

Preliminary Phytochemical Studies and Evaluation of Antibacterial Activity of *Psoralea corylifolia* Seed Extract

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ABSTRACT

Present study aims to evaluate antibacterial efficacy of Bakuchi (*Psoralea corylifolia*) seed extracts prepared in two different solvents. Antibacterial assay was performed by agar well diffusion method against Gram positive skin pathogens, like *Staphylococcus aureus*, *Staphylococcus epidermidis* *Pseudomonas aeruginosa* and Gram negative skin pathogens, which included *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris* and *Escherichia coli*. Bakuchi seed extract in methanol and diethyl ether exhibited broad spectrum antibacterial activity against skin pathogens, however comparatively bakuchi seed extract in methanol was found to be more promising with maximum zone of inhibition against *K. pneumoniae* with zone of inhibition 21mm in diameter and the minimum inhibitory concentration (MIC) 2.5mg/ml. These results confirmed the potential of the Bakuchi seed extract in the development of ayurvedic topical skin formulations.

Keywords: Bakuchi seed extract; Antibacterial Activity; Phytochemical analysis.

INTRODUCTION

The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic system of medicine. As per WHO report 80% of world population, presently use herbal medicines for some aspect of primary health care.¹ Owing to global trend towards improved “Quality of Life” there is considerable evidence of an increase in demand of plant derived medicines. Since antiquity, many

plants species reported to have pharmacological properties as they are known to be the source of several secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, terpenes etc. which can be utilized to combat against the disease causing pathogens.²

The clinical efficacy of many existing antibiotic is being threatened by evolving Multi Drug Resistance strains of several pathogenic bacteria.³ The emergence

of antimicrobial resistance is a global problem today, and it has created immense clinical problem in treatment of infectious diseases. In this scenario, there is continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for infectious diseases which leads to the screening of several medicinal plants in search of new chemical component with a promising antimicrobial activity.³

Psoralea corylifolia Linn. belong to the family *Fabaceae*, commonly known as 'Bakuchi' by Indian traditional medicine and 'Bu Ghuzhi' by traditional Chinese medicine. It is a medicinally important plant, indigenous to tropical and subtropical regions of the world. It is reported in Indian pharmaceutical codex, Chinese, British and the American pharmacopoeias and in different traditional system of medicines such as Ayurveda, Unani and Siddha.⁴

In Ayurvedic literature⁵ the properties of *Bakuchi* plant is documented as

तत्फलपित्तलक्ष्णकफानिलहरकटु। केश्यत्वच्यकामेश्वासकासशोथामपाण्डनृत॥⁵

The Sanskrit shloka states the use of *Bakuchi* in various Ayurvedic treatments as in Kushtha (skin disorders); Keshya and Tvchya (skin and hair treatments); Krumi (as a germicidal); Shwasa & Kasa (Bronchial Asthma and Cough); Pandu (Anaemia); and Shotha (Oedema).

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 (bakuchiol and 3-hydroxybakuchiol).⁶ The *Psoralea corylifolia* extracts have been reported to possess antibacterial,⁷ antifungal,⁸ antioxidant,^{9,10} anti-inflammatory,¹¹ antileishmanial,¹² estrogenic,¹³ antitumour,¹⁴ and immunomodulatory activity¹⁵. Moreover, the plant

is conventionally used in ayurvedic system of medicine in some skin diseases and disorders such as psoriasis, leucoderma and leprosy in the form of internal¹⁶ as well as external applications¹⁷.

Considering the therapeutic efficacy of *Psoralea corylifolia* in skin diseases a scientific investigation was undertaken to screen the antibacterial activity of different extracts of *Psoralea corylifolia* seeds using agar well diffusion method against major skin pathogens.

MATERIALS AND METHODS

Chemicals

Methanol and Diethyl ether used for extractions were of HPLC grade, and were purchased from M/S Merck Ltd. Mumbai.

Plant material

The seeds of *Psoralea corylifolia* plant were procured from local ayurvedic medicine supplier. The dried 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.

Extraction of seeds of *Psoralea corylifolia*

The fine powder of each of the seed (25g), with 250ml of methanol and diethyl ether 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 they were filter sterilized. All extracts were stored at -20°C in air tight bottle and used within 1 week after preparation.

Test Microorganisms

The microorganisms used in the present study include were obtained from the

NCIM, National Chemical Laboratory, Pune, and MTCC Institute of Microbial Technology, and Chandigarh, India. Gram positive bacteria such as *Staphylococcus aureus* (NCIM- 2079), and *Staphylococcus epidermidis* (NCIM-2493) and Gram negative bacteria such as *Klebsiella pneumoniae* (NCIM-2719), (*Proteus mirabilis* MTCC425), *Proteus vulgaris* (NCIM-2027), *Pseudomonas aeruginosa* (NCIM-2074) and *Escherichia coli* (NCIM- 2256) were used to test the antimicrobial activity.

Culture Maintenance & Inoculum preparation

The pure stock cultures were maintained on the agar at 37°C and subsequently sub-cultured into newly prepared nutrient agar slants in the laboratory. Inoculums of bacteria were prepared by suspending a loop full of bacterial cultures into 100mL of nutrient broth and were incubated at 37°C for 24 h. Next day 10mL of the broth inoculated till obtained the microbial count as 1×10^6 cells/ml of each pathogenic microorganism.

Antibacterial Activity Assay

The antibacterial activity of the methanol and diethyl ether extract of *Psoralea corylifolia* seed was determined by the agar well-diffusion method on Nutrient agar (Hi Media, India) medium. Using a cork borer, wells (6 mm in diameter) were punched out in the agar medium and inoculums containing 1×10^6 cfu/ mL of the test bacteria were spread plated onto the surface of the medium with a sterile spreader. 50 µl of the extract was pipette into the wells. The agar plates were incubated at 37°C for 24 h and the diameter of the zone of inhibition surrounding the wells was measured. The diameters of zone of inhibition due to extracts were compared with those produced by the commercial control antibiotics, Cephalosporin (30µg/ml). Antibacterial tests were performed

in triplicates and observed values of zone of Inhibition were expressed as average value.¹⁸

Detection of Minimum Inhibitory Concentration

Based on the antimicrobial assay the most promising extract of *Psoralea corylifolia* seeds with significant antimicrobial activity against all tested microorganisms was subjected for the analysis of Minimum Inhibitory Concentration (MIC). Assay with a potent seed extract with concentrations (2- 5 mg/ml) was prepared in methanol.¹⁹ each nutrient broth test tube with seven bacterial test inoculums (10^6 cfu/ml) was inoculated with various concentrations of the test sample. Test tube containing nutrient broth and bacterial culture was used as control. These sets of test tubes for seven tested bacteria were incubated for 37°C for 24 h. Based on the visible turbidity, which was representative of a growth of test organism the MIC was noted and confirmed with viable count analysis. The minimum concentration at which the growth was inhibited as compared to the control was confirmed as MIC.

Phytochemical Analysis

The seed extract with promising broad spectrum antimicrobial activity was subjected for phytochemical tests for detection of plant secondary metabolites, tannins, saponins, steroid, alkaloids and glycosides in accordance with J Harborne.²⁰

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

R_f value (for each spot developed was calculated.²¹

RESULT AND DISCUSSION

Antimicrobial Assay

The result of the antimicrobial activity of the tested *Psoralea corylifolia* seed extracts in methanol and petroleum showed antimicrobial activity against both Gram positive and Gram negative bacteria which were summarized in Table 1.

The antimicrobial potential of both the Methanol and Petroleum Ether seed extracts were evaluated according to their zone of inhibition against skin pathogens. Gram positive, Gram negative microorganisms selected were susceptible to the both the extracts, however methanol extract of *Psoralea corylifolia* seeds was found to be comparatively more effective against all pathogens under investigation. The highest zone of Inhibition (zoi) was observed against *K. pneumoniae* (zoi= 21 mm), *E. coli* (zoi =20 mm), followed by *P. vulgaris* (zoi= 20 mm), *S. epidermidis* (zoi= 20 mm), *S. aureus* and *P. mirabilis* (zoi=18mm) respectively and *P. aeruginosa* with least inhibition (zoi=16mm).

Petroleum ether extract of *Psoralea corylifolia* seeds was observed to be effective against Gram negative bacteria *P. mirabilis* (zoi= 19 mm), followed by *K. pneumonia* (zoi= 18 mm). Other gram negative pathogens under study showed moderate antibacterial activity such as *P. vulgaris* (zoi= 17mm), *E. coli* (16 mm) and *P. aeruginosa* (zoi=16mm). With respect to the Gram positive microorganisms, petroleum ether extract was effective against *S. epidermidis* (zoi= 20 mm) followed by *S. aureus* (zoi= 17 mm) of zone of inhibition. Both methanol and petroleum ether extract showed similar antibacterial potential against gram positive microorganisms however, in case of gram negative microorganisms the methanol extract was found to be more effective as compare to petroleum ether extract

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that inhibit the visible growth of a microorganism after overnight incubation. After incubation at 37⁰C for 24 h, each concentration was compared with control for turbidity. The resulted MIC values were summarized in table 2

MIC of methanol extract of *Psoralea corylifolia* seeds for Gram positive skin pathogens *S. aureus* and *S. epidermidis* was 5 mg/ml. MIC value obtained for Gram negative skin pathogens tested was 5 mg/ml and 4mg /ml except *K. pneumoniae* and *P. mirabilis* it was recorded as 2.5mg/ml. These results showed the more susceptibility of some Gram negative bacteria towards the methanol seeds extracts of *Psoralea corylifolia*.

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 3. Presence of these secondary metabolites will be held responsible for antimicrobial activity.

The retention factors (R_f) of ethanol and aqueous extracts in different solvent systems are shown in table 4. The ethanol extracts produces three fraction having R_f 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.

CONCLUSION

The antimicrobial activity of *Psoralea corylifolia* seeds in methanol and petroleum ether extract was explored specifically against

the bacterial skin pathogens under the present study. Result of this antimicrobial assay showed promising antimicrobial activity of methanol extract against both gram positive and gram negative skin pathogens. Methanol extract of *Psoralea corylifolia* seeds reported to have Minimum Inhibitory Concentration (MIC) in the range of 2.5 to 5 mg/ml for most of the pathogens.

The phytochemical analysis and TLC profiling of methanol and aqueous extracts gives confirmatory result that directing towards the presence of number of phytochemical. The TLC method is best choice for the identification of secondary metabolite present in plants. Here the different R_f values indicate the presence of different nature of phytoconstituents in single extracts. The respective R_f values of the phytoconstituents separated also reflects an idea about their polarity. This information will help in selection of appropriate solvent system for further separation of compound from these extract. However, further analysis of the promising extract could be done to allocate the individual phytoconstituent, so that they can be used as bioactive antimicrobial ingredients in various topical skin formulations.

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Table 1. Antimicrobial Activities of *Psoralea corylifolia* seed Extracts

Microorganisms	Diameter of Zone of Inhibition (mm)*		
	Methanol Extract	Petro. Ether Extract	A*
<i>Staphylococcus aureus</i>	18	17	28
<i>Staphylococcus epidermidis</i>	20	20	28
<i>Proteus mirabilis</i>	18	19	27
<i>Pseudomonas aeruginosa</i>	16	16	30
<i>Proteus vulgaris</i>	20	17	25
<i>Klebsiella pneumoniae</i>	21	18	29
<i>Escherichia coli</i>	20	16	29

*A= Antibiotic; *Average of triplicate test

Table 2. Minimum Inhibitory Concentration of Methanol Extracts

Microorganism	MIC (mg/mL)
<i>S aureus</i>	5
<i>S. epidermidis</i>	5
<i>E. coli</i>	4
<i>K. pneumonia</i>	2.5
<i>P. aeruginosa</i>	5
<i>P. vulgaris</i>	4
<i>P. mirabilis</i>	2.5

Table 3. Photochemical analysis of *Psoralea corylifolia* seed extract

Test for	Methanol Extract
Alkaloid	+
Carbohydrates	+
Flavonoids	+
Glycosides	+
Saponins	+
Steroids	-
Terpanoids	-

+ = Present; - = Absent

Table 4. TLC Studies of *Psoralea corylifolia* methanol extract

Extract	Solvent System	No .of Spots	R _f values
Methanol Extract	<i>Ethyl acetate: Methanol (3:7)</i>	04	0.16, 0.33, 0.44, 0.91