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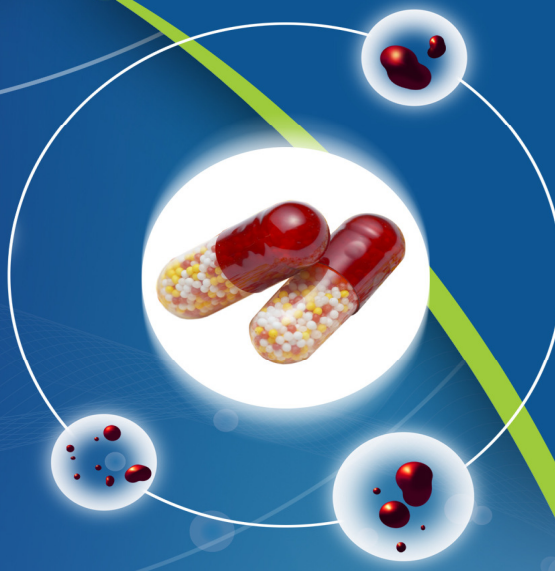

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Research Article

IN VITRO ANTI-BIOFILM ACTIVITY OF THE EXTRACT OF *PILEA CADIEREI*

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ABSTRACT

Anti-biofilm activity of the *Pilea cadierei* on clinical isolated of *Escherichia coli* U 07 was employed using a crystal violet-stained microtiter plate method and microscopic biofilm formation assay. The results indicated that crystal violet-stained microtiter plate method is not suitable to screen for biofilm formation inhibitors from crude plant extracts. Microscopic biofilm formation assay clearly showed the extract of *Pilea cadierei* can inhibit the *Escherichia coli* U 07 biofilm formation at concentration of 4 mg/ml. The results suggest that the *Pilea cadierei* could be a useful source for the development of promising anti-biofilm agents.

Key words: Anti-biofilm, *Plant extract*, *Pilea cadierei*, Traditional Chines medicine

INTRODUCTION

Biofilms have been found to be involved in a wide variety of microbial infections in the body. Within bacteria, a biofilm matrix is able to resist antibiotics at concentrations from 1000 to 1500 times higher than are conventionally used. Disassembly of the biofilm could be exploited to treat infections [1]. However, no specific biofilm inhibitor is commercially available. *Escherichia coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. Uropathogenic *E. coli* (UPEC) is responsible for approximately 90% of urinary tract infections (UTI). Biofilm-producing *E. coli* are recalcitrant to immune factors and antibiotic therapy, and are often responsible for chronic urinary tract infections [2-3].

The discovery of anti-infective agents, which are active not only against planktonic micro-organisms but also against microbial biofilms, represents an important goal [4]. Natural products are supposed as an important source of chemicals with anti-biofilm properties [5-6]. *Pilea cadierei* (Urticaceae) is an herbaceous plant that is used for treatment of urinary tract infections in traditional Chinese medicine. This genus is known to yield saponin, flavones, and cumarins [7]. The extract of *Pilea cadierei* could inhibit the growth of *E. coli* at minimum inhibitory concentration of 6.25 mg/ml [8].

In this paper, we report the anti-biofilm activity of the extract of *Pilea cadierei* based on crystal violet-stained microtiter plate method and microscopic biofilm formation assay. As far as we know, the *Pilea cadierei* has not previously been tested as anti-biofilm agents.

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MATERIALS AND METHODS

Plant material and extraction

The whole plant of *Pilea cadierei* was collected from Leida Valley, Guiyang, Guizhou province, China, and identified by authors. A voucher specimen was maintained in the herbarium of the Pharmacy Laboratory of Guizhou University, China.

The air-dried whole plants (100 g) of *Pilea cadierei* were refluxed with 95% EtOH (200ml × 3). After filtration and evaporation of the EtOH at reduced pressure to afford brown syrup (8 g).

Microplate colorimetric assay

Escherichia coli U 07, a clinical strain, was obtained from the Department of Urinary Surgery of Guiyang Medicinal College Hospital, Guiyang, China. The formation of *E coli* U 07 biofilms were studied in commercially available presterilized, polystyrene, flatbottom 96-well microtitre plates by the method described previously [9]. The concentration of test samples in each well was ranging from 4-0.5 mg/ml (Our previous study showed that the MIC of the extract of *Pilea cadierei* against *E. coli* U 07 is 8 mg/ml). Rifampicin was used as positive control at sub-inhibitory concentration of 1.2µg/ml. Negative controls included wells with only an appropriate volume of Mueller–Hinton broth and inoculum of *Escherichia coli* U 07. Comparing the average of OD of the negative control wells with that of the sample wells, we calculated the inhibition percentages for each concentration of the test samples by the following formula:

$$[(OD_{\text{negative control}} - OD_{\text{sample}})/ OD_{\text{negative control}}]\times 100$$

Microscopic biofilm formation assay

The assay used was a modification of the methods described by Blackman and Frank [10]. The surfaces used for attachment were glass slides, placed in 60 mm Petri dish. The extract of *Pilea cadierei* was dissolved in Mueller-Hinton broth and the concentration in dishes was ranging from 4-0.5 mg/ml. Rifampicin was used as positive control at sub-inhibitory concentration of 1.2µg/ml. Ten milliliters of Mueller-Hinton broth was placed into each dish and inoculated with 0.1 ml of *Escherichia coli* U 07 overnight culture grown in Mueller-Hinton broth (Fig.1). Dishes were incubated at 35°C for 24 h, allowing biofilm development. After incubation, slides were removed from the growth medium, washed with distilled water to remove any loosely attached cells, fixed with 95% ethanol for 45 s, stained with 5% crystal violet, and analyzed by microscope.

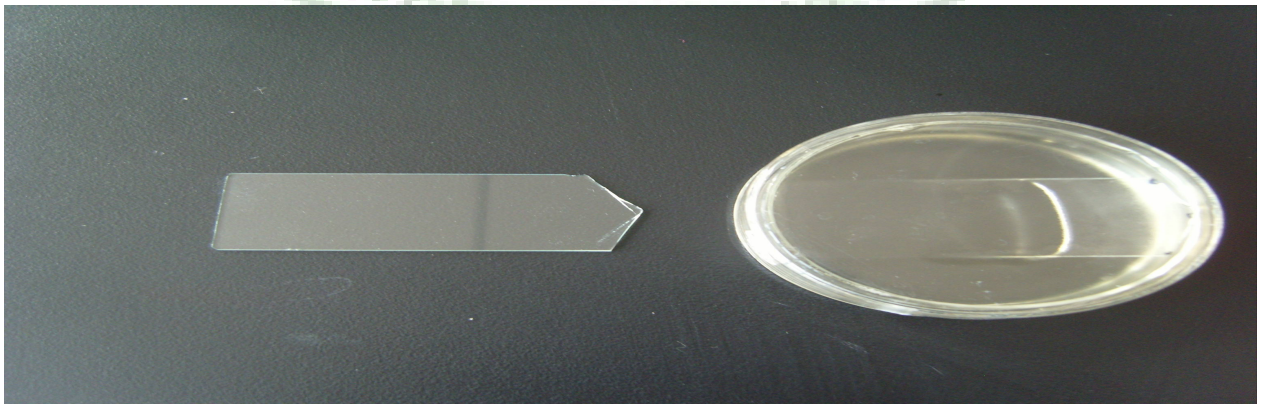


Figure: 1 Part of glass slide is immersed into the Mueller-Hinton broth

RESULTS

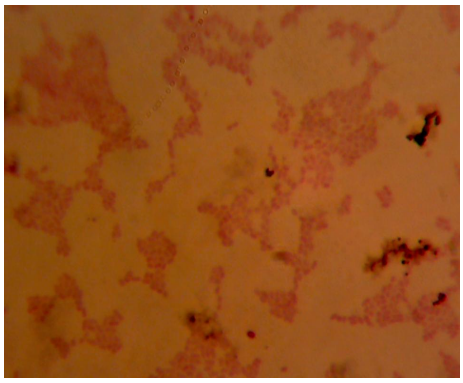
Table 1 show that the extract of *Pilea cadierei* exhibited very weak anti-biofilm activities against *Escherichia coli* U 07 biofilm. It is very interesting that the extract of *Pilea cadierei* at the highest concentration (4 mg/ml) showed the lowest inhibition rate (12%). The extract of *Pilea cadierei* exhibited anti-biofilm activity not in a concentration-dependent manner.

The results of the microscopic biofilm formation assay are not similar to that of the microscopic biofilm formation assay. As shown in Fig.2b, the extract of *Pilea cadierei* showed an obvious anti-biofilm activity at concentration of 4 mg/ml. Only a few bacteria are adherent to the surface of slide. However, many residues are adhering to the surface of the slide. The picture of negative control is characterized by biofilm formation (Fig. 2a). Rifampicin showed a powerful anti-biofilm activity in this experiment (Fig. 2c). No bacterium and only a few residues are adherent to the surface of slide. It is clear that the residues are derived from the extract of *Pilea cadierei*.

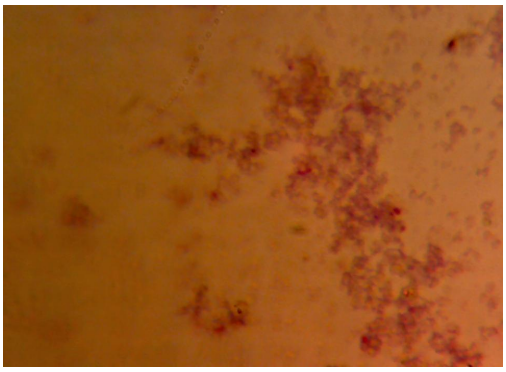
Table 1 Anti-biofilm activity of the extract of *Pilea cadierei* against *Escherichia coli* U 07 biofilm

Samples	Concentration	Inhibition rate (%)*
Extract of *Pilea cadierei*	0.5 mg/ml	26±6
Extract of *Pilea cadierei*	1 mg/ml	28±7
Extract of *Pilea cadierei*	2 mg/ml	24±4
Extract of *Pilea cadierei*	4 mg/ml	12±3
Negative control	-	0
Positive control	1.2µg/ml	94±2

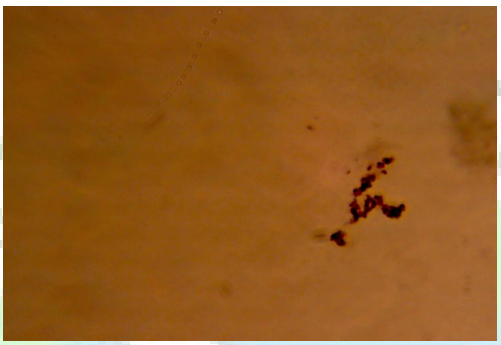
* Data were expressed as the means ± S.D.



2a. Negative control



2b. The extract of *Pilea cadierei* (4 mg/ml)



2c. Positive control

Figure: 2 *Escherichia coli* U 07, grow in Mueller-Hinton broth at 35℃ for 24 h. The fields were observed under a 100× oil-immersion objective (Eclipse E400; Nikon).

DISCUSSION

Several methods have been developed for the cultivation and quantification of biofilms. Among them the microtiter plate method is the most frequently used assays for investigation of biofilm. In this study, the results indicate that this method is not suitable to screen for biofilm formation inhibitors from crude plant extracts. The crude plant extract contains various components which are usually adherent to the surface of plate. Crystal violet stains not only cells, but essentially any material adhering to the surface of the plate (e.g. matrix components), and therefore, crystal violet staining may overestimate the number of adherent bacteria. Djordjenvic et al. [11] compared the biofilm formation ability by microtiter plate assay and quantitative epifluorescence microscopy and reported that former assay revealed greater differences in biofilm production than did the microscopy biofilm assay. So microscopy could be a precious and invaluable tool for analyzing the growth of biofilm. In conclusion, the anti-biofilm effect of *Pilea cadierei* may explain its use in treatment of urinary tract infection in traditional Chinese medicine. Further research should be done to investigate the anti-biofilm activity of *Pilea cadierei* in animal model.

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REFERENCES

1. *Kim L. Riddle of biofilm resistance. Antimicrob Agents Chemother 2001; 45: 999–1007.*
2. *Justice S, Hunstad D, Seed P, Hultgren S. Filamentation by Escherichia coli subverts innate defenses during urinary tract infection. Proc Natl Acad Sci U S A 2006; 103: 19884-19889.*
3. *Ehrlich G, Hu F, Shen K, Stoodley P, Post J. Bacterial plurality as a general mechanism*

driving persistence in chronic infections. Clin Orthop Relat Res 2005; 437: 20-24.

4. *Projan S.J., and Youngman P.J., Antimicrobials: new solutions badly needed. Curr Opin Microbiol 2002; 5:463–465.*
5. *Khodavandi A, Harmal N.S., Alizadeh F, Scully OJ, Sidik SM, Othman F, Sekawi Z, Ng K.P, Chong P.P., Comparison between allicin and fluconazole in Candida albicans biofilm inhibition and in suppression of HWP1 gene expression. Phytomedicine 2011; 19: 56-63.*
6. *Khan R, Adil M, Danishuddin M, Verma PK, Khan A.U., In vitro and in vivo inhibition of streptococcus mutans biofilm by trachyspermum ammi seeds: an approach of alternative medicine. Phytomedicine 2012; 19: 747-755.*
7. *Sun C.L., Du W, Li H.Q., Antimicrobial activity of the extract of Pilea cadierei. Journal of mountain agriculture and biology 2009; 28 : 468-470.*
8. *Niu Y.H., Liang Z.Y., Gan X.M., The preliminary examination of the chemical constituents of Pilea cadierei. Journal of Guizhou normal college 2010; 26: 26-28.*
9. *Hu J-F, Garo E, Goering M.G., Pasmore M, Yoo H-D, Esser T, Sestrich J, Cremin P A, Hough GW, Perrone P, Lee Y-S, Le N-T, O'Neil-Johnson M, Costerton JW, and Eldridge GR. Bacterial biofilm inhibitors from diospyros dendo. J. Nat. Prod 2006; 69:118-120.*
10. *Blackman I.C., and Frank J.F., Growth of Listeria monocytogenes as a biofilm on various food-processing surfaces. J. Food Prot 1996; 59:827-831.*
11. *Agarwal R.K., Singh S, Bhilegaonkar K.N., and Singh V.P., Optimization of microtitre plate assay for the testing of biofilm formation ability in different Salmonella serotypes.International Food Research Journal 2011;18: 1493-1498.*

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