME against CAB strains. Combined form of extracts was evaluated in the range of 1 μgmL^{-1} to 60 μgmL^{-1} for TAEO and TFME, 0.5 μgmL^{-1} to 60 μgmL^{-1} for NSEO and TVME, and 10 μgmL^{-1} to 100 μgmL^{-1} for TAEO and ICME in a 1:1 ratio. To determine MBC, 100 µL of the MICs were spread on a nutrient agar plate and incubated at 37℃ for 24 h.

2.11 Determination of the time kinetics of biofilm formation assay

The test tube method was used to determine the time kinetics of biofilm formation with slight modifications of Liaqat et al.[19]. Sterilized catheter segments coated with human blood plasma were incubated at 37℃ for 3, 5, and 7 days in 3 mL of fresh broth inoculated with 30 µL of CABs. After incubation, the culture was discarded, and the segments were washed with saline solution to remove planktonic cells. The segments were then stained with 1% crystal violet solution. After discarding the crystal violet, the washing step was repeated, and the segments were air-dried at room temperature. To dissolve the biofilm, 33% glacial acetic acid was added. The optical density (OD) was measured at 580 nm using a visible spectrophotometer. The process was repeated with the remaining two sets of test tubes.

2.12 Determination of the anti-biofilm of plant extracts alone and in combination

Following biofilm formation on catheters, various concentrations (20, 30, 40 and 50 μgmL^{-1}) of plant extracts (alone and combined) were added to test tubes containing mature biofilm of CABs. After 18 h of incubation, the biofilm was quantified using a crystal violet assay as discussed above. The percentage of anti-biofilm activity was calculated using the formula given below:

$$\text{Percentage antibiofilm (Pre-formed biofilm disruption)} = \frac{\text{Absorbance of control} - \text{Absorbance of treated biofilm}}{\text{Absorbance of control}} \times 100$$

2.13 Determination of the biofilm inhibitory activity

Biofilm inhibitory activity was determined using a protocol similar to that used for biofilm time kinetics. Catheter segments were added to test tubes containing fresh nutrient broth and 30 µL of CAB inoculum. Different concentrations of plant extracts (alone or combined) at 20, 30, 40 and 50 μgmL^{-1} were added to the test tubes prior to incubation. The samples were incubated at 37℃ for the duration required for CABs to form mature biofilms. After incubation, the amount of biofilm was quantified using a crystal violet assay. Biofilm inhibitory activity was calculated using the following formula:

$$\text{Percent biofilm inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of treated biofilm}}{\text{Absorbance of control}} \times 100$$

2.14 Statistical analysis

Data were presented as mean and standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by a post hoc Tukey test was applied to establish the level of significance ($p \leq 0.05$) using SPSS version 20.0.

3 Results

3.1 Isolation and characterization of CAB

A total of 18 CAB strains were isolated and characterized morphologically and biochemically to identify these strains up to the species level. Out of these, 13 strains showed negative results while 5 showed positive results for Gram staining. Twelve strains were rod-shaped, while six were spherical. Similarly, 11 were motile, while 7 were non-motile (Table S1). Morphologically, most of the colonies were round-shaped, with entire margins, flat elevation, and opaque opacity (Table S2). Biochemically, most of the CAB strains showed positive results for carbohydrate fermentation and the catalase test, while negative for the MRVP test. The biochemical results are summarized in Table S3.

16S rRNA gene sequencing revealed that the isolated CAB strain MT1 showed 100% homology with *K. pneumoniae* (OK669223), MT2 with *Enterobacter cloacae* (OK669224), MT3 with *Achromobacter xylosoxidans* (OK669225), MT4 with *Pseudomonas aeruginosa* (OK669226), MT5 with *S. aureus* (OK669227), MT6 with *Staphylococcus saprophyticus* (OK669228), AA1 with *Klebsiella* sp. (OK561619), AA2 with *Enterobacter* sp. (OK561620), AA3 with *Klebsiella* sp. (OK561621), AA4 with *Enterococcus* sp. (OK561622), AA5 with *Klebsiella aerogenes* (OK561623), AA6 with *E. coli* (OK561624), CAB1 with *Enterococcus* sp. (OK360623), CAB2 with *Proteus mirabilis* (OK360619), CAB3 with *Klebsiella* sp. (OK360618), CAB4 with *S. aureus* (OK360620), CAB5 with *Pseudomonas aeruginosa* (OK360621), and strain CAB6 showed 100% homology with *E. coli* (OK360622). A phylogenetic tree was constructed using MEGA software, and genetic distance among species was measured (Fig. 1).

3.2 Antibiotic susceptibility test

The antibiotic susceptibility evaluation showed that *K. aerogenes* was resistant to all antibiotics except ciprofloxacin. *Klebsiella* sp., *Enterococcus* sp., and *E. coli* were resistant to rifampicin. All strains, except *P. aeruginosa*, were susceptible to ciprofloxacin, with zones of inhibition ranging from 15 to 32.5 mm (Fig. 2).

3.3 Determination of antibacterial activity

The TFME and TAEO showed a concentration-dependent increase in the zone of inhibition (ZOI) against isolated CAB strains *Enterococcus* sp., *Klebsiella* sp., *P. aeruginosa*, *S. aureus*, *A. xylosoxidans*, and *S. saprophyticus*. Both TFME and TAEO demonstrated the highest antibacterial activity with ZOI of 30.0 ± 0.1 mm and 30.3 ± 0.1 mm, respectively, against *S. aureus* at 100 $\mu g mL^{-1}$, and in combination, they achieved a ZOI of 32.0 ± 0.2 mm (Fig. 3A). The TAME and ICME exhibited promising antibacterial activity against *Klebsiella* sp., *Enterobacter* sp., *Enterococcus* sp., *K. aerogenes*, and *E. coli*. The TAME, alone and in combination with the ICME, showed the highest antibacte-

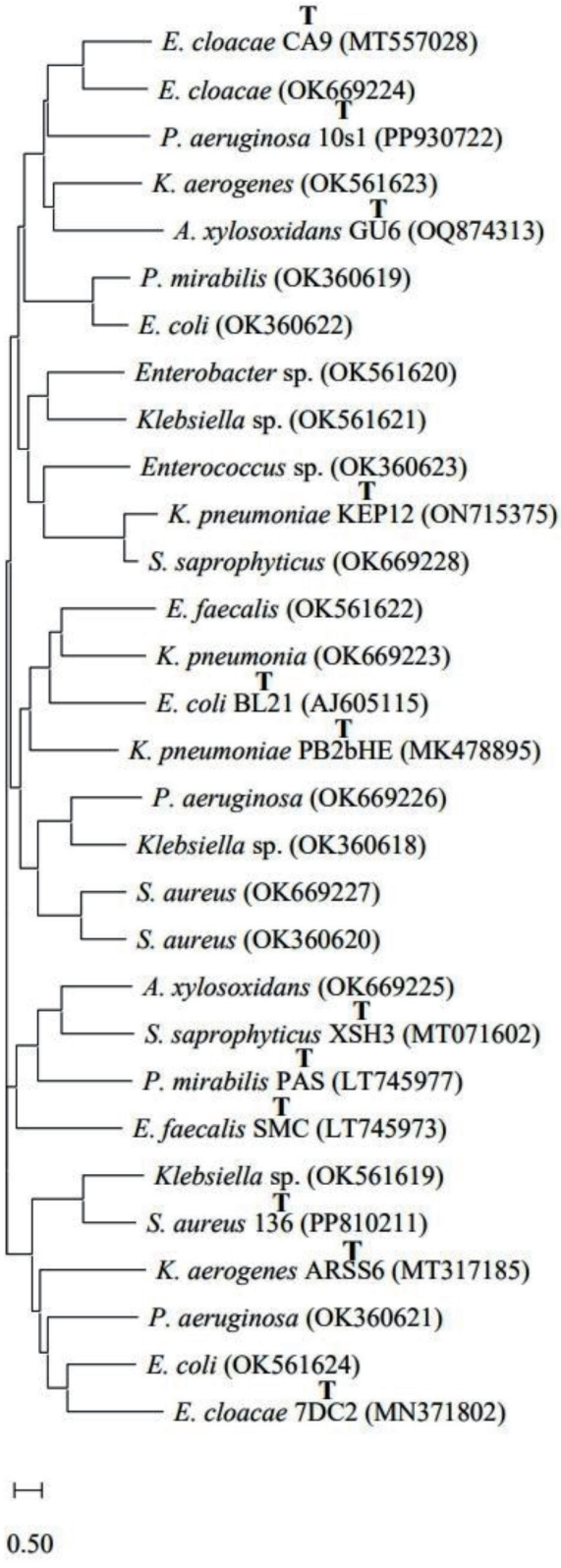


Fig. 1 A phylogenetic based on 16S rRNA gene sequence was constructed to measure genetic variability among CAB species using MEGA software and neighbor joining tree method.

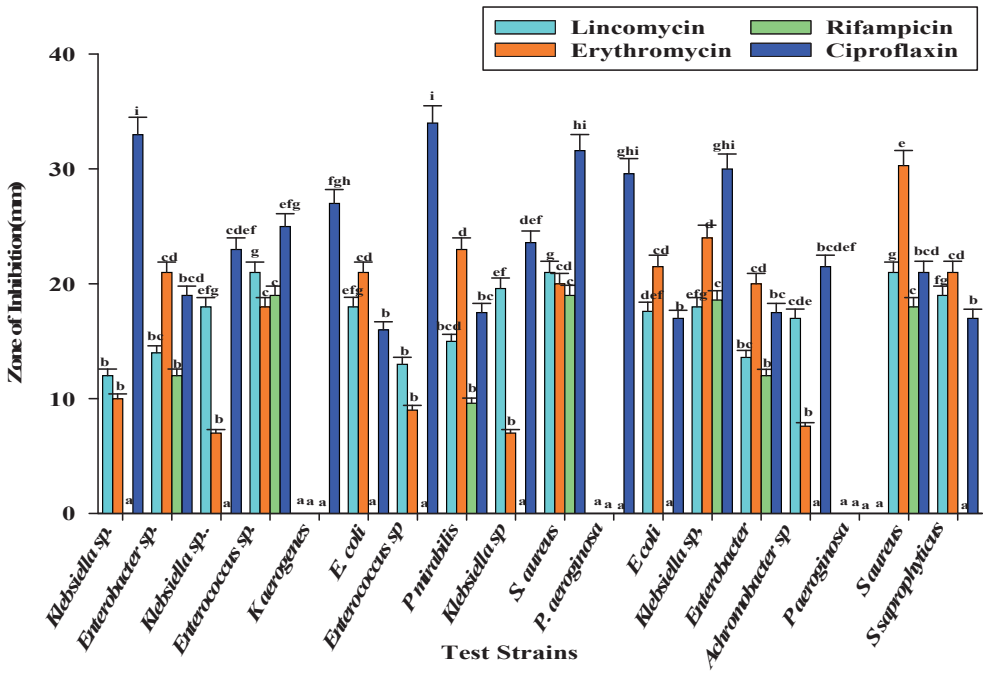


Fig. 2 Antibiotic susceptibility test of four antibiotics (lincomycin, 100 μgmL^{-1}; erythromycin 20 μgmL^{-1}; rifampicin, 100 μgmL^{-1} and ciprofloxacin, 40 μgmL^{-1}) against Catheter associated bacteria (CAB) strains. *P. aeruginosa* was highly resistant to all the tested antibiotics. Ciprofloxacin was most effective against all CAB. Results were expressed as mean ± standard error of mean (SEM) of triplicates. $p \leq 0.05$.

rial activity with ZOI of 23.0 ± 0.1 mm and 24.0 ± 0.2 mm, respectively, against *Klebsiella* sp. The ICME alone showed the highest activity with a ZOI of 23.3 ± 0.2 mm against *Enterococcus* sp. at 100 μgmL^{-1} (Fig. 3B).

The NSEO and the TVME displayed strong concentration-dependent activity against *Enterococcus* sp., *P. mirabilis*, *Klebsiella* sp., *S. aureus*, *P. aeruginosa*, and *E. coli*. The highest ZOI observed were 31.0 ± 0.5 mm against *P. mirabilis* at 100 μgmL^{-1} by the NSEO and 28.6 ± 0.8 mm against *E. coli* by the mixture of both (Fig. 3C). Results are expressed as mean ± SEM. One-way ANOVA followed by post hoc Tukey tests showed that the results were statistically highly significant within groups ($p \leq 0.05$).

3.4 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentrations (MIC) of TAEO and TFME were observed in the range of 2-15 μgmL^{-1}, while the minimum bactericidal concentrations (MBC) were between 2-45 μgmL^{-1} against CAB strains (Fig. 4A). The MIC of TAME and ICME alone ranged between 15-135 μgmL^{-1}, while the MBC ranged from 25-145 μgmL^{-1}, as shown in Fig. 4B. Similarly, the MIC and MBC of NSEO and TVME ranged between 5-55 μgmL^{-1} and 5-65 μgmL^{-1}, respectively, as depicted in Fig. 4C. Results are expressed as mean ± SEM. The results were statistically significant ($p \leq 0.05$) as revealed by one-way ANOVA.

3.5 Time kinetics of biofilm formation assay

Out of 18 CAB strains, 13 strains showed mature biofilm formation on the 5th day, 3 strains on the 3rd day, and 2 strains on the 7th day of incubation (Fig. 5). The biofilm time kinetics results were statistically significant ($p \leq 0.05$).

3.6 Antibiofilm activity

The TAEO showed the highest antibiofilm activity (78.0 ± 0.0%) against *Enterobacter* sp. The TFME was most effective against *Enterobacter* sp. (85.0 ± 0.0%). The combination of TAEO and TFME was the most effective (87.7 ± 0.0%) against *S. aureus* at 50 μgmL^{-1} (Fig. 6A). The TAME showed the highest antibiofilm activity (91.0 ± 1.4%) against *Klebsiella* sp., while ICME showed 69.0 ± 1.5% activity against *Enterococcus* sp. The combination of TAME and ICME demonstrated increased effectiveness, with the highest antibiofilm activity being 90.1 ± 1.0% against *Klebsiella* sp. at 50 μgmL^{-1} (Fig. 6B).

The NSEO showed the highest antibiofilm activity (90.0 ± 0.5%) against *S. aureus*, while the TVME showed the highest activity (76.0 ± 0.9%) against *P. mirabilis*. The combination of NSEO and TVME was also highly effective (87.6 ± 0.9%) against *P. mirabilis* at 50 μgmL^{-1} (Fig. 6C). Results are expressed as mean ± SEM. One-way ANOVA revealed that these results were statistically significant ($p \leq 0.05$).

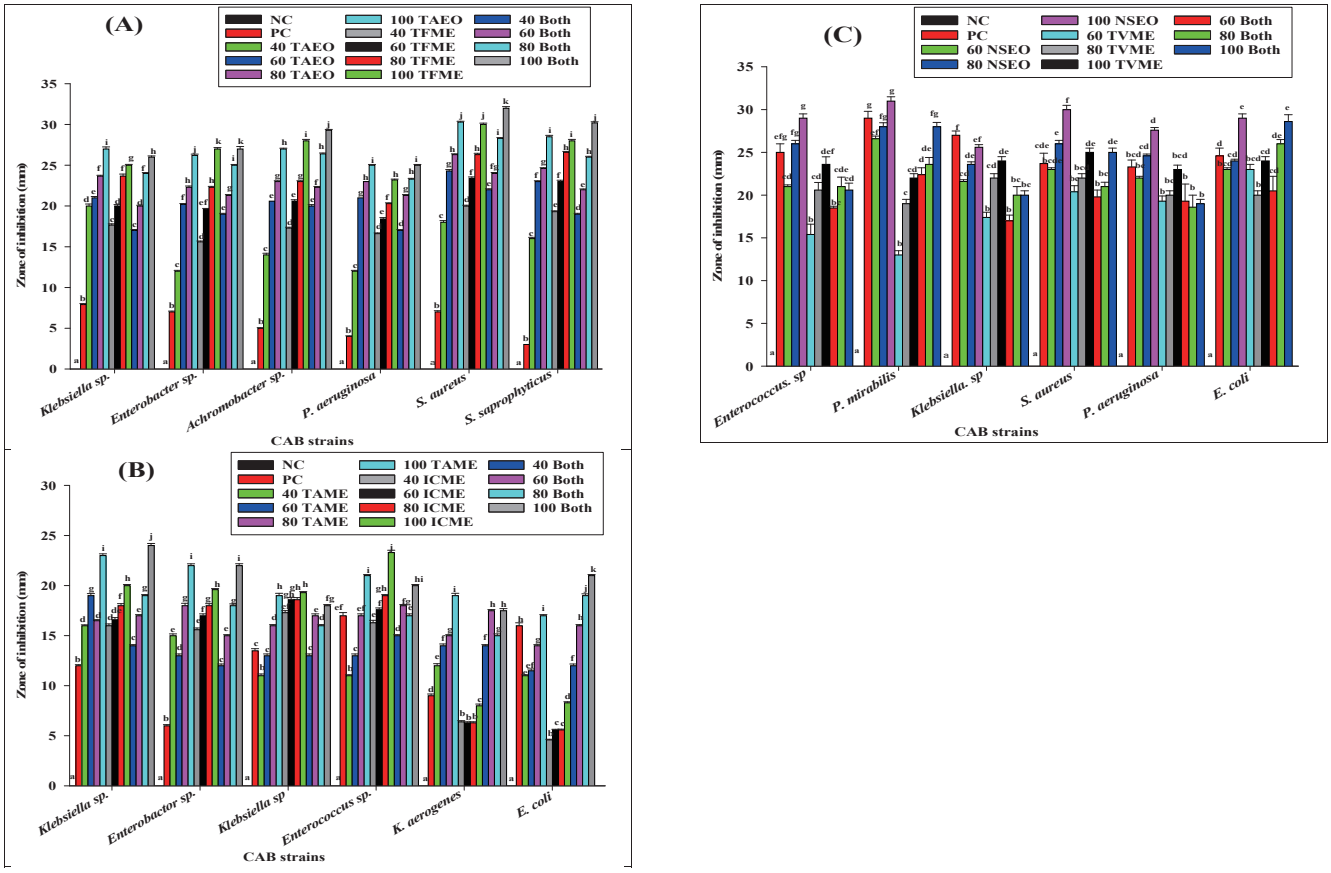


Fig. 3 Antibacterial activity of methanolic extracts and essential oils of medicinal plants against CAB. (A) antibacterial activity *T. arjuna* essential oil (TAEO) and *T. foenum-graecum* methanolic extract (TFME) *against* CAB strains (*Klebsiella* sp., *Enterobacter* sp., *Achromobacter* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*). (B) antibacterial activity of *T. arjuna* methanolic extract (TAME) and *I. carnea* methanolic extract (ICME) against CAB strains (*Klebsiella* sp., *Enterobacter* sp., *Klebsiella* sp, *Enterococcus* sp., *Klebsiella aerogenes*, *Escherichia coli*). (C) antibacterial activity of *N. sativa* essential oil (NSEO) and *T. vulgaris* methanolic extract (TVME) against CAB strains (*Enterococcus* sp., *P. mirabilis*, *Klebsiella*. sp, *S. aureus*, *P. aeruginosa* and *E. coli*). Results were expressed as mean ± SEM of triplicates. $p \leq 0.05$. NC = Negative control (DMSO/Methanol) and PC = Positive control (rifampicin (100 μgmL^{-1})).

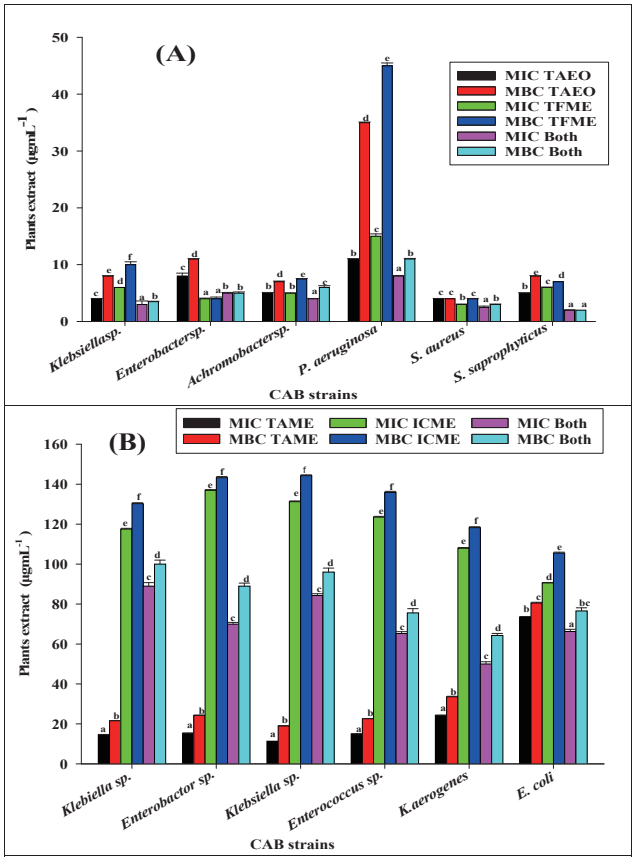
3.7 Biofilm inhibitory activity

The TAEO showed the highest biofilm inhibition (83.3 ± 0.3%) against *Pseudomonas* sp., while TFME showed the highest inhibition (89.7 ± 0.0%) against *P. aeruginosa*. Combination of TAEO and TFME was highly effective (93.4 ± 0.0%) against *Klebsiella* sp. at 50 μgmL^{-1} (Fig. 7A). The TAME showed the highest biofilm inhibitory activity (90.5 ± 0.7%) against *K. aerogenes*, while ICME showed 92.4 ± 0.9% inhibition against *Klebsiella* sp. The combination of TAME and ICME demonstrated the highest biofilm inhibitory activity (93.1 ± 0.0%) against *K. aerogenes* at 50 μgmL^{-1} (Fig. 7B).

The NSEO showed the highest biofilm inhibitory activity (94.1 ± 1.0%) against *S. aureus*, while TVME showed 83.3 ± 1.7% inhibition against *P. mirabilis*. The combination of NSEO and TVME demonstrated the highest biofilm inhibitory activity (84.3 ± 0.5%) against *S. aureus* at 50 μgmL^{-1} (Fig. 7C). Even lower concentrations showed promising inhibition for most bacteria. Results are expressed as mean ± SEM. One-way ANOVA revealed that these results were statistically significant ($p \leq 0.05$).

4 Discussions

Catheters are commonly used as medical devices in urinary systems. Many types of bacteria grow on these catheters and cause urinary tract infections (UTIs). These bacteria produce biofilms on catheters that increase their pathogenicity and protect them from antibiotics thus responsible for antibiotic resistance against commercial antibiotics. About 98% UTIs are caused by these catheters associated bacterial (CAB) strains[23]. Because of antibiotic resistance, the cost to cure UTIs has increased up to 1.82



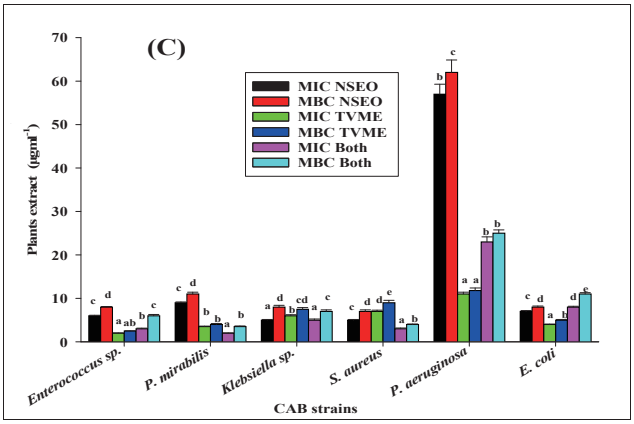


Fig. 4 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extract against CAB strains. (A) Combination of TAEO and TFME inhibited *S. saprophyticus* growth at 2.0 ± 0.05 µgmL^{-1} and also showed MBC at the same concentration. (B) TAME showed lowest MIC and MBC against *Klebsiella* sp. at 11.3 ± 0.0 and 19.0 ± 0.1 µgmL^{-1} respectively. (C) TVME showed lowest MIC and MBC against *Enterococcus* sp. at 2.0 ± 0.04 and 2.5 ± 0.05 µgmL^{-1}, respectively while combination of both was most effective against *P. mirabilis* at 2.0 ± 0.06 and 3.5 ± 0.14 µgmL^{-1}, respectively. Data was presented as mean ± SEM of triplicates.

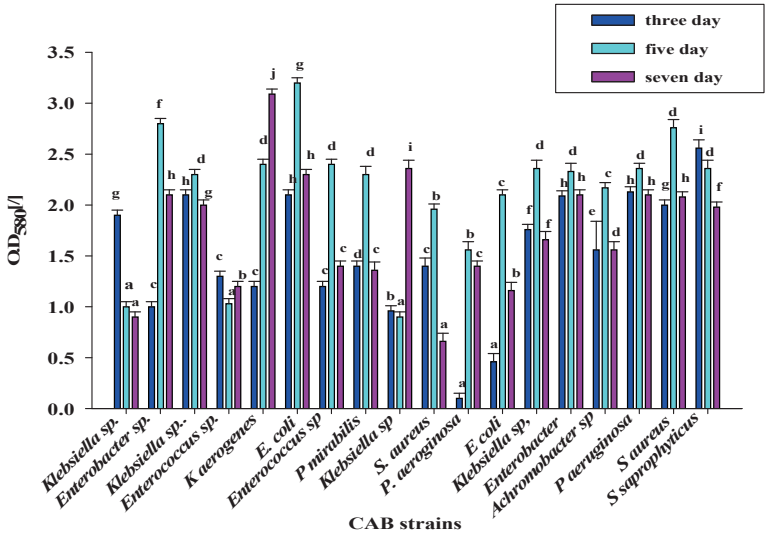


Fig. 5 Biofilm time kinetics. Thirteen CAB strains showed mature biofilm formation on 5th day, two showed on 7th day while three CAB strains showed mature biofilm formation on 3rd day. Optical density (OD) was measured at 580 nm while data was presented as mean ± SEM. The experiment was conducted in triplicates to get reliable results.

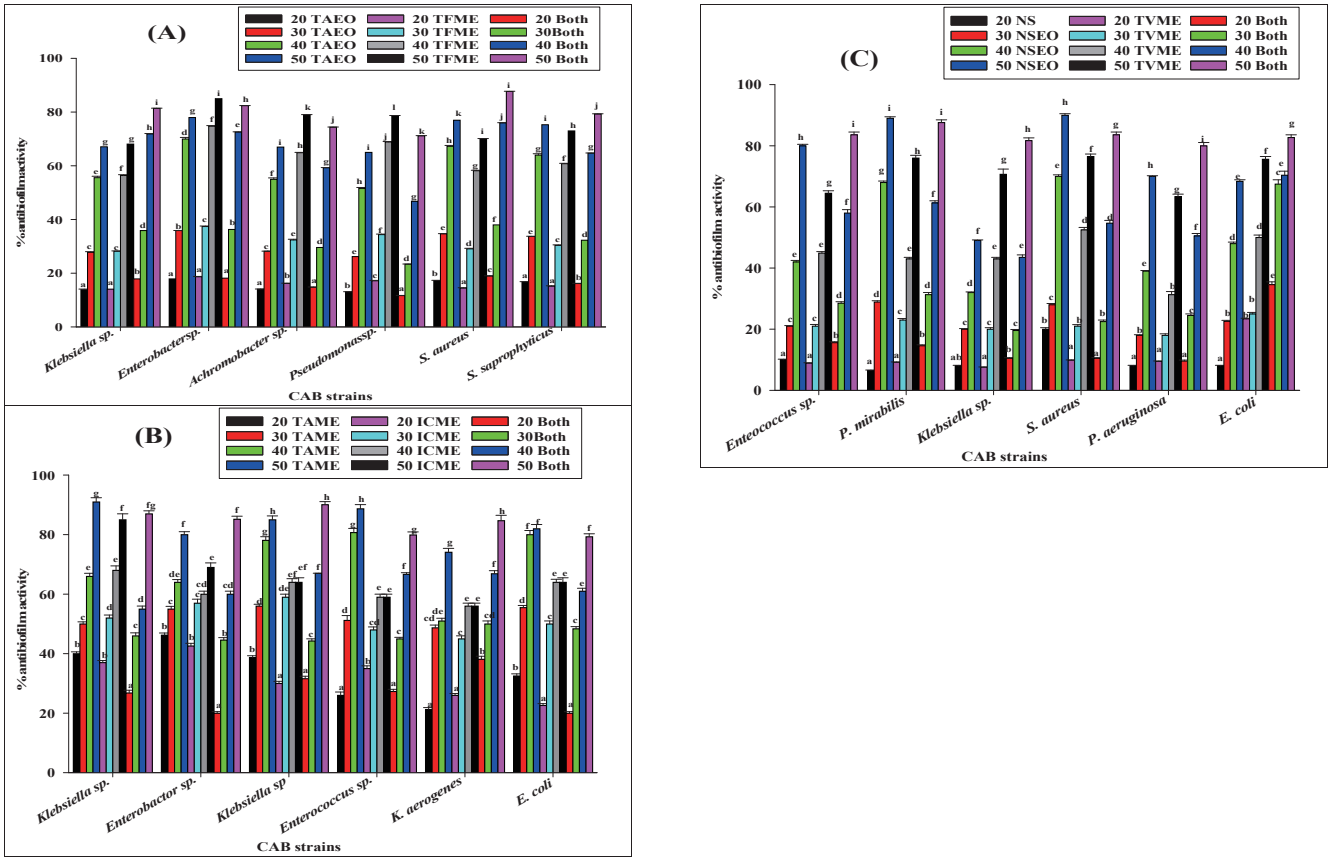


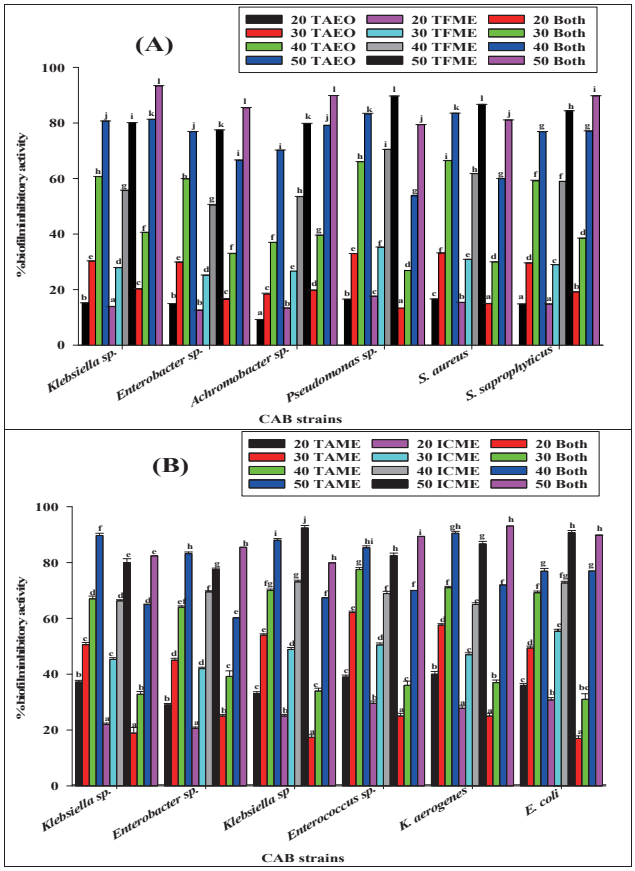
Fig. 6 Percent antibiofilm activity. (A) TAEO and TFME showed up to 80% antibiofilm activity (alone) while in combine form up to 88% antibiofilm activity was observed. (B) TAME and ICME showed up to 90% antibiofilm activity alone as well as in combine form and a concentration dependent activity was observed. (C) NSEO showed up to 90% antibiofilm activity against *S. aureus* and *P. mirabilis* at 50 μgmL^{-1} concentration. Data was presented as mean ± SEM of triplicate.

billion dollars annually[24]. Therefore, the present study was designed to assess the antibacterial, antibiofilm, and biofilm inhibitory activity of *N. sativa*, *T. ammi*, *T. vulgaris*, *T. arjuna*, *I. carnea*, and *T. foenum-graecum* against CAB strains. Different strains were isolated and characterized on morphological, biochemical, and genetic basis.

Genetic characterization by 16S rRNA gene sequencing showed that the strains belong to *Enterobacter* sp., *Achromobacter* sp., *S. aureus*, *S. saprophyticus*, *K. aerogenes*, *K. pneumonia*, *E. coli*, *E. cloacae*, *E. faecalis*, *P. mirabilis*, and *P. aeruginosa*. The results were supported by Gunardi et al.[25], who reported *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, *S. aureus*, *Enterobacter* sp. and *P. mirabilis* as the main dwelling microorganisms in catheters.

The plant extracts (*N. sativa* EO, *T. ammi* EO, *T. vulgaris* ME, *T. arjuna* ME, *T. foenum-graecum* ME, and *I. carnea* ME) effectively inhibited the growth of CAB strains associated with catheter infections. In this study, TFME showed up to 30.0 ± 0.0 zone of inhibition (ZOI) at 100 μgmL^{-1}. These results can be compared with the study of Odelola et al.[26] in which they reported the antibacterial activity (ZOI 24.4 ± 2.51 mm) of corn plant extract at 100 mgmL^{-1}. In our case the ZOI was bigger at lower concentration which indicate that TFME has more antibacterial potential as compared to corn extract. Results of TAME and ICME showed effective antibacterial activity against six CAB strains. Maximum antibacterial activity in case of *T. arjuna* was measured as ZOI (23.0 ± 1.7 mm) against *Klebsiella* sp. at 100 μgmL^{-1} while in case of *I. carnea* the maximum activity was observed as ZOI (23.3. ± 0.04 mm) against *Enterococcus* sp. at 100 μgmL^{-1}. These results were supported by Premalatha et al.[27], where the bark extract of *T. arjuna* showed highest antibacterial activity against four isolated strains i.e., *K. aerogenes*, *Enterococcus* sp. and *K. pneumonia* measured as ZOI (15.0 ± 0.03 mm; 18.5 ± 0.57; 21.0 ± 0.01 mm) respectively.

The MIC and MBC for the plant extracts were consistent with previous studies. The TFME's values were supported by Hilles & Mahmood[28] while TAEO's results were similar to Zomorodian et al. with minor variations[29]. In a study by



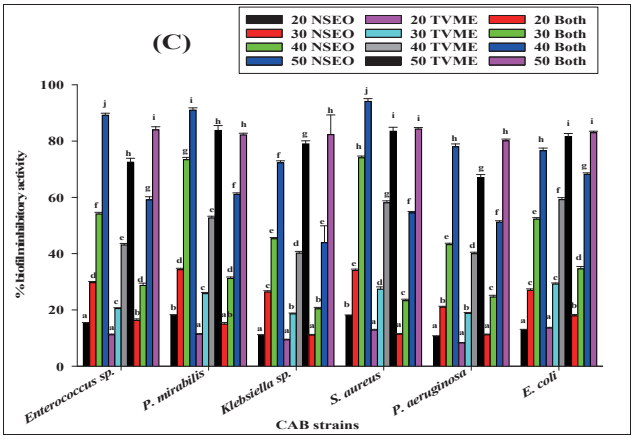


Fig. 7 Percent biofilm inhibition. (A) TAEO and TFME in combine form showed up to 90% activity against *Klebsiella* sp., *Achromobacter* sp. and *S. saprophyticus* while TFME alone showed 90% biofilm inhibition activity against *Pseudomonas* sp. (B) TAME and ICME showed 80-90% biofilm inhibition activity against six CAB strains at 50 μgmL^{-1}. (C) NESO showed highest (90%) activity against *S. aureus*, *P. mirabilis* and *Enterococcus* sp. while NSEO and TVME in combine form showed around 80% activity against six CAB strains at 50 μgmL^{-1}.

Basri & Fan, MIC and MBC value of TAME against *K. pneumonia*, and *E. coli*. were 5-15 μgmL^{-1} and 60-70 μgmL^{-1}, respectively[30]. These values are close to our TAME and ICME results (MIC and MBC of *E. coli* and *K. pneumonia* were 15-25 μgmL^{-1} and 120-130 μgmL^{-1}, respectively).

In order to check the antibiofilm activity of plant extracts, crystal violet assay was performed as per instructions of Liaqat et al.[19]. Khan et al. reported that the antibiofilm potential of *T. ammi* and other plant extracts is mainly due to the presence of naphthalene derivate compound[31]. In another research conducted by Kryvtsova et al. highest antibiofilm activity of plant extract was observed against *Staphylococcus* sp. which is similar to our results[32]. Similarly, Singh et al. reported the antibiofilm potential of *T. ammi* and the results were similar to our findings[33]. Likewise, Romero et al. reported the percent antibiofilm activity of *T. arjuna* against *E. coli* and *K. pneumonia* and *Staphylococcus* sp. in between 50 to 65%[34]. Another study by Schito et al. confirmed *T. arjuna* methanolic extract as an antibiofilm candidate against *E. faecalis* was measured in percent biofilm disruption as 70% and supported our results in which the antibiofilm activity was observed as 75% against *E. faecalis*[35]. Similarly, Elkhouly et al. reported the antibiofilm activity of *I. carnea* against *Klebsiella* sp. and *E. coli* ranged from 60 to 80 %, quite close to our findings which showed up to 70% biofilm disruption against *Klebsiella* sp. and *E. coli*[36]. Another study by Sharma & Bachheti reported the antibiofilm activity of *I. carnea* methanolic extract as 80% and 70% against *E. coli* and *K. pneumonia*, respectively[37]. Similar results were obtained from our findings which showed biofilm disruption as 79.5% and 75% against *E. coli* and *K. pneumoniae*.

Followed by antibiofilm activity, biofilm inhibitory activity of TAME and ICME were also checked. According to our study, TAME was able to inhibit biofilm ranged from 70-90 % against *K. aerogenes*, *E. coli* and *K. pneumonia* and similar to data presented by TA & Arnason where TAME inhibited biofilm up to 80% against *K. aerogenes*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*[38]. Similarly, Khan et al. studied the biofilm inhibitory potential of *T. ammi*

extract against *Streptococcus* sp. and identified active components like alkanoids, flavonoids and phytochemicals responsible for the biofilm inhibitory activity of *T. ammi*[39]. In a most recent study (Perveen et al. nanoparticles of *T. ammi* seed extracts were used to check the biofilm inhibitory and anti-cancerous activity of the aforementioned plant. According to the study, *T. ammi* inhibited the biofilm formation up to 73%[40]. The least activity observed was against *Listeria monocytogenes* (up to 58%). Likewise, Khan et al.[41] studied the biofilm inhibitory activity of silver nanoparticles in combination with *T. foenum-graecum* extract and significant results were obtained for *Pseudomonas* sp.[41].

5 Conclusion

The antibacterial and antibiofilm activity of six different plants extracts was evaluated against eighteen strains of CAB isolates. The combination of antibacterial and antibiofilm activity of six different plants extracts was evaluated against eighteen strains of Catheter associated bacteria (CAB) isolates. The combination of *T. arjuna* essential oil (TAEO) with *T. foenum-graecum* methanolic extract (TFME) exhibited the highest ZOI (32.0 ± 0.2 mm) at a concentration of 100 $\mu g mL^{-1}$ against *S. aureus*. *N. sativa* essential oil and *T. vulgaris* methanolic extract exhibited the most promising results, achieving up to 88% biofilm inhibition against CAB strains. Concentration-dependent inhibition was observed against most CAB strains including *P. mirabilis*, *Klebsiella* sp., and *P. aeruginosa*. Our study suggests that the tested extracts, alone or combined, can be effective against bacterial and biofilm-associated catheter-caused infections. Based on results, it is suggested that TAEO and TFME serve as the best possible natural remedy against infectious CAB.

Overall, this study presents compelling evidence for the potential of plant extracts as promising agents for preventing and treating catheter-associated bacterial infections. Their dual action of directly targeting bacteria and hindering biofilm formation makes them potential candidates for future advancements in healthcare. However, further research regarding extraction of bioactive components is needed and in progress in our lab to pinpoint the specific effects.

Supporting Information

This material is available free of charge via the Internet at doi: 10.5650/jos.ess24212

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Data Availability

All data related to article is available in manuscript.

Competing Interest

All authors declare no conflict of interests.

Ethical Approval

Not applicable.

Informed Consent

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Consent for Publications

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Research Involving Human and Animals Participants

Not applicable.

References

1) Rubi, H.; Mudey, G.; Kunjalwar, R. Catheter-associated urinary tract infection (CAUTI). *Cureus* **14**, e30385 (2022). doi: 10.7759/cureus.30385
2) Li, Z.; Ding, H.; Liu, X.; Zhang, J. Ultrasound-guided percutaneous peritoneal dialysis catheter insertion using multifunctional bladder paracentesis trocar: A modified percutaneous PD catheter placement technique. *Sem. Dialysis* **33**, 133-139 (2020). doi: 10.1111/sdi.12862
3) Safety, P. National Healthcare Safety Network (NHSN) Overview. (2017).
4) Leelakrishna, P.; Karthik, R. A study of risk factors for catheter associated urinary tract infection. *Int. J. Adv. Med.* **5**, 334-339 (2018). doi: 10.18203/2349-3933.ijam20180525
5) Edmiston, C.E., Jr.; McBain, A.J.; Roberts, C.; Leaper, D. Clinical and microbiological aspects of biofilm-associated surgical site infections. *Adv. Exp. Med. Biol.*

830, 47-67 (2015). doi: 10.1007/978-3-319-11038-7_3
6) Sabir, N.; Ikram, A.; Zaman, G.; Satti, L.; Gardezi, A. et al. Bacterial biofilm-based catheter-associated urinary tract infections: Causative pathogens and antibiotic resistance. *Am. J. Infect. Control* **45**, 1101-1105 (2017). doi: 10.1016/j.ajic.2017.05.009
7) Hobbs, T.; Schultz, L.N.; Lauchnor, E.G.; Gerlach, R.; Lange, D. Evaluation of biofilm induced urinary infection stone formation in a novel laboratory model system. *J. Urol.* **199**, 178-185 (2018). doi: 10.1016/j.juro.2017.08.083
8) Nicolle, L.E. Catheter associated urinary tract infections. *Antimicrob. Resist. Infect. Control* **3**, 23 (2014). doi: 10.1186/2047-2994-3-23
9) Odoemene, C.A.; Adiri, C.O. Catheter associated urinary tract infection offending pathogens, antimicrobial sensitivity, and complications. *Niger. J. Surg.* **29**, 6-13 (2019). doi: 10.4103/njss.njss_6_20
10) Singh, A.; Amod, A.; Pandey, P.; Bose, P.; Pingali, M.S. et al. Bacterial biofilm infections, their resistance to antibiotics therapy and current treatment strategies. *Biomed. Mater.* **17**, (2022). doi: 10.1088/1748-605X/ac50f6
11) Banerjee, D.; Shivapriya, P.M.; Gautam, P.K.; Misra, K.; Sahoo, A.K. et al. A Review on basic biology of bacterial biofilm infections and their treatments by nanotechnology-based approaches. *Proc. Natl. Acad. Sci. India Sect. B: Biol. Sci.* **90**, 243-59 (2020). doi: 10.1007/s40011-018-01065-7
12) Balikci, E.; Yilmaz, B.; Tahmasebifar, A.; Baran, E.T.; Kara, E. Surface modification strategies for hemodialysis catheters to prevent atheter-elated infections: A review. *J. Biomed. Mater. Res. B: Appl. Biomater.* **109**, 314-327 (2021). doi: 10.1002/jbm.b.34701
13) Mahmood, S.; Bashir, S.; Farzana, K.; Akram, M.; Abrar, M. et al. Differential inhibition of common bacterial species by extracts of three fruits using different solvents. *Philipp. Agric. Sci.* **95**, 169-174 (2012).
14) Malik, A.R.; Sharif, S.; Shaheen, F.; Khalid, M.; Iqbal, Y. et al. Green synthesis of RGO-ZnO mediated Ocimum basilicum leaves extract nanocomposite for antioxidant, antibacterial, antidiabetic and photocatalytic activity. *J. Saudi. Chem. Soc.* **26**, 101438 (2022). doi: 10.1016/j.jscs.2022.101438
15) Gălăţanu, M.L.; Panţuroiu, M.; Popescu, M.; Mihăilescu, C.M. Plant extracts with antibiotic effect. in *Handbook of research on advanced phytochemicals and plant-based drug discovery*. IGI Global, pp. 49-72 (2022).
16) Khalate, S.; Chandgude, P.; Kambale, M.; Dhawan, S.; Mane, S. et al. Biofilm inhibition of UTI pathogens using *Terminalia arjuna* and *Ipomea carnea* plant extract. *Indian J. Sci. Technol.* **13**, 2452-2562 (2020). doi: 10.17485/IJST/v13i24.725
17) Asif, H.M.; Sultana, S.; Akhtar, N. A panoramic view on phytochemical, nutritional, ethanobotanical uses and pharmacological values of *Trachyspermum ammi* Linn. *Asian Pac. J. Trop. Biomed.* **4**, S545-S53 (2014). doi: 10.12980/APJTB.4.2014APJTB-2014-0242
18) Khatoon, F.; Azahar, M.; Uddin, Q.; Husain, N.; Moin, M.S. et al. A Comprehensive Review on *Trigonella foenum-graecum* L. with special reference to unani medicine. *Comb. Chem. High Throughput Screen.* **27**, 187-202 (2024). doi: 10.2174/1386207326666230504112848
19) Liaqat, I.; Muhammad, N.; Ara, C.; Hanif, U.; Andleeb, S. et al. Bioremediation of heavy metals polluted environment and decolourization of black liquor using microbial biofilms. *Mol. Biol. Rep.* **50**, 3985-3997 (2023). doi: 10.1007/s11033-023-08334-3
20) Liaqat, I.; Muhammad, N.; Mubin, M.; Arshad, N.; Iftikhar, T. et al. Antibacterial and larvicidal activity of ethyl acetate extract of actinomycetes from soil samples. *Pak. J. Zool.* **55**, 2075-2083 (2023). doi: 10.17582/journal.pjz/20200526130518
21) Sadat Noori, S.A.; Norouzi, M.; Karimzadeh, G.; Shirkool, K.; Niazian, M. Effect of colchicine-induced polyploidy on morphological characteristics and essential oil composition of ajowan (*Trachyspermum ammi* L.). *Plant Cell Tissue Organ Cult.* **130**, 543-551 (2017). doi: 10.1007/s11240-017-1245-0
22) Saxena, S.N.; Kakani, R.K.; Sharma, L.K.; Agarwal, D.; John, S. et al. Genetic variation in seed quality and fatty acid composition of fenugreek (*Trigonella foenum-graecum* L.) genotypes grown under limited moisture conditions. *Acta Physiol. Plant* **39**, 1-10 (2017). doi: 10.1007/s11738-017-2522-6
23) Faustino, C.M.C.; Lemos, S.M.C.; Monge, N.; Ribeiro, I.A.C. A scope at antifouling strategies to prevent catheter-associated infections. *Adv. Colloid. Interface Sci.* **284**, 102230 (2020). doi: 10.1016/j.cis.2020.102230
24) Werneburg, G.T. Catheter-associated urinary tract infections: Current challenges and future prospects. *Res. Rep. Urol.* **14**, 109-133 (2022). doi: 10.2147/rru.S273663
25) Gunardi, W.D.; Karuniawati, A.; Umbas, R.; Bardosono, S.; Lydia, A. et al. Biofilm-producing bacteria and risk factors (gender and duration of catheterization) characterized as catheter-associated biofilm formation. *Int. J. Microbiol.* **2021**, 8869275 (2021). doi: 10.1155/2021/8869275
26) Odelola, O.S.; Oyetayo, V.O.; Ogundare, A.O.; Omoya, F.O.; Ajayi, O.E. Antibacterial activity of zea mays silks and husks crude extract on biofilm producing multidrug resistant bacteria from urinary catheters. *Int. J. Pathog. Res.* **12**, 37-51 (2023). doi: 10.9734/ijpr/2023/v12i2224

27) Premalatha, S.; Jeyasankar, A.; Elumalai, K. Screening of some selected indigenous plants for antibacterial activity. *Int. J. Interdisci. Res. Revs.* **1**, 65-72(2012).
28) Hilles, A.R.; Mahmood, S. Therapeutic uses and applications of fenugreek. *Fenugreek: Biology and Applications* 503-522(2021).
29) Zomorodian, K.; Ghadiri, P.; Saharkhiz, M.J.; Moein, M.R.; Mehriar, P. et al. Antimicrobial activity of seven essential oils from Iranian aromatic plants against common causes of oral infections. *Jundishapur J. Microbiol.* **8**, e17766(2015). doi: 10.5812/jjm.17766
30) Basri, D.F.; Fan, S. The potential of aqueous and acetone extracts of galls of *Quercus infectoria* as antibacterial agents. *Indian J. Pharmacol.* **37**, 26-29 (2005). doi: 10.4103/0253-7613.13851
31) Khan, A.; Ahmad, A.; Akhtar, F.; Yousuf, S.; Xess, I. et al. *Ocimum sanctum* essential oil and its active principles exert their antifungal activity by disrupting ergosterol biosynthesis and membrane integrity. *Res. Microbiol.* **161**, 816-823(2010). doi: 10.1016/j.resmic.2010.09.008
32) Kryvtsova, M.; Salamon, I.; Koscova, J.; Bucko, D.; Spivak, M. Antimicrobial, antibiofilm and biochemichal properties of *Thymus vulgaris* essential oil against clinical isolates of opportunistic infections. *Biosyst. Divers.* **27**, 270-275(2019). doi: 10.15421/011936
33) Singh, R.; Sahore, S.; Kaur, P.; Rani, A.; Ray, P. Penetration barrier contributes to bacterial biofilm-associated resistance against only select antibiotics, and exhibits genus-, strain- and antibiotic-specific differences. *Pathog. Dis.* **74**, ftw056(2016). doi: 10.1093/femspd/ftw056
34) Romero, C.M.; Vivacqua, C.G.; Abdulhamid, M.B.; Baigori, M.D.; Slanis, A.C. et al. Biofilm inhibition activity of traditional medicinal plants from Northwestern Argentina against native pathogen and environmental microorganisms. *Rev. Soc. Bras. Med. Trop.* **49**, 703-712(2016). doi: 10.1590/0037-8682-0452-2016
35) Schito, A.M.; Piatti, G.; Stauder, M.; Bisio, A.; Giacomelli, E. et al. Effects of demethylfruticuline A and fruticuline A from *Salvia corrugata* Vahl. on biofilm production in vitro by multiresistant strains of *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Enterococcus faecalis*. *Int. J Antimicrob. Agents* **37**, 129-134(2011). doi: 10.1016/j.ijantimicag.2010.10.016
36) Elkhouly, H.I.; Sidkey, N.M.; Ghareeb, M.A.; El hosainy, A.M.; Hamed, A.A. Bioactive secondary metabolites from endophytic *Aspergillus terreus* AH1 isolated from *Ipomoea carnea* growing in Egypt. *Egypt. J. Chem.* **64**, 7611-7620(2021). doi: 10.21608/ejchem.2021.85908.4161
37) Sharma, A.; Bachheti, R. A review on *Ipomoea carnea*. *Int. J. Pharma. Bio. Sci.* **4**, 363-377(2013).
38) Ta ChieuAnhKim, T.C.; Arnason, J. Mini review of phytochemicals and plant taxa with activity as microbial biofilm and quorum sensing inhibitors. *Molecules* **21**, 29(2016). doi: 10.3390/molecules21010029
39) Khan, R.; Adil, M.; Danishuddin, M.; Verma, P.K.; Khan, A.U. In vitro and in vivo inhibition of *Streptococcus mutans* biofilm by *Trachyspermum ammi* seeds: An approach of alternative medicine. *Phytomedicine* **19**, 747-755(2012). doi: 10.1016/j.phymed.2012.04.004
40) Perveen, K.; Husain, F.M.; Qais, F.A.; Khan, A.; Razak, S. et al. Microwave-assisted rapid green synthesis of gold nanoparticles using seed extract of *Trachyspermum ammi*: ROS mediated biofilm inhibition and anticancer activity. *Biomolecules* **11**, 197(2021). doi: 10.3390/biom11020197
41) Khan, F.; Manivasagan, P.; Lee, J.W.; Pham, D.T.N.; Oh, J. et al. Fucoidan-stabilized gold nanoparticle-mediated biofilm inhibition, attenuation of virulence and motility properties in *Pseudomonas aeruginosa* PAO1. *Mar. Drugs* **17**, 208(2019). doi: 10.3390/md17040208