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Anti – Biofilm Activity and Bioactive Component Analysis Associated With Psoralea Corylifolia Seed Extracts

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Abstract – The aim of the present study was to determine the biofilm inhibition properties associated with the Psoralea corylifolia (Bakuchi) seed extract with the preliminary evaluation of bioactive components contributing for its biofilm inhibition properties. Klebsiella Spp. is an uropathogen and forms a thick biofilm on the urinary catheters in patients with Urinary Tract Infections (UTIs). The biofilm inhibition activity of the Psoralea corylifolia seed extracts in methanol and diethyl ether were investigated against Klebsiella Spp. The methanol extract showed maximum inhibition of biofilm almost (61.35%) as compare to that of diethyl ether extract (52.98%). Thin Layer Chromatography (TLC) and Gas Chromatography/Mass Spectroscopy (GC/MS) analysis of the methanol extract was performed to ascertain the major chemical component present. The GC/MS analysis confirmed that the methanols extract present with major phytochemicals as 1-(+) -Ascorbic acid 2, 6-dihexadecanoate (8.76%), Coumarin –Psoralen (7.67%), Angecin- Isopsoralen (6.34%), caryophyllene oxide (2.82%) with several other components in trace portion as bioactive components ,may be responsible for anti-biofilm properties of bakuchi seeds. The results of this study confirmed the possibility of using Bakuchi seed extract or some of bioactive components against the formation of biofilm by Klebsiella Spp.

Keywords – Psoralea corylifolia(Bakuchi), Seed extracts , Klebsiella Spp., antibiofilm.

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INTRODUCTION

Plants have been an important source of medicine for thousands of years. As per World Health Organization estimates, up to 80 percent of people still depend on traditional remedies such as herbs for their medicines. Today, Ayurvedic, and Unani physicians utilize numerous species of medicinal plants as a source for their drugs [1]. The twenth century set a trend for introducing a new generation of botanical therapeutics that includes plant derived pharmaceuticals, multicomponent botanical drugs; dietary supplements, functional foods and plant produced recombinant proteins. Many of these products will soon complement conventional pharmaceuticals in the treatment, and prevention of diseases.

Psoralea corylifolia Linn. belongs to the family *Fabaceae*, commonly known as '*Bakuchi'* by Indian traditional medicine. It is a medicinally important plant, indigenous to tropical and subtropical regions of the world. [2] Several chemical compounds were identified and documented from the *Psoralea corylifolia* including flavonoids (bavachalcone, bavachinin, bavachin, corylin, and 6-prenylnaringenin etc.), coumarins (psoralidin, psoralen, isopsoralen and angelicin) and meroterpenes bakuchi-ol and 3-hydroxybakuchiol). [3] The Psoralea corylifolia extracts have been reported to possess antibacterial,[4]antifungal,[5] anti-oxidant,[6,7] anti-inflammatory,[8] and immunomodulatory activity[9].

In continuation with the earlier studies and considering the therapeutic efficacy of *Psoralea corylifolia* in skin diseases a scientific investigation was undertaken to screen the antibiofilm activity of extracts of *Psoralea corylifolia* seed against major uropathogen. The aim of the present study was to assess and confirm the antibiofilm activity of bakuchi seed extract against catheter associated urinary tract (CAUTI) pathogen, *Klebsiella Spp.* The in-vitro study was performed on longitudinal section of catheter surface, along with the phytochemical investigation of the most promising extract using TLC profiling and GC/MS analysis.

MATERIAL AND METHODS

Materials

Bacterial culture

Klebsiella Spp. was a clinical isolate was obtained from patient suffering from urinary tract infection in local hospital from Mumbai, India. The bacterial culture was further sub-cultured and stored on Tryptone Soy Agar (TSA). Tryptone Soy Broth (TSB) media was used for conducting further experiments.

Chemicals

Methanol and Diethyl ether used for extractions were of HPLC grade, and were purchased from M/S Merck ltd. Mumbai. The Chemicals such as 1% Crystal Violet was obtained from Biolab Diagonostics (I) Pvt. Ltd., 95% Ethanol was prepared from absolute alcohol made by S D Fine Chem Ltd. and phosphate buffer saline (pH 7.3) was prepared in the laboratory itself.

Plant material

The seeds of *Psoralea corylifolia* plant were procured from local ayurvedic medicine supplier. Thedried seeds were cleaned and disinfected with 15% H2O2, crushed into powder sample using an electronic blender. The powdered sample was stored in bottle at room temperature prior to subject for an extraction and ensure to use in a day for extraction.

Catheter

Sterile urethral catheter which was used to test the biofilm was manufactured by Romsons Scientific & Surgical Industries Pvt.ltd. Nonpyrogenic, 15ml High clarity, screw cap Polypropylene Conical tubes, manufactured by BD Falcon were used.

METHODS

Extraction of seeds of Psoralea corylifolia

The fine powder of each of the seed (25g), with 250ml of methanol and diethyl ether solvents respectively were taken in a round bottom flask. For successive extraction with these solvents, seed powder was allowed incubate for 48 h with intermittent shaking at room temperature. The liquid extracts so obtained were filtered with whatman filter paper and were filter sterilized. and labeled as M1, D2, respectively. Both extracts were stored at -20°C in air tight bottle and used within 1 week after preparation.

Biofilm inhibition assay

The anti-biofilm activity of two extracts of the bakuchi seeds (M1 and D2) were tested against *in* catheter tube assay [10] in which 100 µl /ml of bacterial inoculums was inoculated in two tubes containing sterile LB broth. Sub-MIC concentration of 200µl/ml of each extract were added in each of the test tube with LB. Respective controls with only LB media as well as LB media with each extract was prepared and was incubated for 96 h at 37^0C. The amount of biofilm produced in each tube was then quantified and measured. This test was performed in triplicate and the final readings were taken as an average of the three readings. The percentage reduction in biofilm formation was measured using the modified formula [11]

$$\% \text{ Reduction} = \frac{\text{Control OD- Test Sample OD}}{\text{Control OD}} \times 100$$

Preliminary Phytochemical Analysis

The seed extract with promising broad spectrum biofilm inhibition activity was subjected for phytochemical tests for detection of plant secondary metabolites, tannins, saponins, steroid, alkaloids and glycolsides in accordance with prior studies. [12]

Thin Layer Chromatography (TLC)

The most promising extract was spotted in preparative TLC plates coated with silica gel G. The plates were developed in TLC chamber previously saturated with Ethyl acetate: Methanol (3:7) solvent system. The solvent was allowed to evaporate and different spots developed were identified by means of UV light at λ max 254 nm and the Rf value (for each spot developed) was calculated. [13]

GC/MS Analysis

The most promising extract of *Psoralea corylifolia* seeds (M1) was subjected for gas chromatography-mass spectrometry analysis with the GC MS–QP 2010 plus system (Shimadzu). The methanol seed extract with most promising activity was subjected for the GC /MS analysis. 2µl of the sample was injected to the GC-MS with the oven temperature programmed as 80°-280°C with increment of 5°C/min with Helium as a carrier gas. The injection size was 0.1 µL, and detector temperature of 270^0 C. The eluted peaks were identified using NIST library by comparing with the mass spectral data and retention indices in the literature.

RESULTS

Effect on Biofilm formation on catheter tubes

The results obtained in the present study relieved that the tested two extracts of bakuchi seeds possess considerable reduction in biofilm formation on catheter tube. The optical density was measured for all test tubes under investigation. The final biofilm inhibition was calculated by subtracting the readings of the inhibition of biofilm by the two extracts in

methanol (M1) and diethylether (D2) respectively, with the readings obtained from the control. The percentage reduction of biofilm was calculated and documented. Among the two extract subjected for biofilm inhibition activity on the surface of the urinary catheter tube, methanol extract showed maximum inhibition of biofilm almost (61.35%) as compare to that of diethyl ether extract (52.98%). Methanol extract with the maximum inhibition activity was selected and was subjected for further analysis.

Preliminary Phytochemical Screening

The preliminary phytochemical screening of methanol extracts of the *Psoralea corylifolia* seeds showed the presence of alkaloids, carbohydrates, flavonoids, glycosides and saponin. However the steroids and terpanoids were absent as represented in table 1.

Table 1: Phytochemical analysis of *Psoralea corylifolia* seed extract

Tests for	Methanol Extract
Alkaloid	+
Carbohydrates	+
Flavonoids	+
Glycosides	+
Saponins	+
Steroids	-
Terpenoids	-

+ = Present; - =Absent

Thin Layer Chromatography (TLC) Analysis

Presence of the secondary metabolites will be held responsible for antibiofilm activity. The retention factors (Rf) of methanol extract in different solvent systems are shown in table 2. The methanol extracts produces four fraction having Rf 0.16, 0.33, 0.44 and 0.91 under ethyl acetate: methanol (3:7) solvent system. The results of TLC indicate that methanol extracts has number of chemical constituents.

Table 2: TLC Analysis of *Psoralea corylifolia* seed methanol extract

Extract	Solvent System	No. Of Spots	R_f Values
Methanol	Ethyl acetate: Methanol(3:7)	04	0.18,0.36, 0.42,0.94

GC/MS Analysis

The GC-MS analysis confirmed the presences of several important phytoconstituents in the bioactive methanol extract .A total of 15 compounds were identified in methanol extract of *P. corylifolia* seeds. The major components identified were, 1-(+) -Ascorbic acid 2, 6-dihexadecanoate (8.76%), Coumarin – Psoralen (7.67%), Angecin- Isopsoralen (6.34%), caryophyllene oxide (2.82%). Several other components in trace amounts were also present. In earlier findings by S. Shreenivasan et al also reported the presence of the phenyl derivative of pyranocoumarin (PDP) in bakuchi oil with a potent antifungal activity against *Fusarium* species. And antimicrobial activities. [14]

CONCLUSION

In the present study, the antibiofilm activities of *Psoralea corylifolia* seed extract in methanol and diethylether extract were explored specifically against major bacterial urinary skin pathogen. The results clearly demonstrate that, the methanol seed extract of *P. Corylifolia* comprise of a promising antibiofilm activity against selected pathogen as compare to diethylether extract. The phytochemical and TLC analysis of the methanol extract followed by GC- MS screening confirmed the presence of various phytochemicals. Major identified phytochemicals in methanol extract of *P. corylifolia* seeds are phenol derivatives and coumarin – psoralen, isopsoralen which may be accountable for its antibiofilm activity. In Ayurvedic system of medicine, *Psoralea corylifolia* seeds are commonly used in various dermatological conditions [15- 19]. The present study supports the use of bakuchi seed in the development of ayurvedic skin formulations. Present study explored the additional benefits associated with its traditional applications. Additional in vitro studies would be needed to justify and further the potential of this extract as an effective biofilm inhibitor in UTIs. Further individual phytochemical evaluation with each identified chemical fractions responsible for these inhibitory activities is essential. Thus, the effect of these extract and its derived molecules could be of considerable interest in future new drug developments.

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