

Antimicrobial Activity of *Psoralea corylifolia* Linn. (Baguchi) Seeds Extracts by Organic Solvents and Supercritical Fluids

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ABSTRACT

Objective: To determine the antimicrobial activity of organic and supercritical fluids extracts of *Psoralea corylifolia* seeds against both Gram +ve bacteria (*S. aureus* ATCC- 29213 and MRSA -15187) and Gram -ve bacteria (*E. coli* ATCC-6739 and *P. aeruginosa* ATCC- 9027).

Methods: The supercritical fluid extracts of *Psoralea corylifolia* were prepared by using pure CO₂ and CO₂ with co-solvent ethyl acetate. On the other hand, organic solvents extracts were prepared sequentially by using petroleum ether, dichloromethane, ethyl acetate, methanol, hydro alcohol (50%) and water. The antibacterial activities of all prepared extracts (PCE 1- PCE 11) were studied along with different marker compounds (bakuchiol, psoralen, isopsoralen and psolaridin) using Broth Dilution method.

Results: The results clearly indicates that all prepared seeds extracts and the marker compound from *Psoralea corylifolia* shows significant antibacterial activity against Gram +ve bacteria, whereas the extracts (PCE 6 - PCE 11) and the compound psolaridin were found to inactive against Gram -ve bacteria. Out of eleven prepared extracts, two extracts namely PCE 1 and PCE 2 along with bakuchiol was found to be more active against all tested micro-organisms, especially against Gram +ve *S. aureus* ATCC- 29213 and MRSA – 15187 bacteria. On the other hand, the extract (PCE 1 - PCE 5) along with marker compounds (bakuchiol, psoralen and isopsoralen) shows significant inhibitory effect of against Gram -ve bacteria.

Conclusion: It was observed that the extract of *Psoralea corylifolia* seeds were active against both Gram +ve bacteria and Gram -ve bacteria. Moreover, the present work clearly demonstrates that the presence bakuchiol has a key role for antimicrobial activity of *Psoralea corylifolia*.

Keywords: *Psoralea corylifolia*, Antimicrobial, Supercritical fluid extraction, Bakuchiol.

INTRODUCTION

Infectious microbial diseases remain pressing problems world-wide, because resistance to a number of antimicrobial agents (β -lactam antibiotics, macrolides, quinolones, and vancomycin) among variety of clinically significant species of microorganisms has become an important global health problem¹. One way to battle with this challenge is the conscious usage of the currently marketed antibiotics; the other is the development of novel anti-microbial agents². Medicinal plants have always been a good source to find new remedies for human health problems. The usage of medicinal plants as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects³⁻⁴. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources.

Psoralea corylifolia, also known as Babachi, is an erect annual herb, belonging to the largest families of flowering plants – Leguminosae used in Ayurvedic medicine as well as in traditional Chinese medicine

almost throughout India⁵. The plant extracts has been reported to possess antibacterial⁶, estrogenic⁷, antitumour⁸⁻⁹, antioxidant¹⁰⁻¹¹, anti-inflammatory¹², antiparasitic¹³, antifungal¹⁴ and immunomodulatory activity⁸. Besides this, it is also used as laxative, diuretic and as a remedy for skin diseases¹⁵⁻¹⁶. Considering the vast potentiality of plants as sources for antibacterial agents, a systematic investigation was undertaken to screen the antibacterial activity of different extracts of *Psoralea corylifolia* using Broth Dilution method. Moreover, the antimicrobial activities of different extracts were compared with ciprofloxacin.

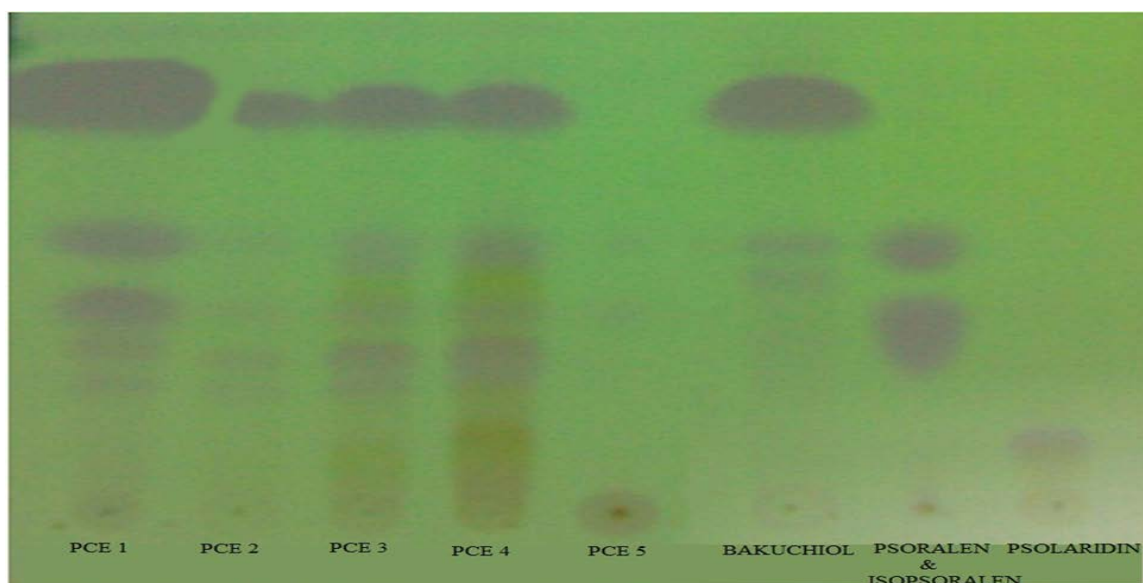
In the SFE, Pure CO₂ and CO₂ in a mixture with ethyl acetate as co-solvents was applied at different conditions of temperature and pressure. On the other hand, sequential solvent extracts were prepared by using different organic solvents such as petroleum ether, dichloromethane, ethyl acetate, methanol, hydro alcohol (50%) and water by maceration.

MATERIALS AND METHODS

Plant material: The seeds of *Psoralea corylifolia* plant were purchased from local market of Jammu Tawi and well authenticated by Dr B. K. Kapahi Taxonomist of the Indian Institute of Integrative Medicine (CSIR). A sample was also submitted to the institute herbarium for

Table 1: The extractive value of different supercritical extracts, Weight of the plant material taken-2 kg

S.No.	Name of the extract	Weight of the extract (g)	Extractive Value (%)
1	PCE 1	15	1.5
2	PCE 2	62.5	6.25
3	PCE 3	8.625	0.86
4	PCE 4	15.25	1.52
5	PCE 5	147.5	14.75



1. TLC of supercritical extracts (PCE 1-PCE 5) along with isolated marker compounds.

Table 2: The extractive value of different sequential organic extracts, Weight of the plant material taken-250g

S.No.	Name of the extract	Weight of the extract (g)	Extractive Value (%)
1	PCE 6	28.54	11.41
2	PCE 7	25.23	10.09
3	PCE 8	7.24	2.89
4	PCE 9	16.07	6.43
5	PCE 10	37.40	14.96
6	PCE 11	7.80	3.12

preservation. The seeds were grounded in a domestic blender immediately before the extractions.

Drug and chemicals: Ciprofloxacin was purchased from Sigma Chemical Co. (St Louis, MO, USA). Carbon dioxide (99.9%) was used as a solvent for supercritical fluid extraction. All other solvents used were of LR grade, and were used as received. The marker compounds (bakuchiol, psoralen, isopsoralen and psolaridin) were provided by Indian Institute of Integrate Medicine, CSIR, Jammu (J&K).

Test Organisms: The antimicrobial activity was carried out at Clinical Microbiology Division, Indian Institute of Integrate Medicine, Canal road, Jammu (J&K) India using the following microorganism: *Staphylococcus aureus* ATCC-29213, methicillin-resistant *Staphylococcus aureus* (MRSA ATCC-15187), *Escherichia coli* ATCC-6739 and *Pseudomonas aeruginosa* ATCC-9027.

Supercritical Fluid Extraction (SFE): The extractive values of different extracts were given in Table 1. The powdered seeds of *P. corylifolia* (1 kg) were first extracted statically for 2 hours with CO₂ at 45°C temperature and 100 bar pressure (PCE 1), the dried marc

was again extracted for additional 2 hours with CO₂ at 55°C temperature and 200 bar pressure (PCE 2). The marc left behind was subjected to further extraction with CO₂ and EtOAc as co-solvent at same conditions of temperature and pressure mentioned above for additional 2 - 2 hours respectively (PCE 3 and PCE 4). For complete removal of polar constituents the marc was re-extracted with distilled water for 24 hrs (3 times) at room temperature by maceration. The extracts obtained were combined and lyophilized (PCE 5).

Thin layer Chromatography: Thin-layer chromatography (TLC) was carried out on Merck Silica gel 60 F254 with a thickness of 0.2 mm on aluminum sheet using 30% ethyl acetate in petroleum ether as mobile phase. The TLC of super critical fluid extracts (PCE 1 - PCE 5) along with marker compounds is shown in figure 1. TLC optimization was carried out under ultraviolet light and by using sulphuric acid as visualizing agent.

Sequential Solvent Extraction: The extractive values of different solvents were given in Table 2. The powdered seeds of *P. corylifolia* (250 g) were macerated successively at room temperature using different organic solvents in respective order of their increasing polarity.

The extraction process yielded in n-hexane (PCE 6), dichloromethane (PCE 7), ethyl acetate (PCE 8), methanol (PCE 9), hydroalcoholic solution 50% (PCE 10) and water (PCE 11) extracts which were then evaporated to dryness using by vacuum rotary evaporator under reduced pressure below 50°C. Different extracts were kept in the dark at 4°C until tested.

Antimicrobial activity: MIC of bacterial pathogens was determined by Broth Dilution method using 96-well micro-titre plates. Brain heart infusion was used for culturing the bacteria and incubated for 24 hrs with 5% CO₂ at 37°C using NCCLS M11- A3 and NCCLS M7-A5. All cultures were preserved in 50% glycerol at -70°C. The stock solutions of compounds were prepared in 100% dimethyl sulphoxide (DMSO); the highest final concentration of DMSO used in assays (1%, v/v) caused no inhibition of bacterial growth. Two fold concentration of the solution were prepared in micro-centrifuge tube and then transferred to micro-titre plates. Each microorganism suspension was then added into the wells. The last two columns containing 100 µL and 200 µL of medium, without drug served as growth and medium control, respectively. After incubation at 37°C for overnight the first well showing no turbidity of growth was determined as minimal inhibitory concentration (MIC). Ciprofloxacin was used as control at a MIC of 0.25µg/ml.

RESULTS

The antimicrobial activities of all the prepared extracts (PCE 1 – PCE 11) and the marker compounds (bakuchiol, psoralen, isopsoralen and psolaridin) were shown in Table 3. Among the eleven prepared extracts, the supercritical extracts (PCE 1 and PCE 2) and the marker compound bakuchiol was found to be more active against all tested micro-organisms, especially against Gram +ve bacteria. The supercritical fluid extracts PCE 1, PCE 2, PCE 3 and PCE 4 shows remarkable growth inhibitory effects against *S. aureus* ATCC- 29213 and MRSA – 15187 at a conc. of 4µg/ml, 4µg/ml, 8µg/ml and 16µg/ml respectively. Whereas, the inhibitory effect of all four

extracts against *E. coli* ATCC-6739 and *P. aeruginosa* ATCC- 9027 were shown at a conc. of >128µg/ml. Although, the organic extracts PCE 6, PCE 7, PCE 8 and PCE 11 showed significant growth inhibitory effects against Gram +ve *S. aureus* ATCC- 29213 and MRSA – 15187 at a conc. of >128µg/ml yet they were found inactive against Gram -ve *E. coli* ATCC-6739 and *P. aeruginosa* ATCC- 9027. Moreover, the extracts PCE 9 and PCE 10 were also found inactive against Gram -ve bacteria but these showed significant growth inhibitory effects against Gram +ve *S. aureus* ATCC- 29213 and MRSA – 15187 at a conc. of 8µg/ml. On the other hand, the marker compounds Bakuchiol, Psoralen, Isopsoralen and Psolaridine were also evaluated for their antibacterial activity against Gram +ve and Gram -ve bacteria. Out of the four compounds bakuchiol was found to possess significant anti bacterial activity. The inhibitory effect of first three compounds (i.e. bakuchiol, psoralen and isopsoralen) against *E. coli* ATCC-6739 and *P. aeruginosa* ATCC- 9027 were shown at a conc. of >128µg/ml whereas the compound psolaridine was found inactive against the same bacterial strains.

However the marker compounds psoralen, isopsoralen and psolaridine shows significant inhibitory effect against Gram +ve *S. aureus* ATCC- 29213 and MRSA – 15187 at a conc. of >128µg/ml, >128µg/ml and 256 respectively.

DISCUSSION

In the present study, the coarse powdered seeds of *Psoralea corylifolia* were extracted using different methods (organic solvents and supercritical fluids), which were evaluated for their antimicrobial activity. The results clearly demonstrate that the extracts (PCE1 and PCE2) and the marker compound bakuchiol show similar antibacterial activity (Table 3). Furthermore, the TLC of the prepared extracts (PCE 1- PCE 5) along with marker compounds (bakuchiol, psoralen, isopsoralen and psolaridine) demonstrates that the extracts (PCE 1 - PCE 4) possess bakuchiol as main constituent (figure 1). Therefore, this can be concluding that antimicrobial activity of the extracts of *Psoralea corylifolia* was due to

Table 3: Antibacterial activity of all prepared extracts along with isolated compounds.,Antibacterial Activity MIC µg/ml

S.No.	Code of Extract	<i>S.aureus</i> ATCC-29213	MRSA- 15187	<i>E.coli</i> ATCC-6739	<i>P.aeruginosa</i> AICC-9027
1	PCE 1	4	4	>128	>128
2	PCE 2	4	4	>128	>128
3	PCE 3	8	8	>128	>128
4	PCE 4	16	16	>128	>128
5	PCE 5	>128	>128	>128	>128
6	PCE 6	>128	>128	-	-
7	PCE 7	>128	>128	-	-
8	PCE 8	>128	>128	-	-
9	PCE 9	8	8	-	-
10	PCE 10	8	8	-	-
11	PCE 11	>128	>128	-	-
12	BAKUCHIOL	4	4	>128	>128
13	ISOPSORALEN	>128	>128	>128	>128
14	PSORALEN	>128	>128	>128	>128
15	PSOLARIDIN	256	256	-	-

the presence of bakuchiol.

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