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Original Article

Histopathological Changes in Animal Models by Catheters Coated with Saudi Medicinal Herb *Evolvulus alsinoides* L. Extract in Urinary Tract Infections by *Klebsiella pneumoniae*

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ABSTRACT

Background: Catheter-Associated Urinary Tract Infections (CA-UTIs) pose a significant danger especially when they are caused by multi-drug resistant strains of *Klebsiella pneumoniae*. As elsewhere in the world, Saudi Arabia also shows a higher prevalence of *Klebsiella pneumoniae* which complicates the treatment and management of the CA-UTIs. To tackle this, it is urgent to develop novel antibacterial compounds from natural sources and, phytochemicals have always been potential candidates. *Evolvulus alsinoides* is a traditional medicinal plant found in Saudi Arabia possesses proven activities against CA-UTI causing bacteria including *Klebsiella pneumoniae*. **Aim:** The present study was aimed to investigate the antibacterial, anti-inflammatory and anti-biofilm activities of *Evolvulus alsinoides* plant extract against multidrug resistant *Klebsiella pneumoniae*. **Materials and Methods:** The study was designed to coat the methanolic extract of *Evolvulus alsinoides* on catheters and evaluate its efficacy on Guinea pigs (*Cavia porcellus*). Inflammatory marker concentrations, SOD (reactive oxygen species), and the MPO (neutrophil recruitment) were also evaluated to understand the effect of this plant extract on tissue damage. **Results:** *Evolvulus alsinoides* displayed the presence of alkaloids, flavonoids, phenols, saponins, tannins and fixed oils. *Klebsiella pneumoniae* was resistant to four of the five antibiotics tested. The extract showed anti-*Klebsiella pneumoniae* activity and the MIC was 4.61 mg/mL. Showing its anti-biofilm activity, 31.23 µg/mL of the extract reduced the catheter biofilm to 16.23%±2.4. At 5 µg/mg, the extract displayed anti-inflammatory markers as MPO degranulation 49.36%±1.4, MDA 0.142±0.001 nmol/mg protein and SOD 58.97±1.6 units/mL. **Conclusion:** In the light of these findings, it could be assessed that the *Evolvulus alsinoides* extract possesses antibacterial, anti-biofilm and anti-inflammatory activities, and the extract should further be studied to develop it for its clinical usage.

Keywords: Biofilms, CA-UTIs, Guinea pigs, Inflammatory markers, *Klebsiella pneumoniae*, MDR strains, Urinary catheters, Evolvulus *alsinoides*.

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INTRODUCTION

Urinary Tract Infections (UTIs) contribute 30-40% of Hospital-Acquired Infections (HAIs), and about 80% of them are due to urinary catheters.[1] As a routine procedure in the ICUs (Intensive Care Units), the urinary catheters are used in 15-25% of all the hospitalized patients who would stay for 2-4 days.[1,2] This cause a huge prevalence of Catheter-Associated Urinary Tract Infections (CA-UTIs), and in Saudi Arabia, between 2004 and 2011, it was found that the rate of occurrence of CA-UTIs in adult ICUs was 8.18 for every 1000 catheter days.[1] Other researcher have also reported a high prevalence of UTIs in Saudi Arabia, which could lead to hospitalization and CA-UTIs. Menyfah Q. Alanazi in 2018[3] estimated that the CA-UTI incidents contributed 25% of total infections recorded in Saudi Arabia. Another study conducted in 1,000 Saudi diabetic patients at the of King Saud University Diabetes Centre, Riyadh, Kingdom of Saudi Arabia during June 1993 and December and 2009 showed a 25.3% prevalence of UTIs.[4] in all the HAIs. If left untreated, CA-UTIs can lead to infections in the kidneys (pyelonephritis) and the bloodstream (septicemia),[5] which can lead to sepsis or, in the worst-case scenario, even to death. The usage of antibiotics which can destroy the biofilms formed by the stubborn bacteria in urinary catheters is a typical treatment for UTIs.

Klebsiella pneumonia is a major Multi-Drug Resistant (MDR) bacterial pathogen that has been declared as 'the urgent threat' by various international organizations and agencies, including the WHO (World Health Organization), the UK Department of Health and the US Centre for Disease Control and Prevention

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(CDC).[6,7] In a healthcare system, infections by various strains of *Klebsiella pneumoniae* are dangerous and, in many cases fatal, especially when it comes to immunocompromised patients, neonates, and aged patients with urinary catheterization.[8,9] *Klebsiella pneumonia* causes catheter-associated urinary tract infections and lower respiratory tract infections.[10] Together with *Escherichia coli*, they pose a growing pathological danger as they are found in both hospital and community settings around the world.[11] So, tackling the issue of MDR *Klebsiella pneumoniae* in cases of CA-UTIs is a challenge.

The prevalence and complications due to drug resistance associated with *Klebsiella pneumoniae* causing UTIs and urinary catheter infections are high in Saudi Arabia. Different studies underline the seriousness of this situation. A review report by Shadi Ahmed Zakai, 2019[12] mentioning the prevalence of urinary tract infections leading to CA-UTIs in Middle Eastern countries showed that the ciprofloxacin-resistant *Klebsiella pneumoniae* have remarkably increased in hospital-acquired isolates in Saudi Arabia and, surprisingly, with a jump in the rate of resistance in just six years, from 2.6 to 23%. Another study conducted at the King Salman Armed Forces Hospital, Tabuk showed that the *Klebsiella pneumoniae* was the second most prevalent UTI causing bacteria in children, and they were all Ampicillin-resistant with an increased rate of resistance of 42.9% to Ceftriaxone.[13] In 2016, Zowawi, HM[14] reported that treating common bacterial infections like UTIs is becoming more difficult because of the steady increase in the global prevalence of MDR strains. The report also noted that the occurrence of *Klebsiella pneumoniae* isolates producing ESBL (extended-spectrum beta-lactamase) in Saudi Arabia has increased up to 65% since the 1990s. Consequently, these rising rates have been linked to numerous outbreaks and mortality rates ranging from 11 to 40%. A report by Misfer Al-Ghamdhi, 2011,[15] based on a study conducted at the Al-Kharj military hospital, Saudi Arabia, in 1076 Mid-Stream Urine (MSU) samples from out-patients showed a high presence of *Klebsiella pneumoniae* (30.7%) isolates. All these findings underline the risks behind the CA-UTIs, especially when it is caused by MDR *Klebsiella pneumoniae.*

Evolvulus alsinoides is a medicinal plant traditionally used in various parts of Saudi Arabia and is widely seen in the Fayfa mountains and in the Asir mountains of the northern side of Jizan province.[16] This plant has proven antibacterial activities against MDR *Klebsiella pneumoniae* causing CA-UTIs and the fact has been reported by many researchers, including Kumar *et al.*, 2004[17] and Gollen and Mehla, 2018.[18] So, the present study was a trial to utilize the antibacterial activity of the plant against the major CA-UTI causing *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Duration and Setting

This research work was conducted at the Department of Basic Medical Sciences, College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia during the period between October 17, 2021, and August 27, 2022, recognized by the institutional ethical committee.

Collection of the herb

Evolvulus alsinoides leaves were collected and after being washed with distilled water they were dried in the shade at room temperature. For extraction, the dried leaves were ground into fine powders and kept in a sterile container.

Extraction of bioactive compounds

Evolvulus alsinoides leaf powder was packaged and put in a Soxhlet device. Methanol solvent solution was added to the extractor, which was then heated to 60°C and left for 6 hr. After the solvent vaporized the extract was collected. In sterile containers, the dried extracts were stored.

Phytochemical analysis of plant extracts

The qualitative phytochemical analysis was performed (as per the protocols explained by Jyothiprabha and Venkatachalam, 2016;[19] Boggula and Peddapalli, 2017[20]) with the powdered crude extracts of different plant parts.

Alkaloids

To 3 mL of the test solution, a few drops of dil. iodine solution (I_2) were added. Blue colour indicated the presence of alkaloids.

Flavonoids

To understand the qualitative presence of flavonoids, the alkaline reagent test was performed. Few drops of diluted Sodium Hydroxide (NaOH) were added to the crude, powdered extract. The presence of flavonoids was confirmed by the formation of a bright yellow colour that became colourless when a few drops of diluted HCl were added.

Tannins

0.5 g of aqueous extract was added with 10 mL of bromine water. Decolorization of the bromine water was considered as an indication for the presence of tannins in the extract.

Terpenoids

Concentrated sulfuric acid was added to the crude powder's chloroform extract. The presence of terpenoids was indicated by a reddish-brown ring appearance.

Steroids To confirm the qualitative steroid presence, the Liebermann-Burchard reaction was used. The crude powder in its chloroform solution was added to the con. sulfuric acid down

the sides of the test tube. The formation of a blue-green ring was the indication of steroid compounds.

Saponins

Frothing test was used to identify the presence of saponins. Following a vigorous shake with distilled water and a 10-min stand time, the crude powder was analysed for the presence of saponin. If no froth was there, it was considered as an indication of the absence of saponins and a stable froth of 1.5 cm or higher was considered as the indication of saponins.

Cardiac glycosides

The Keller-Kiliani test was used to determine whether cardiac glycosides were present. 1 mL mixture of $FeCl_3$ (5%) and glacial acetic acid in 1:99 v v-1 ratio was used to treat the crude extract with. Few drops of con. sulfuric acid was added to this solution and the development of a greenish blue colour in few minutes of addition was considered as the indication of the presence of cardiac glycosides. To 2 mL of test solution, $FeCl_3$ (0.5 mL, w/v) was added. The development of an intense colour was considered as the indication of phenols. When treated with concentrated hydrochloric acid, the development of green colour was regarded as the indication for quinone presence.

Test for fixed oil

A small quantity of the extracts was forced between two filter papers. The presence of fat and fixed oils was indicated by the formation of oil stain on the paper.

Isolation and identification of clinical pathogens

The clinical species of *Klebsiella pneumoniae* was collected form the PSAU hospital lab. It was further confirmed by standard microbiological procedures including Gram staining and growth on MacConkey agar.

Antibiotic sensitivity of the bacterial pathogen against commercial drugs (Jorgensen and Turnidge, 2007)[21]

Using Muller-Hinton Agar (MHA) by disc diffusion method (Kirby-Bauer), the bacterial susceptibility to antibiotics was analysed against five standard antibiotics *viz.,* Amoxicillin, Methicillin, Ampicillin, Tetracycline and Streptomycin procured from Oxoid Ltd.UK. The clinical isolates of the test bacterium-*Klebsiella pneumoniae* were tested against the five selected antibiotics separately. The results were interpreted by the disk diffusion method as explained in the NCCLS2000 (National Committee for Clinical Laboratory Standards).[22] Different antibiotics were taken as ampicillin (25 mcg), tetracycline (30 mcg), methicillin (5 mcg), streptomycin (30 mcg), and amoxicillin (30 mcg). By measuring the inhibition zone size (in mm) and comparing it to the zone interpretation chart as per CLSI, the pattern of resistance/susceptibility was determined.

Antibacterial activity using the good diffusion method

Using standard methods (Wayne, 2019),[23] the antibacterial potentials of *Evolvulus alsinoides* extract against the clinical isolate of *Klebsiella pneumoniae* were determined. The sterile Muller-Hinton Agar (MHA) was swabbed with overnight cultures of the test bacterium and 6 mm bore wells were made on the agar surface. To each of the wells, the samples in 100 µl were added and the agar plates were kept for a period of 48 hr at a temperature of 37°C for incubation. The anti-bacterial activity of the extract was determined by measuring the diameters (in mm) of the possible zones of growth inhibition around the wells in the incubated plates.

Determination of the MIC (Minimum Inhibitory Concentration) of the plant extracts

The dilution method was performed to determine the MIC (Minimum Inhibitory Concentration). In a series of 1 mL of sterile Muller-Hinton Broth (MHB) test tubes, 1 mL of *Evolvulus alsinoides* extract was diluted into different concentrations as 1.95 mg/mL, 3.90 mg/mL, 7.80 mg/mL, 15.60 mg/mL, 31.50 mg/mL, 62.50 mg/mL, 1250 mg/mL and 250 mg/ml. A 100 µl of *Klebsiella pneumoniae* culture at 0.5 McFarland standard (Eucast, 2003) was inoculated to the tubes, which were then incubated for 24 hr at 37°C. The test tubes were analysed for growth/turbidity by naked eye (CLSI, 2012).[24]

Biofilm inhibition assay

The biofilm inhibition assay was performed as per the protocols described by Stepanović *et al.,* 2007.[25] Using polystyrene tube assay (which is based on the crystal violet staining method), the biofilm inhibition ability of extracts was determined. For the studies, 96 well titter plates were used. 10 µl of fresh pathogen (OD 0.4) was poured in the well and various concentration of extracts (10 µL, 20 µL, 30 µL, 40 µL) were inoculated and fresh MHB was added making up to 200 µl. The plates were incubated at 37°C for 48 hr. After discarding the liquid media, with Phosphate-Buffered Saline (PBS) the adherent cells were rinsed for two times. They were then stained for 30 min with 0.5% of crystal violet. The stain was then eluted from the adherent cells by vertexing for 5 min using ethanol solvent. Absorbance was measured at 590 nm using an ELISA reader (Shishin, SH-U830, Taipei, Taiwan, ROC). Using fresh samples each time, the assay was repeated thrice.

Animals used

1-2-month-old Guinea pigs were purchased from Riyadh. Under closely monitored hygienic conditions in galvanized cages with a 12: 12 hr light and dark cycle, the Guinea pigs were acclimatized to the animal house environment. Vitamin C (ascorbic acid, 50 mg in one litre of drinking water) was also supplied as the daily requirement throughout the experiment as described by

Sarah and Maggie, (2003).[26] The experiments were approved by the institution, with the number 2021/03/18542.

Catheter implantation

The Guinea pigs were anesthetized by intraperitoneal injection (1 mg/kg) of a 1:2 (v/v) solution of xylazine (20 mg/mL) and ketamine (100 mg/mL) prior to catheter insertion. Each animal's lower back was clipper-shaved before being cleaned with 0.5% chlorhexidine in 70% alcohol. Longitudinally, an incision of 2 mm was created and the dissection of the subcutis was performed. Aseptically, into the subcutaneous space, 6 one-centimeter segmented polyurethane intravenous catheters (BD VialonTM 16G, 1.7×45 mm, Becton Dickinson and Co., Canada) were implanted. Before implantation, the catheters were dipped in cerium nitrate (1 mg/mL) for 24 hr. and the *Evolvulus alsinoides* extract at various concentrations corresponding to prior calculated MIC, 2×MIC and 3×MIC for each species. The positive controls were those catheters which were incubated in plain PBS. In each of the experiments, five animals were used. After that, PBS control or 300 mL of each standardized bacterial suspensions were injected into the pockets. After closing the incision with a monofilament suture, the area was disinfected with 0.5% chlorhexidine in 70% alcohol. On day seven, the animals were sacrificed to aseptically remove the catheters. The biofilms were spectrophotometrically quantified by Crystal Violet (CV) assay by measuring the biofilm total biomass at 590 nm. This subcutaneous foreign body infection on mouse model is as explained by Rupp *et al*., (1999).[27]

In vivo anti-inflammatory activities of *Evolvulus alsinoides* extracts

The *in vivo* anti-inflammatory activity of the plant extract was carried out by a modified method explained by Rauf *et al*., 2014.[28] Guinea pigs were randomly grouped into four with five animals in each one. Group 1 was the control and received 0.4 mL of distilled water. Group 2 was given 0.5 µg/mg and Group 3 was given 1 µg/mg of the *Evolvulus alsinoides* extracts. For Group 4, it was 2.5 µg/mg and for group 5, the extract given was 5 µg/mg, and all were dosed orally for a period of 14 days. The animals were closely monitored for daily changes and for other signs or symptoms of toxicity or death throughout the study period. At the end of the dosing period, after an overnight fast through cardiac puncture with mild anesthesia by diethyl ether, the blood samples were taken from the animals. The blood samples were kept in specimen bottles without anticoagulant. Liver, kidney and testes were quickly excised, perfused with normal saline, and in phosphate buffer (0.2 M, PH 7.4), they were homogenized in 0.25 M sucrose.

Superoxide Dismutase (SOD) Activity

The assay for determining SOD potential was performed as per the protocol explained by Kakkar *et al*., (1984).[29] The final 3 mL volume was adjusted by 186 MM Phenozinemetho-Sulphate (PMS), 0.052 M sodium pyrophosphate buffer with a PH of 8.3, 780 MM NADH, sonicated enzyme preparation, 300 MM Nitroblue Tetrazolium (NBT) and water. The reaction was begun after adding NADH, which was followed by an incubation for 90 sec at 37°C. The reaction was halted after the incubation period by adding 1.0 mL of glacial acetic acid, and the mixture was then vigorously mixed with 4.0 mL of n-butanol. After centrifuging the mixture and separating the butanol layer, the mixture was let to stand for 10 min. Using a spectrophotometer, the colour intensity of the chromogen in butanol was evaluated at 560 nm versus butanol. As the control, a mixture of cell suspension without enzyme was used.

Malondialdehyde (MDA) Activity

The assay for Malondialdehyde was performed as per Okhawa *et al*. (1979).[30] 1 mL of tissue homogenate was added with 1 mL of normal saline and 2.0 mL of 10% TCA and the whole mixture was shaken well. To separate the proteins, the mixture was centrifuged for 10 min at 3000 g. Then, 2 mL of supernatant was taken and 0.5 mL of 1.0% TBA was poured to it and heated for 60 min at a temperature of 95°C and a pink colour was generated, which was of MDA. Using a spectrophotometer, the OD of the samples was measured at a wavelength of 532 nm.

Myeloperoxidase (MPO) Activity

The test for MPO was performed in accordance with the procedures illustrated by Luo *et al.*, 2012.[31] Animals were grouped into five of four in each as mentioned elsewhere in this article. *Evolvulus alsinoides* extract was administered by IP injection. After 24 hr, 0.5 mL blood was drawn from the animal and the RBCs were lysed with the help of ammonium chloride. The neutrophils were counted and sonicated in PBS 0.2% CTAB. The MPO activity was found out as explained earlier, with H_2O_2-dependent TMB oxidation assay at a wavelength of 655 nm.

RESULTS

Analysis for phytocompounds from the plant extract

The crude methanolic extracts from the leaves of the herb-*Evolvulus alsinoides* were analysed for qualitative phytochemical compounds and the results were as shown in the Table 1. The extract showed the presence of compounds like alkaloids, flavonoids, saponins, phenols, tannins and fixed oils. But the others including terpenoids, steroids, cardiac glycosides and quinones were absent.

Antibiotic sensitivity of the bacterial pathogen against commercial drugs

The clinical isolate showed resistance to the four of the tested, commercial antibiotics *viz* Amoxicillin, Methicillin, Ampicillin and Tetracycline. Streptomycin was the only antibiotic against

Table 1: 1 The phytochemical compound analysis of *Evolvulus alsinoides* extract. (+) sign indicates the presence; (-) sign indicates the absence.

Test	Result
Alkaloids	+
Flavonoids	+
Saponins	+
Phenol	+
Tannins	+
Steroids	-
Terpenoids	-
Oil	+
Quinine	-
Glycosides	-

Table 2: Antibiotic sensitivity test for the isolated clinical pathogen-*Klebsiella pneumoniae*.

Sl. No.	Antibiotics	Inference
1	Amoxicillin	Resistant
2	Methicillin	Resistant
3	Ampicillin	Resistant
4	Tetracycline	Resistant
5	Streptomycin	Intermediate

Table 3: Antibacterial activity of Evolvulus alsinoides extract against *Klebsiella pneumoniae*.

Sl. No.	Concentration of the extract (in mg/mL)	Zone of inhibition (in mm)
1	125	-
2	250	-
3	500	11
4	1000	15

which the *Klebsiella pneumoniae* showed intermediate sensitivity. The results are shown in the Figure 1 and Table 2.

Antibacterial activity of the plant extract

Antibacterial efficacy of the *Evolvulus alsinoides* methanolic extract against the clinical isolate of *Klebsiella pneumoniae* is shown in Figure 2 and in Table 3. Extracts at concentration 125 mg/mL and 250 mg/mL showed no zone of inhibition whereas at concentration 500 mg/mL and 1000 mg/mL produced zones of inhibition with 11 mm and 15 mm respectively.

The Minimum Inhibitory Concentration (MIC) of *Evolvulus alsinoides* extract against *Klebsiella pneumoniae*

The Minimum Inhibitory Concentration (MIC) of *Evolvulus alsinoides* extract against *Klebsiella pneumoniae* was found to be 4.61 mg/mL as shown in Figure 3.

Biofilm formation and inhibition assay

The procured catheters which were first subjected to biofilm formation showed the results as shown in the graph Figure 4 and Table 4. The control catheter (incubated in plain PBS) shown 100% biofilm formation on it by *Klebsiella pneumoniae* whereas the one with cerium nitrate coated shown 58.78%±8.9. the catheter coated with 3X MIC (Extract 31.23 µg/mL) showed the lowest percentage (16.23±2.4) of biofilm formation.

Anti-inflammatory activities of *Evolvulus alsinoides* extracts

The anti-inflammatory markers of *Evolvulus alsinoides* extract detected by Superoxide Dismutase (SOD) Activity Analysis, Malondialdehyde (MDA) Activity Analysis and Myeloperoxidase (MPO) activity analysis was produced results as shown in graphs Figures 5, 6 and in Table 5.

Table 4: *Klebsiella pneumoniae* biofilm formation and inhibition assay using *Evolvulus alsinoides* extract.

Sl. No.	Compounds	Biofilm formation (in %)
1	Control	100
2	Cerium nitrate	58.78±8.9
3	Extract (10.41 µg/mL)	52.04±4.8
4	Extract (20.82 µg/mL)	45.65±6.1
5	Extract (31.23 µg/mL)	16.23±2.4

Table 5: Anti-inflammatory markers of *Evolvulus alsinoides* extract by Superoxide Dismutase (SOD) Activity Analysis, Malondialdehyde (MDA) Activity Analysis and Myeloperoxidase (MPO) activity.

Concentration (µg/mg)	MPO degranulation (%)	MDA (nmol/mg protein)	SOD (units/mL)
Distilled water	99.99±0.01	0.405±0.004	12.00±0.58
0.5	86.19±1.6	0.291±0.002	24.13±1.8
1	71.82±1.2	0.263±0.06	38.68±4.2
2.5	56.44±3.5	0.189±0.002	46.39±2.9
5	49.36±1.4	0.142±0.001	58.97±1.6

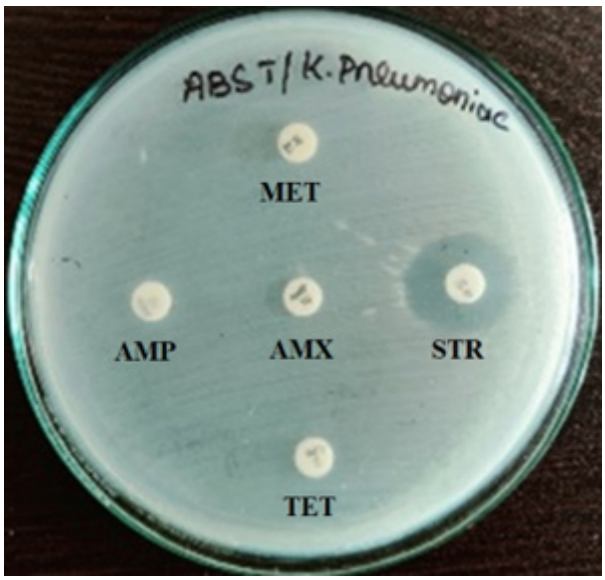

Figure 1: Antibiotic sensitivity test for the isolated clinical pathogen-*Klebsiella pneumoniae*. AMP-Ampicillin; MET-Methicillin; AMX-Amoxicillin; STR-Streptomycin; TET-Tetracycline.

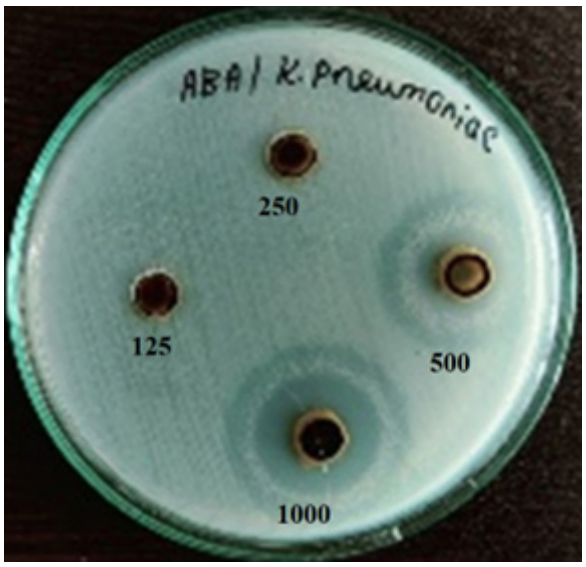

Figure 2: Antibacterial activity of the *Evolvulus alsinoides* extract against *Klebsiella pneumoniae*. The numbers indicate the concentration of the extract as: 125 mg/mL, 250 mg/mL, 500 mg/mL and 1000 mg/mL.

Antibacterial and Anti-biofilm activities of the extract-coated catheters

The anti-bacterial and anti-biofilm activities of *Evolvulus alsinoides* extract coated catheters were observed as shown in Figures 7 and 8. The biofilm inhibitory action of *Evolvulus alsinoides* extract clearly shown that that the concentrations higher than 6.26 mg/mL, 125 mg/mL and 250 mg/mL could suppress the biofilms completely.

DISCUSSION

The present study was planned to understand the antibacterial and anti-biofilm activities of the plant *Evolvulus alsinoides* against the major, most prevalent Catheter-Associated Urinary Tract Infection (CAUTI) causing multi-drug resistant bacterium *Klebsiella pneumoniae*. The study is of greater significance as it has been calculated that 65 to 80% of total bacterial infections were as a result of biofilms.[32] There have been many scientific investigations like that of Townsend *et al.*, 2020,[33] in which they demonstrated an effectivealternative based on a combined

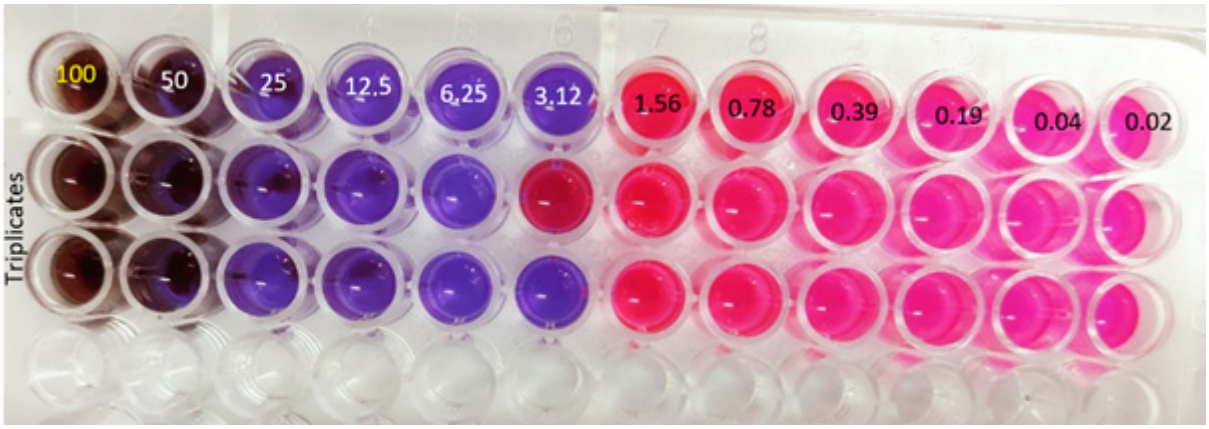

Figure 3: Minimum Inhibitory concentration of the *Evolvulus alsinoides* extract. Violet colour-no growth; pink colour-bacterial growth MIC-4.61 mg/mL.

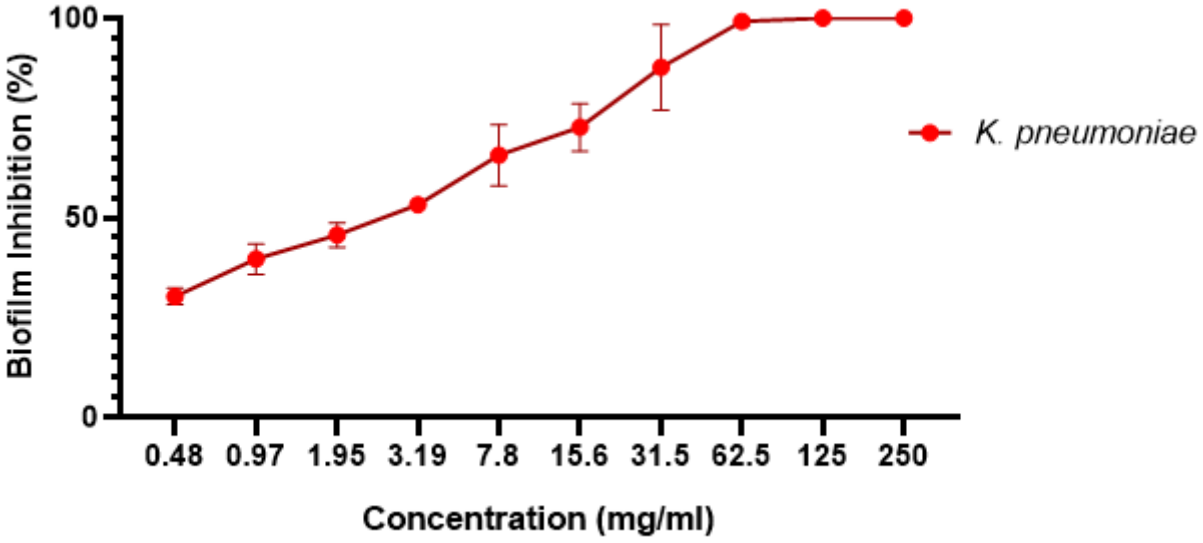

Figure 4: Graph showing the percentage of *Klebsiella pneumoniae* biofilm inhibition by *Evolvulus alsinoides* extract.

action of bacteriophages and antimicrobials in combating the CAUTIs by *Klebsiella pneumoniae*. Another study[34] used the possibilities of green synthesized silver nanoparticle (AgNPs) coatings on urinary catheters which have also reduced the extent of colonisation significantly. As the current investigation is solely focused on the antibacterial and anti-biofilm potentials of the selected plant, we had to analyse the plant phytochemically too.

Many plants have been analysed for their phytochemical compounds in order to understand different biochemical properties including antimicrobial activities.[35] The present investigation for the phytochemical analysis of the leaf extracts of the selected *Evolvulus alsinoides* plant revealed that it contained components like alkaloids, flavonoids, saponins, phenols, tannins and fixed oils. Different studies conducted by various researchers like (Omogbai and Eze, 2011;[36] Zahir and Kumaresan, 2014;[37] Mohanasundari *et al.*, 2021;[38] Srinivasan *et al.*, 2021)[39] showed that the *Evolvulus alsinoides* contained alkaloids, glycosides, tannins, saponins, flavonoids and volatile oil were more effectively extracted in ethanol than in water. So, all these studies in principle underline the findings in our study.

The CA-UTIs formed caused by *Klebsiella pneumoniae* (up to 30.7% of all the total UTIs) are the worst scenario as they have a number of Multi-Drug Resistant (MDR) strains, and untreated or antibiotic resistant biofilm CA-UTIs may result in pyelonephritis (infections in the kidneys) and septicaemia (bloodstream infections).[5] In this study, we could find that the clinical isolate was resistant to the four of the tested antibiotics *viz* Amoxicillin, Methicillin, Ampicillin and Tetracycline. Streptomycin was the only antibiotic against which the *Klebsiella pneumoniae* was intermediately susceptible. Other studies by various researchers (including Lin *et al.*, 2022)[40] have also found that the rates of resistance cefazolin, tobramycin, gentamicin,

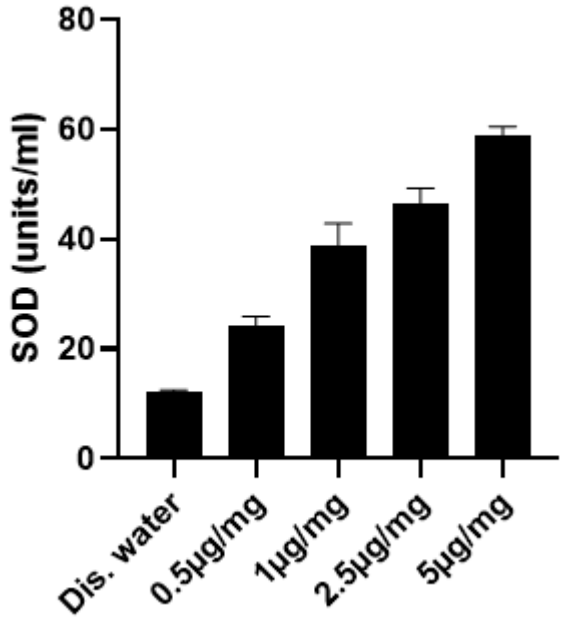

Figure 5: Graph showing Superoxide Dismutase (SOD) Activity Analysis, by *Evolvulus alsinoides* extract. The x-axis shows the concentration of the extract.

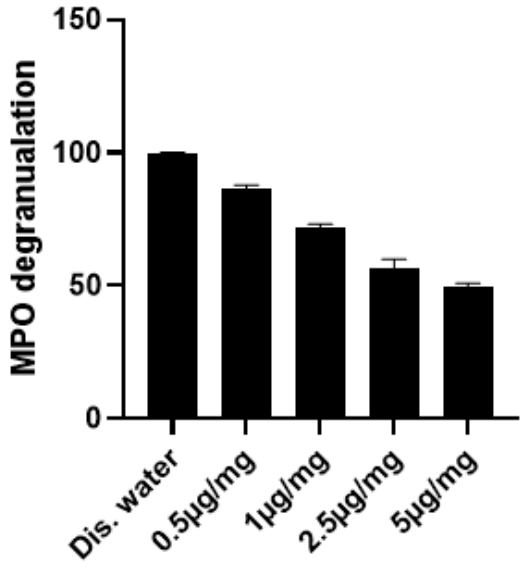

Figure 6: Graph showing Myeloperoxidase (MPO) denaturation activity by *Evolvulus alsinoides* extract. The X-axis shows the concentration of the extract.

imipenem, ceftazidime and ciprofloxacin were 40.82%, 25.07%, 21.57%, 12.83%, 17.78% and 44.61%. Ibrahim *et al.*, 2020[41] found that a rate of resistance against antibiotics was shown by *Kebsiella pneumoniae* clinical isolates as 65.8% against ceftriaxone, 96.9% against ampicillin and 60.8% against cefepime (60.8%). Thus, the antibiotic resistance in clinical isolates is a wide spread and concerning.

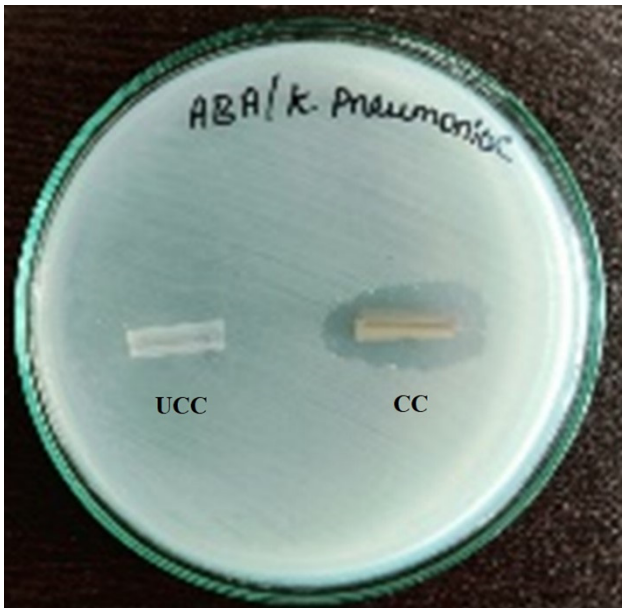

Figure 7: Antibacterial activity of *Evolvulus alsinoides* extract coated catheters against *Klebsiella pneumoniae.* UCC-uncoated catheter, CC-coated catheter.

As this study displayed, antibacterial and anti-biofilm efficacies of the *Evolvulus alsinoides* extract against the clinical isolate of *Klebsiella pneumoniae* are enough to provide hope for scientists who search for potential antibiotics of natural origin. Even though we couldn't find any report on the activity of *Evolvulus alsinoides* plant extract against CA-UTI *Klebsiella pneumoniae*, the plant had been screened by different researchers against clinical and other isolates of the bacterium. Mohanasundari *et al.*, 2021;[39] Nazanin *et al.*, 2017;[42] Zahir and Kumaresan, 2014;[37] and Omogbai and Eze, 2011[36] have shown potential inhibitory activities of the plant extract against Gram-negative bacteria including *Klebsiella pneumoniae* and against many of the Gram-positive bacteria. The anti-bacterial activities of this plant and other plant materials against clinical isolates of *Klebsiella pneumoniae* biofilms and other urinary biofilms were also investigated by Olawuwo *et al.*, 2022;[43] Srinivasan *et al.*, 202[39] Mitra *et al.*, 2016;[44] Adesina *et al.*, 2015;[45] Mohsenipour *et al.*, 2015[46] Ezeonu *et al.*, 2009,[47] Gomes *et al.*, 2019;[48] etc. and concluded that the plants possessed potential activity as anti-biofilm and antibacterial agents. All these reports are in agreement with our findings.

This study also investigated the anti-inflammatory activities of *Evolvulus alsinoides* extracts by evaluating the anti-inflammatory markers detected by superoxide dismutase (SOD-which is an effective enzyme that can be used in treatments against reactive oxygen (RO) species-mediated disorders)[49] activity analysis, Malondialdehyde (MDA-the final product of lipid peroxidation, and the increase of which indicates the higher free radicals)[50] analysis and myeloperoxidase (MPO-which is an important

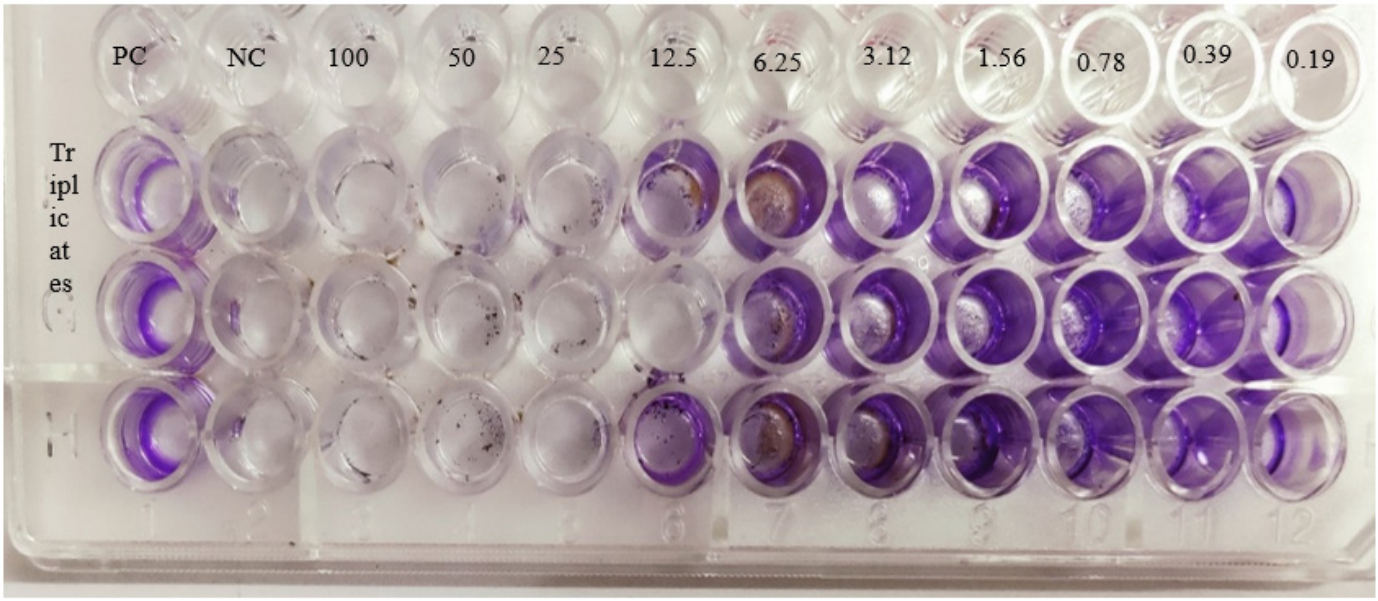

Figure 8: The anti-biofilm activity of the *Evolvulus alsinoides* extract against *Klebsiella pneumoniae*. Concentrations higher than 6.26 mg/mL could suppress the biofilms completely.

inflammatory enzyme acting as a triggering agent for oxidative stress and neuroinflammation)[51] activity analysis. As shown elsewhere in this article, our study could report that all these markers were shown positive tendencies. There are a number of studies (like that performed by Duraisamy *et al.*, 2013;[52] Bhattacharya *et al.*, 2000[53] etc) showing the quantification and analysing the tendencies of these markers using plants including *Evolvulus alsinoides* and *Bacopa monniera* Linn. both these studies reported the facts found by our investigation.

CONCLUSION

The present investigation was conducted in the wake of the increasing prevalence of incidents of multi-drug resistant infections in CA-UTIs and biofilm formation on urinary catheters by the bacterial pathogen *Klebsiella pneumoniae*. As the study could find that the *Evolvulus alsinoides* plant extract is affective against the bacterium both in culture and in biofilms, we recommend further studies to analyse the use of the standardised phytocompounds form the plant that could replace the common antibiotics in use. Also, the anti-inflammatory activities of the plant extract further promises that *Evolvulus alsinoides* could be one of the potential candidates for drug development.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CA-UTIs: Catheter-associated urinary tract infections; **SOD:** Superoxide dismutase; **MPO:** Myeloperoxidase; **MIC:** Minimum inhibitory concentration; **MDA:** Malondialdehyde; **MDR:** Multi-drug resistant; **HAIs:** Hospital-acquired infections; **ICUs:** Intensive care units; **CDC:** Centre for disease control and prevention; **ESBL:** Extended-spectrum beta-lactamase; **MSU:** Mid-stream urine; **NaOH:** Sodium hydroxide; **HCl:** Hydrochloric acid; **$FeCl_3$:** Ferric chloride; **Con.:** Concentrated; **mL:** Milliliter; **w/v:** Weight/volume; **MHA:** Muller-Hinton agar; **NCCLS:** National committee for clinical laboratory standards; **CLSI:** Clinical and laboratory standards institute; **mm:** Millimetre; **µL:** Microliter; **MHB:** Muller-Hinton broth; **mg:** Milligram; **OD:** Optical density; **PBS:** Phosphate-buffered saline; **ELISA:** Enzyme-linked immunosorbent assay; **CV:** Crystal violet; **PMS:** Phenozinemetho-sulphate; **NADH:** Nicotinamide adenine dinucleotide hydrogen; **NBT:** Nitroblue tetrazolium; **RBCs:** Red blood cells; **TBA:** Thiobarbituric acid; **TCA:** Trichloroacetic acid; **CTAB:** Cetyltrimethylammonium bromide; **H_2O_2:** Hydrogen peroxide; **TMB:** Tetramethylbenzidine; **RO:** Reactive oxygen.

SUMMARY

Catheter-associated urinary tract infections or CA-UTIs are one of the major nosocomial infections in the Kingdom of Saudi Arabia and elsewhere in the world. When these infections are caused by biofilm-forming multidrug resistant bacterial pathogens like *Klebsiella pneumoniae*, they can result in serious patient sufferings due to increased morbidity, financial burden and higher mortality. A medicinal plant - *Evolvulus alsinoides* - which is known to possess anti-bacterial activity has been found to have potential anti-inflammatory and anti-biofilm properties also. When urinary catheters were coated with the mentioned plant extract, it also showed potential biofilm inhibition capabilities, suggesting that this plant extract could be further studied and developed as a catheter coating agent.

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