



ABSTRACT

Catheter associated bacteria (CAB) are major problem and almost inevitable after long-term catheterization. Approximately 75% of the urinary tract infections (UTIs) are linked with the use of catheters. The current study was planned to isolate and characterize CAB and to evaluate the antibacterial, antibiofilm and biofilm inhibitory potential of two medicinal herbs viz., *Trachyspermum ammi* essential oil (EO) and *Trigonella foenum-graecum* methanolic extract (ME). Total six strains were isolated from the infected catheters and characterized on morphological, biochemical, and genetic basis. The strains were identified up to species level using 16S rRNA gene sequencing and identified as *Klebsiella pneumoniae* (OK669223), *Enterobacter cloacae* (OK669224), *Achromobacter xylosoxidans* (OK669225), *Pseudomonas aeruginosa* (OK669226), *Staphylococcus aureus* (OK66927) and *Staphylococcus saprophyticus* (OK669228). *T. ammi* EO and *T. foenum-graecum* ME were prepared by hydrodistillation and maceration extraction methods, respectively. The antibiotic susceptibility profile of CAB was checked by disc diffusion method using four antibiotics (lincomycin 100 $\mu\text{g mL}^{-1}$, erythromycin 20 $\mu\text{g mL}^{-1}$, rifampicin 100 $\mu\text{g mL}^{-1}$ and ciproflaxin 40 $\mu\text{g mL}^{-1}$). *P. aeruginosa* showed maximum resistance against all the tested antibiotics. Time kinetics was used to check the biofilm forming ability of CAB at 72h, 120h and 168h. Maximum biofilm formation was observed at 120h for all the strains. Antibacterial activity of *T. ammi* EO, *T. foenum-graecum* ME alone and in combination was evaluated by agar well diffusion method. Maximum zone of inhibition (32 ± 0.8) was recorded for the combined activity of *T. ammi* EO and *T. foenum-graecum* ME against *S. aureus*. Zone of inhibition (30.3 ± 2.3 , 30.0 ± 1.3) were recorded against *S. aureus* when *T. ammi* EO and *T. foenum-graecum* ME were applied alone. Antibiofilm and biofilm inhibitory activity of *T. ammi* EO and *T. foenum-graecum* ME was evaluated by crystal violet assay in test tubes. Statistically significant concentration dependent increase ($p < 0.05$) was observed in percentage inhibition of biofilm formation on catheters using *T. ammi* EO and *T. foenum-graecum* ME alone and in combination against CAB. Maximum percent antibiofilm activity (87.7 ± 0.02) was observed against *S. aureus* by using the combination of *T. ammi* EO and *T. foenum-graecum* ME. When applied alone, *T. ammi* EO and *T. foenum-graecum* ME showed maximum percent antibiofilm activity against



Enterobacter sp. (78 ± 0.01 , 85 ± 0.25) respectively. Similarly, the combined activity of *T. ammi* EO and *T. foenum-graecum* ME showed the maximum potential of percent biofilm inhibition against *K. pneumoniae* (93.4 ± 0.00). When used alone, *T. ammi* EO and *T. foenum-graecum* ME inhibited percent biofilm inhibition against *Pseudomonas* sp. Up to (83.5 ± 0.02 , 89.7 ± 0.03) respectively. There was a concentration dependent increase in the antibacterial, antibiofilm and biofilm inhibitory activity of *T. ammi* EO and *T. foenum-graecum* ME against CAB. It was observed that *T. ammi* EO and *T. foenum-graecum* ME when used in combination were more potent against all the strains as compared to when used alone. It is recommended that *T. ammi* EO and *T. foenum-graecum* ME could serve as the best possible natural remedy against the infectious CAB. However, still there is need to conduct more research to identify the bioactive compounds of *T. ammi* and *T. foenum-graecum*. These results can be utilized for the further study of antimicrobial, antibiofilm and biofilm inhibitory potential of *T. ammi* and *T. foenum-graecum* and in the production of natural drugs against the resistant microbial pathogens. These plants can serve as the cheap source of drugs as they are easily accessible and of natural origin.