Antimicrobial and antibiofilm activities of *Gymnema sylvestre* methanolic extract against catheter associated urinary tract infections and their anti-oxidant activity

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KEYWORDS

G. sylvestre, antimicrobial, Catheter coating, Urinary tract infection

ABSTRACT:

The catheter associated urinary tract infections (CAUTI) are gaining much attraction due to their high rate of morbidity and mortality. Therefore, urgent for alternative antimicrobial agent to fight against CAUTI. However, in the study *Gymnema sylvestre* methanolic crude extracts antimicrobial activity and antibiofilm activity were investigated against *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* and *Candid albicans.* The antimicrobial activity was studied through agar diffusion method which showed potent antimicrobial activity against test pathogens. The very minimal inhibitory concentrations of *G. sylvestre* methanolic crude extract was detected using micro dilution method and exhibited 1 mg/ml was required for *S. aureus, E. coli, E. faecalis* inhibition and 2 mg/ml against *C. albicans*. Moreover, the anti-biofilm activity was quantified through crystal violet assay wherein the *G. sylvestre* methanolic crude extract effectively inhibited the test pathogens biofilm formation upto their MIC level. Also, the effect of *G. sylvestre* methanolic crude extract was investigated on test pathogens mature biofilms and efficiently eradicated the 85%, 88%, 81% and 84% of *S. aureus, E. faecalis, E. coli* and *C. albicans* biofilms correspondingly. The *G. sylvestre* methanolic crude extract coated catheter tube antimicrobial activity was studied through *in vitro* bladder model and exposed potent activity against test pathogens. The *G. sylvestre* methanolic crude extract antioxidant property was studied and the extract was not revealed any cytotoxic effect to normal cells. Altogether, the antimicrobial and anti-biofilm activity of *G. sylvestre* methanolic crude extract suggested that it can be an alternative agent for CAUTI treatment.

1. Introduction

The medical devices are played a major role in medical field to ensure the health care support for hospitalized patients who admitted for many reasons.[1] In particular, catheter is a one of the significant medical devices used to drain the urine from urinary system during the patient's hospitalization.[2,3] The catheter usage may be a short term or long term it depends on patient's need and the catheter usage create the suitable environment of microbial growth in sterile area.[4] In some circumstances, long term catheter usage may give the chance for microbial entry via catheter lumen and get attached to the surface of catheter to form the colonization on catheter surface.[5] During the microbial entry, the urine leftover in the catheter tube act as a growth medium that ensure the microbial attachment followed by colonization leads to form a strong polymicrobial structure on inner and outer surface of catheter.[6] The mature polymicrobial structure able to cause catheter associate urinary tract infection (CAUTI) which responsible for 40% of hospital related infection[7-9] resulting mild to strong complications such as septicaemia, pyelonephritis and so on. The polymicrobial structure include bacteria and fungi *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Candida albicans* and ability to form biofilms. The biofilm formation helps the microbes to protect themselves by altering the target site, expel of antibiotics, escaping from extreme environment makes treatment failure resulting resistant organisms' development which challenges the physician.[10,11] Generally, the proper treatment and catheter replacement makes CAUTI preventable but the inappropriate treatment and filed to change the catheter procedure enhance the infection severity by biofilm formation, incontinency in urine drainage and age of particular patient and giving raise to greater concern.[7] Keeping all the evidence, the requirement of different antimicrobials are urgently needed to eliminate the biofilm formation on catheter surface.

In the present scenario, among the several nature sources, the plants have been known for its enormous biomolecule's discovery for various pharmaceutical purposes. Hence, plant-based compounds identified were used in the folk as well as traditional medicine for treating various diseases and conditions such as antimicrobial, anticancer and anti-inflammatory etc. Consequently, *Gymnema sylvestre* is an ethno-medicinal plant which extensively spread across India and this is widely used for treating type II diabetes.[12,13] The phytochemicals present in *G. sylvestre* shown different activities including anti-inflammatory, hypoglycemic, antimicrobial and anti-cancer activities. Therefore, in the study the methanolic extracted *G. sylvestre* antimicrobial activity and antibiofilm activities were investigated against CAUTI causing organisms.

2. Methods

a. Preparation of inoculum

The *E. coli, S. aureus C. albicans* and *E. faecalis* overnight cultures were preprepared in Mueller Hinton broth (MHB), Brain Heart Infusion (BHI) broth and Sabouraud dextrose broth (SDB) and the adjusted (0.5 MacFarland unit) cultures used for all the studies. Rifampicin, ampicillin and nystatin used as positive controls and the vehicle control was methanol.

b. Methanolic crude extract preparation of *G. sylvestre*

The locally purchased 20 g of *G. sylvestre* filled in cellulose thimble was placed in the Soxhlet apparatus as referred earlier.[14] The methanol added to the flask to start the reaction at 60oC and it was running for another 6-7 hours until the clear solution obtained. The solvent evaporated end product was used for further studies.

***c. G. sylvestre* methanolic crude extract antimicrobial activity**

The antimicrobial activity of *G. sylvestre* methanolic crude extract was examined against above mentioned test pathogens such as *E. coli, S. aureus C. albicans* and *E. faecalis* vi the well diffusion method as dictated previously.[15] In short, the overnight test pathogenic cultures were lawned over the sterile respective petri plates and the air dried lawned plated were drilled for well which received two different concentrations of *G. sylvestre* methanolic crude extract and plates were incubated for zone formation.

d. MIC determination of *G. sylvestre* methanolic crude extract

The *G. sylvestre* methanolic crude extract MIC was determined against *E. coli, S. aureus C. albicans* and *E. faecalis* using micro dilution method as mentioned earlier.[16] In brief, two-fold serially diluted 4 mg/ml of *G. sylvestre* methanolic crude extract from to 0.031mg/ml followed by test cultures were added in the proper broth and incubated for turbidity observation. The *G. sylvestre* methanolic crude extract MIC was determined after measuring the each and every well at 600 nm.

e. Impact of *G. sylvestre* methanolic crude extract on biofilm formation

To study the impact of *G. sylvestre* methanolic crude extract on *E. coli, S. aureus C. albicans* and *E. faecalis* biofilm formation, the crystal violet staining method was employed as cited earlier.[17] Briefly, the respective broth containing serially diluted *G. sylvestre* methanolic crude extract from 4 mg/ml to 0.031 mg/ml along with test pathogens were allowed for biofilms formation upto 5 days. The formed biofilms in the presence of extracts were fixed using methanol and stained with crystal violet. The stained biofilms were destained by ethanol acetone mixture and the resultant was read at 570nm.

f. Effect of *G. sylvestre* methanolic crude extract on mature biofilm

To quantify the effect of *G. sylvestre* methanolic crude extract on *E. coli, S. aureus C. albicans* and *E. faecalis* mature biofilm, the crystal violet staining method was adopted as shown before.[16] In brief, the test pathogens were permitted to form biofilm in respective broth for 96 hours and the formed biofilms were treated with 1X, 2X and 3XMIC concentrations of *G. sylvestre* methanolic crude extract for 24 hours. The attached biofilms after treatment were fixed through methanol followed by crystal violet staining. The ethanol and acetone mixture were destained the stained biofilms and the obtained final product was measured at 570 nm.

***g. G. sylvestre* methanolic crude extract coated catheter antimicrobial activity**

The catheter coated with *G. sylvestre* methanolic crude extract was examined for their antimicrobial activity against *E. coli, S. aureus C. albicans* and *E. faecalis* using *invitro* bladder as documented before previously.[18] Briefly, sterile catheter tube cut into small piece was soaked into the *G. sylvestre* methanolic crude extract for 30 mins and the air-dried tube was landed on to the test pathogens lawned petriplates and incubated. The zone formation observed surrounding the tube indicates the antimicrobial of *G. sylvestre* methanolic crude extract against test pathogens.

h. **Antioxidant property of *G. sylvestre* methanolic crude extract**

To explore antioxidant property possessed by *G. sylvestre* methanolic crude extract, the DPPH (2, 2-diphenyl-1-picryhydrazyl) free radical scavenging assay was performed as indicted before.[19] Shortly, the *G. sylvestre* methanolic crude extract concentrations such as 0.5 mg/ml, 1mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml and 5 mg/ml were reacted to DPPH solution for 30 min. The radical scavenging activity percentage was calculated after the resultant read at 517 nm.

***i.* Cytotoxicity of *G. sylvestre* methanolic crude extract**

The *G. sylvestre* methanolic crude extract cytotoxicity was investigated on L929 through MTT assay as cited before (Meiyazhagan *et al*., 2015).[16] For the assay, the seeded confluent cells were treated with 0.5 mg/ml, 1mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml concentrations of *G. sylvestre* methanolic crude extract for 24 hours and the MTT solution was added after incubation to allow for formazan product. The dissolved formazan product was measured t 570 nm for call viability percentage calculation.

j. Statistical analysis

For statistical analysis was performed to calculate error bars using Mean and standard deviations from all the experiments.

3. Results

a. G. sylvestre methanolic crude extract antimicrobial activity

The *G. sylvestre* methanolic crude extract antimicrobial activity determined against test pathogens are presented in figure 1. The figure shows, the growth inhibition was observed as zone formation exhibited by two different *G. sylvestre* methanolic crude extract concentrations against test pathogens. The results revealed the *G. sylvestre* methanolic crude extract concentrations antimicrobial activity is dose dependent which was evidenced through increased zone size when increasing *G. sylvestre* methanolic crude extract concentrations against test pathogens.

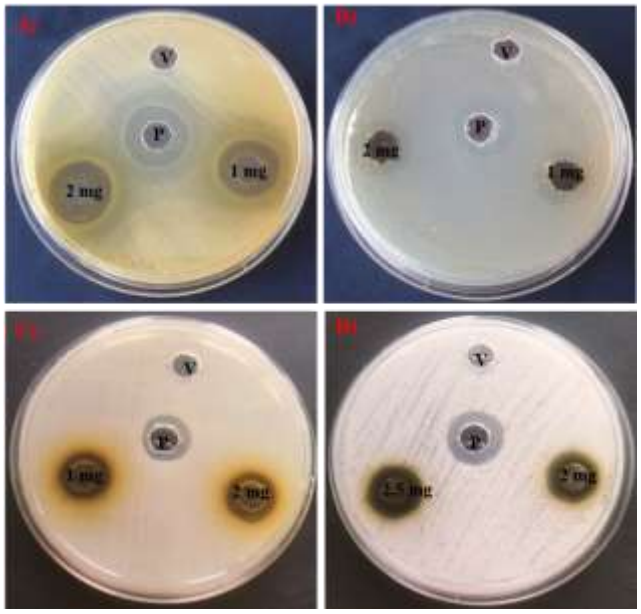

Figure 1: *G. sylvestre* methanolic crude extract zone of inhibition against test pathogens. A) *S. aureus* B) *E. faecalis C) E. coli* and *D) C. albicans. note:* P-positive controls and V-vehicle control.

b. MIC determination of *G. sylvestre* methanolic crude extract

The least inhibitory concentrations of *G. sylvestre* methanolic crude extract required to inhibit the test pathogens growth calculated is displayed in figure 2. As noticed in figure, the graph mentioned that 1 mg/ml of *G. sylvestre* methanolic crude extract was important to stop or inhibit the *E. coli, S. aureus* and *E. faecalis* growth. However, to inhibit the *C. albicans* growth, 2 mg/ml of *G. sylvestre* methanolic crude extract was needed.

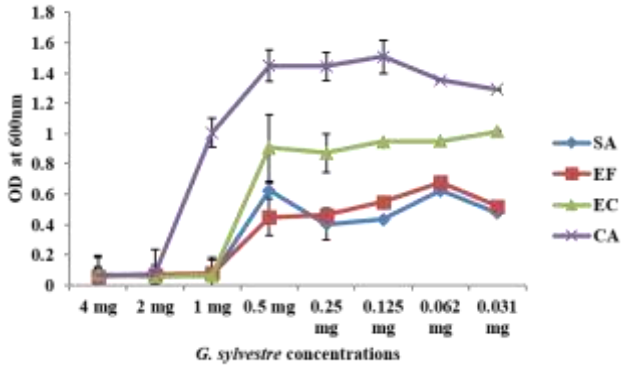

Figure 2: MIC determination of *G. sylvestre* methanolic crude extract against *E. coli*, *S. aureus*, *C. albicans* and *E. faecalis* using microdilution method. Note: SA-*S. aureus*, EF- *E. faecalis*, EC- *E. coli* and CA-*C. albicans*

c. Impact of *G. sylvestre* methanolic crude extract on biofilm formation

The impact of varying *G. sylvestre* methanolic crude extract concentrations on *E. coli*, *S. aureus C. albicans* and *E. faecalis* biofilm formation quantified using crystal violet method is represented in figure 3. As seen in figure, the graph represented the percentage of test pathogens biofilm formation after exposure to *G. sylvestre* methanolic crude extract concentrations. The *G. sylvestre* methanolic crude extract concentration's ability to inhibiting biofilm formation was noticed till their MIC concentration of all the test pathogens which indicates anti biofilm activity of extract. Whereas, after its MIC, the gradual biofilm formation increase was detected which represent very minimal concentration can slow down the process of biofilm formation.

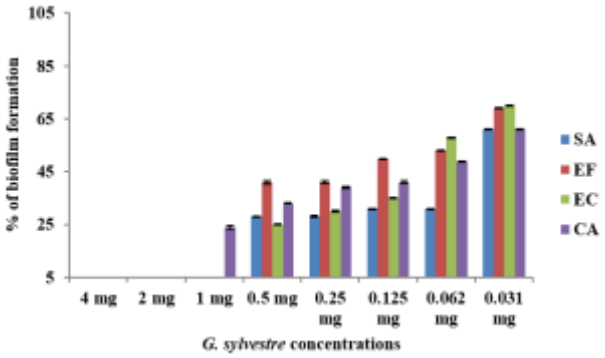

Figure 3: Impact of *G. sylvestre* methanolic crude extract on biofilm formation. Note: SA-*S. aureus*, EF- *E. faecalis*, EC- *E. coli* and CA-*C. albicans.*

d. Effect of *G. sylvestre* methanolic crude extract on mature biofilm

The different *G. sylvestre* methanolic crude extract concentrations effect on mature biofilms of *E. coli*, *S. aureus C. albicans* and *E. faecalis* was quantified and the calculated test pathogens biofilm reduction after treatment is mentioned in figure 4. The figure denotes the 1X, 2X and 3XMIC of *G. sylvestre* methanolic crude extract ability in eliminating or reducing the 5 days mature biofilms of test pathogens and the reduction was noted in all the concentrations. However, the 3XMIC of *G. sylvestre* methanolic crude extract treatment reduced 85% of *S. aureus* biofilm, 88% of *E. faecalis* biofilm, 81% of *E. coli* biofilms as well as 84% of *C. albicans* biofilms.

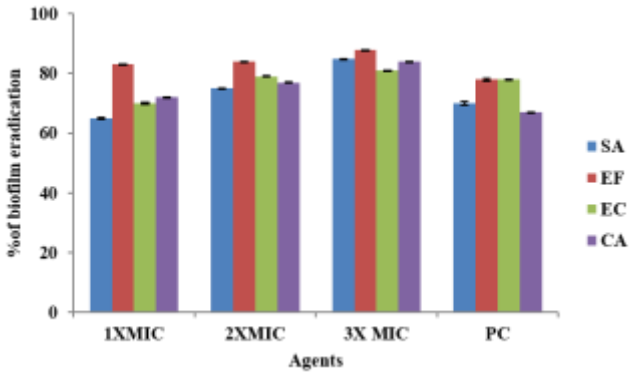

Figure 4: Effect of *G. sylvestre* methanolic crude extract on mature biofilm. Note: SA-*S. aureus*, EF-*E. faecalis*, EC- *E. coli* and CA-*C. albicans.* PC-positive controls.

e. G. sylvestre methanolic crude extract coated catheter antimicrobial activity

The antimicrobial activity of *G. sylvestre* methanolic crude extract coated catheter against test pathogens evaluated in *invitro* bladder model which imitate the appropriate environment is presented in figure 5. As seen in figure, *G. sylvestre* methanolic crude extract coated catheter exhibited the growth inhibition around the tube suggests the antimicrobial activity against test pathogens.

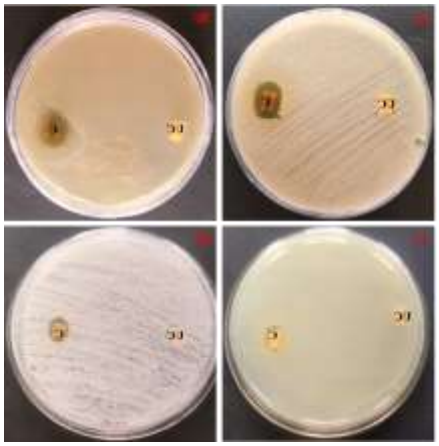

Figure 5: *G. sylvestre* methanolic crude extract coated catheter antimicrobial activity against *E. coli, S. aureus, C. albicans and E. faecalis*. A) *S. aureus* B) *E. faecalis* C) *E. coli* and D) *C. albicans.* Note: UC- uncoated, C- catheter coated with *G. sylvestre* methanolic crude extract.

f. Antioxidant property of *G. sylvestre* methanolic crude extract

The antioxidant property exhibited by wide range of *G. sylvestre* methanolic crude extract is displayed in figure 6. The figure exposed the free radical scavenging percentage of *G. sylvestre* methanolic crude extract different concentrations 0.5 mg/ml, 1mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml and 5 mg/ml as 11%,18%, 20%,29%,37% and 46% after DPPH exposure respectively.

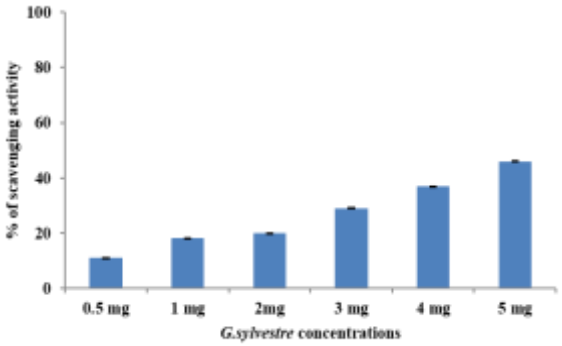

Figure 6: Antioxidant property of *G. sylvestre* methanolic crude extract

g. Cytotoxicity of *G. sylvestre* methanolic crude extract

G. sylvestre methanolic crude extract studied for their cytotoxic effect on L929 is displayed in figure 7. As observed in figure, the graph indicted the *G. sylvestre* methanolic crude extract different concentrations including 0.5 mg/ml, 1mg/ml, 2 mg/ml, 3 mg/ml and 4 mg/ml cell viability percentage after treatment as 94%,91%,84%, 71% and 40% respectively.

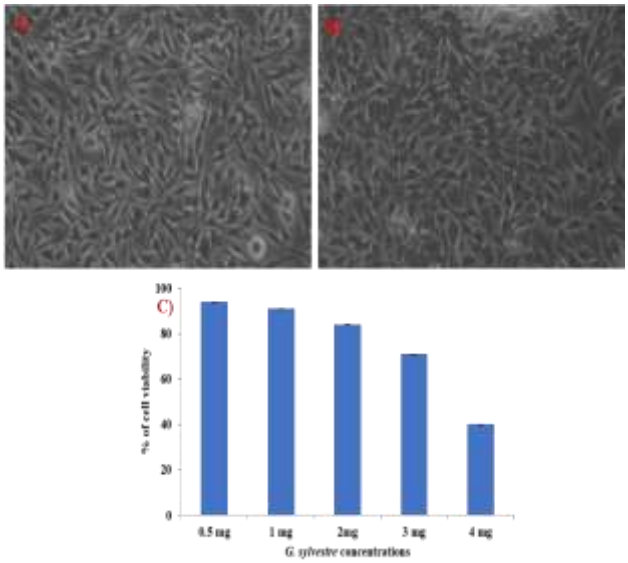

Figure 7: Cytotoxicity of *G. sylvestre* methanolic crude extract was studied on L929 cells. A) Untreated L929 cells B) L929 cells with *G. sylvestre* methanolic crude extract C) Cell viability percentage after treatment with various concentrations of *G. sylvestre* methanolic crude extract

4. Discussion

The antibiotic resistant due to biofilm formation is gaining much interest in medical device related infection. In particular, the CAUTI causes serious complication in hospital admitted patient resulting high morbidity and mortality. Hence, to overcome the biofilm related antibiotic resistant, new antimicrobials are urgently needed. Therefore, in the study evaluated the *G. sylvestre* methanolic crude extract antimicrobial and antibiofilm activities against *E. coli, S. aureus C. albicans* and *E. faecalis* predominantly reported organisms in CAUTI. The study revealed the antimicrobial activity of *G. sylvestre* methanolic crude extract against test pathogens and the activity was noted at 1-2 mg/ml of methanolic crude extract against test pathogens. Our study supported by other group wherein the phytochemicals derived from methanolic fractions of *G. sylvestre* leaf evaluated for their antibacterial activity against *E. coli, S. aureus*, and *P. aeruginosa* and found the potent activity due to the presence of two flavonoids, one alkaloid and six terpenoids. These phytochemicals induced membrane permeabilization among all the test organisms at 0.5, 0.35, and 0.1 mg/ml resulting intercellular component leakage leads cellular proteins loss indicating *G. sylvestre* can be used as novel antibacterial agent to treat bacterial infections.[20] Similarly, various solvent extracted *G. sylvestre* was studied for their antibacterial activity against *E. coli* and *B. cereus* and showed excellent efficacy in herbal formulations which used for treating infections related to microbes. [21.22] Sameway, the ethanolic and methanolic extracted *G. sylvestre* showed good antibacterial activity towards *B. subtilis, S. aureus, Bacillus pumilus* and *P. aeruginosa* which indicates the ethanolic and methanolic extracts possess significant activity. [23.14]

Besides the antimicrobial activity, the *G. sylvestre* methanolic crude extract was investigated for the anti-biofilm activity on polystyrene surfaces as well as on catheter surface. The biofilm related CAUTI poses significant during hospitalization. The CAUTI was initiated and allowed biofilm formation on catheter surface following several steps such as attachment, colonization and maturation. The complex biofilms are able to protect the microbes from various environmental condition that makes treatment challenges. [24,25] Therefore, our study proved the antibiofilm concentrated every biofilm formation step and *G. sylvestre* methanolic crude extract effectively inhibited the test pathogens biofilm through biofilm formation inhibition and mature biofilm eradication. Further, the coating of catheter with

antimicrobial agent is an outstanding model to prevent the biofilm on catheter surface. Hence, our study demonstrated the anti-adhesive property of *G. sylvestre* methanolic crude extract by catheter coating against test pathogens and showed potent activity which proved the anti-adhesive property. The earlier studies reported the different catheter coting with many antibacterial agents like zinc oxide, silver, Fosfomycin, some antibiotic combinations, zinc oxide, sertraline against *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *C. albicans*. [26-30] Overall, *G. sylvestre* methanolic crude extract exposed their efficacy and act as a hopeful antimicrobial agent for the treating CAUTI infection.

5. Conclusion

The *G. sylvestre* methanolic crude extract evaluated showed excellent antimicrobial activity with least inhibitory concentrations against test pathogens. The *G. sylvestre* methanolic crude extract exhibited effective antibiofilm activity by test pathogens biofilm formation inhibition and effective mature biofilm eradication. The *G. sylvestre* methanolic crude extract coated catheter exhibited antiadhesive property by showing zone formation around the coted catheter against test pathogens. The extract exhibited better antioxidant activity and it was not showing any cytotoxic to normal cells. Collectively, *G. sylvestre* methanolic crude extract proved the efficacy against pathogens involved in CAUTI and may act as alternative gent for CAUTI treatment.

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