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Novel phytochemical constituents identified from the seeds of *Psoralea corylifolia*

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Abstract
The present study was to isolate and characterize novel phytoconstituents from the seeds of medicinal plant, *Psoralea corylifolia*. The compounds were characterized by IR, ^{1}HNMR, ^{13}CNMR, Mass spectrum, elemental analysis and screening the antimicrobial activity.

Keywords: *Psoralea corylifolia*, novel compounds, spectral analysis, antimicrobial activity

1. Introduction
Psoralea corylifolia belongs to family fabaceae/paplionaceae. It is a herbaceous plant distributed throughout tropical and subtropical regions of the world. It is commonly called babachi, bakuchi, vakuchi or bemchi. In Arabic it is called Loelab el abid and waghchi in Persian.It is an annual herb about one metre in height. It is a climbing bean found throughout India and China in dry and sandy places. Stem is erect and grooved. Leaves are simple, broadly elliptic, inciso-dentate, rounded, white, hairy and dotted. Flowers are dense, auxilary, solitary, bluish purple and clawed. The purple seed pods contain dark elongated seeds, when ripened they are large, solid and black. The seed is brownish-black in color, has soft skin, pleasant odour and pungent bitter taste. The seed consists of a sticky, oily pericarp, a hard seed coat and a kernel. The whole seed cannot be easily powdered owing to the sticky pericarp [84, 85].
Psoralea promotes bone calcification and is useful in the treatment of osteoporosis. According to Ayurveda, root is useful in carries of teeth whereas leaves are good for diarrhoea. Fruit is diuretic and causes biliousness. It is useful in treatment of vomiting, piles, bronchitis, inflammation and anaemia. Seeds are refrigerant, laxative, antipyretic, anthelmentic, alexiteric and good for heart troubles. The seed has been reported to have antiseptic properties either in its fresh form or in the form of watery extracts prepared from it. In the course of inflammatory skin diseases, leucoderma and psoriasis, it is given both as a local application as well as by mouth. The seeds are frequently used in several toilet preparations because of curative properties against skin disorders [139].

2. Experimental
2.1 Plant Material
The seeds of the plant, *Psoralea corylifolia* were purchased from local market of Jammu & Kashmir. The plant material was identified by local Hakeem, Mr. Mubarak Ahmad Shah.

2.2 Solvents and Reagents
The solvents Pet. ether, hexane, benzene, chloroform, ethyl acetate and methanol were provided by chemistry lab. The reagents sodium chloride, agar powder, beef's extract, peptone and Na_2CO_3 were provided by biotechnology lab.

2.3 Apparatus and Equipments
The Soxhlet Apparatus (JSGW) was used for the extraction of plant material. The equipments laminar air flow, incubator and oven were of Yorko Industries whereas, the autoclave of JSGW. Glasswares and heating mantle were from Perfit India.

2.4 Analytical tools
The spectral analysis of various compounds was performed by FT-IR 8400-S spectrophotometer (Instrumentation lab, department of chemistry, Lovely Professional University), Q-ToF micro mass spectrometer (mass range 20000amu), multinuclear FT NMR Avance II (Bruker) spectrophotometer and Hitachi 330 UV-Visible spectrophotometer (Punjab University, Chandigarh).

2.5 Determination of antimicrobial activity using agar disc diffusion method
2.5.1 Preparation of nutrient agar medium
To the 200 ml distilled water, 0.6gm Beefs extract, 1gm NaCl, 1gm peptone and 3 gm of agar powder was added. The mixture was heated to dissolve the components. The nutrient agar medium was then sterilized in an autoclave for 45 min. After cooling it was poured in the sterile petri plates and placed in laminar till solidification.

2.5.2 Antimicrobial activity of plant extract
The antimicrobial activity of different components obtained from column on elution with mixtures of chloroform, ethyl-acetate and methanol, was studied with two different bacterial strains. Homogenous solutions of the components [1.$CHCl_3$: EtOAc (5:1); 2.EtOAc: $CHCl_3$ (5:1); 3.EtOAc: $CHCl_3$ (5:2) and 4.EtOAc: MeOH (5:1)] of plant, *Psoralea corylifolia* were prepared. The stock solutions so obtained were absorbed on the sterile filter paper discs (5mm diameter), which were subsequently placed in inoculated petri plates. The petri plates were then incubated at 38.7ºC for 24 hours.

2.5.3 Bacterial strains
The biological activity of different extracts was tested against bacteria, *Escherichia coli* and *Bacillus subtilis* available in Biotechnology lab. The bacteria were

maintained at 4°C on nutrient agar.

Table 1

Group	Strain	Cultivation condition
Gram (-)	*Escherichia coli*	Nutrient agar/37°C
Gram (+)	*Bacillus subtilis*	Nutrient agar/37°C

3. Extraction of plant material

Hot extraction of seeds of *Psoralea corylifolia* with soxhlet apparatus was repeatedly done with methanol for about 72 hours. The brownish viscous mass was obtained after evaporating the solvent. This was partitioned among solvents hexane, chloroform and EtOAc and methanol. The chloroform and ethyl acetate portion was subjected to column chromatography. Further the biological activity of four different fractions was studied against two bacterial strains: *Escherichia coli* and *Bacillus subtilis*. The schematic representation of extraction procedure is given in figure 2.6.

4. Chromatography

The semisolid brownish mass obtained from chloroform extract (3gm) and ethyl-acetate extract (5gm) was dissolved in small amount of chloroform and ethyl-acetate respectively and each mixed with (5 gm) of silica gel. The slurry was loaded on a column of silica gel and eluted with petroleum ether, benzene, chloroform, ethyl acetate, methanol and their mixtures of different proportions of increasing polarity. Several fractions were monitored by TLC and the fraction showing single spot on TLC were combined together.

Psoralea corylifolia

Weight of dried seeds: 250gm

Extractive solvent	Yield
Methanol	35gm
Hexane	4gm
Chloroform	12gm
Ethyl-acetate	7gm

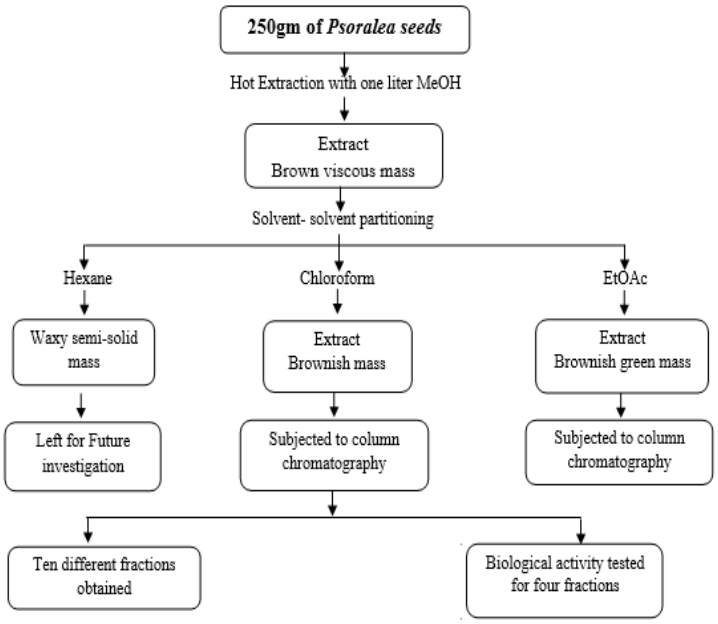
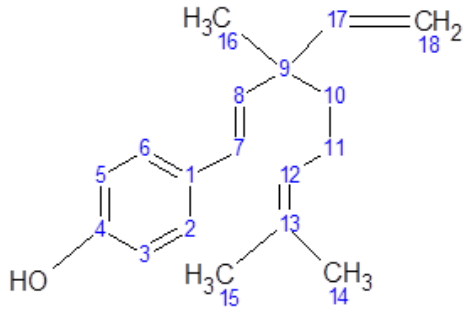


Fig 1: Flow chart showing the seed extraction of *Psoralea corylifolia*

5. Results and Discussion

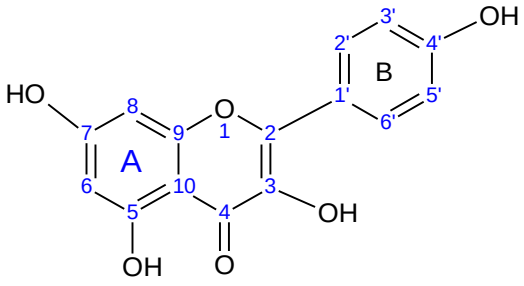
The well dried coarsely powdered plant material was extracted with methanol. On concentration under reduced pressure a brownish viscous mass was obtained which was partitioned between hexane, chloroform and ethyl acetate. The chloroform and ethyl acetate extracts were subjected to column chromatography. The chloroform extract on eluting with solvents of different polarity afforded compound PC-I. Compound PC-II was obtained from ethyl acetate extract.

PC-I: The compound was isolated as a brown liquid from the repeated extraction of seed with chloroform on elution with methanol: benzene (5:1) from the column chromatography. The boiling point of the compound was found to be 200° C. the compound gives positive Libermann-Burchard test[136] confirming its terpenic nature. The IR spectrum of the compound appeared at 3400cm^{-1}, 1625cm^{-1}, 1600cm^{-1}, 980cm^{-1} and 928 cm^{-1} showing the presence of hydroxyl group, olefinic linkages and a side chain in the molecule. Three singlets δ1.26 (3H), δ1.65 (3H) and δ1.1.74 (3H) were observed in the ^{1}HNMR spectrum (Table 2) of the compound showing the presence of three tertiary methyl groups at C-9 and C-13. One proton doublet each appeared at C-7 (δ6.25) and C-8 δ6.11 showing the presence of olefinic linkage between C-7 and C-8. The appearance of two proton doublet signals between δ6.80 (H-3,5) to δ7.28 (H-2,6) confirm the presence of aromatic ring. One double doublet signal appeared at δ5.94 (H-17) showing a methine proton. The presence of olefinic linkages is also supported by the ^{13}CNMR spectrum (Table 3) appearing at δ126.4 (C-7), δ135.8 (C-8), δ124.8 (C-12), δ131.2 (C-13), δ145.9 (C-17), δ111.8 (C-18). The signal at δ154.5 (C-4) confirms the presence of hydroxyl group. The mass spectrum of the compound ($C_{18}H_{24}O$) indicated the presence of a molecular ion peak at (m /z) 256. In addition to this other diagnostically important peaks at 241, 173, 145 and 107 were observed. On the basis of above data the compound was identified as Bakuchiol and following structure was assigned.



Structure of PC-I

PC-II: The compound was isolated as yellow gummy solid (m.p. 170-172^{0}C) from ethyl acetate extract on elution with benzene: ethyl acetate (3:7). It responded positively Shinoda test[137], and showed pink colour with sodium amalgam/HCl [138] confirming its flavonoid nature. The IR spectrum of the compound showed the presence of hydroxyl group and olefinic linkage in the molecule. Its IR absorption bands observed at 3420 cm^{-1} correspond to hydroxyl group and 1640 cm^{-1} corresponded to C=O linkage in the molecule. The ^{1}H NMR spectrum of the compound showed the presence of six protons in aromatic region also in favour of a tetra substituted flavone. The presence of 5, 7 hydroxy groups were confirmed by positive vanillin hydrochloric acid test [139]. The [1] H NMR spectrum showed sharp singlet at δ 6.02 which accounted for two aromatic protons of ring A at C-6 and C-8. The aromatic region also exhibited two distinct peaks at δ 7.13 and δ 7.68 integrating for two protons each assignable to 2',6' and 3',5' protons respectively of ring B [13]. C NMR spectrum (Table 4) exhibited downfield signals at δ187.0 clearly assignable to carbonyl carbon at C-4. The three downfield signals observed in the range of δ 165-160 were assigned to the carbons of aromatic ring
(C-5,7,4') to which OH groups were attached. The fourth OH group attached in pyrrone ring at C-3 was shown by signals at δ 123.8. Further the structure of the compound was established by its mass spectrum which showed the molecular ion peak at m/z [286]$^+$ for $C_{15}H_{10}O_6$. Other diagnostic peaks m/z 193,152,134 and 93 helped in assigning the structure the compound. Thus on the basis of above spectral studies the structure of the compound was assigned as 3, 5, 7, 4' tetra hydroxy flavone.



Structure of PC-II

PC-I: The compound was isolated as a brown liquid (b.p. 200^{o}C) from the repeated extraction of seed with chloroform on elution with MeOH: C_6H_6 (5:1) from the column chromatography.

Anal. Found	**:** C 84.37; H 9.37.
Anal.Calcd.for $C_{18}H_{24}O$	**:** C 84.375; H 9.375.
IRλ max	**:** 3400, 1625, 1600, 980, 928cm^{-1}
Mass Spectrum	[M]$^+$,256,m/z 241, 173, 145, 107.

Table 2: ^{1} H NMR of PC-I

Signals δ(ppm)	No. of protons	Assignments
1.26	3H s	H-16
1.56	2H m	H-10
1.65	3H s	H-15
1.74	3H s	H-14
2.02	2H m	H-11
5.08	2H m	H-18
5.17	1H br	H-12
5.94	1H dd	H-17
6.11	1H d	H-8
6.25	1H d	H-7
6.80	2H d	H-3,5
7.28	2H d	H-2,6

Table 3: ^{13}C NMR of PC-I

Carbon atoms	Signals δ (ppm)
C 1	130.8
C 2	127.3
C 3	115.4
C 4	154.5
C 5	115.4
C 6	127.3
C 7	126.4
C 8	135.8
C 9	42.4
C 10	41.2
C 11	23.3
C 12	124.8
C 13	131.2
C 14	25.6
C 15	17.6
C 16	23.2
C 17	145.9
C 18	111.8

PC-II: The compound was obtained as yellow gummy solid (20 mg), mp 170-172^{0}C from ethyl acetate fraction by eluting with benzene: ethyl acetate (3:7) solvent system.

Anal. Found	**:** C, 62.90; H, 3.54.
Anal.Calcd.for $C_{15}H_{10}O_6$	**:** C, 62.94; H, 3.50.
IR (KBr) **λ** max	**:** 3420, 1640, 886 cm^{-1}.
^{1}H NMR (δ ppm)	**:** δ 6.02(2H,s, H-6,H-8),
δ 7.13(2H,d,H-2',H-6'),	
δ 7.68(2H,d,H-3',H-5').	
Mass Spectrum [M]$^+$,286,m/z 193, 152,134, 93.	

Table 4: ^{13}C NMR of PC-II

Carbon atoms	Signals δ (ppm)
C-2	133.6
C-3	123.8
C-4	187.0
C-5	161.1
C-6	98.7

C-7	164.2
C-8	97.3
C-9	157.2
C-10	102.3
C-1’	121.1
C-2’	127.3
C-3’	116.4
C-4’	161.5
C-5’	114.5
C-6’	27.6

6. Biological Activity of *Psoralea corylifolia* extracts:

The biological activity of following four components was studied against the bacterial strains, *E. coli* and *Bacillus subtilis*. These components were obtained under the column chromatography of chloroform extract of *Psoralea corylifolia* seed.

1. $CHCl_3$: EtOAc (5:1)
2. EtOAc: $CHCl_3$ (5:1)
3. EtOAc: $CHCl_3$ (5:2)
4. EtOAc: MeOH (5:1)

The fractions 1 and 2 showed moderate inhibitory activity whereas the other two fractions showed weak biological activity against *E.coli* (Fig 2.3). The activity of fractions 1 & 2 when studied against *Bacillus subtilis* showed strong activity (Fig 2.4) whereas weak activity was observed for fractions 3 & 4 (Fig 2.5).

Fig 2: Antimicrobial activity of fractions, 1, 2, 3 & 4 of *Psoralea corylifolia* against *Escherichia coli*

Fig 3: Antimicrobial activity of fractions 1 & 2 of *Psoralea corylifolia* Against *Bacillus subtilis.*

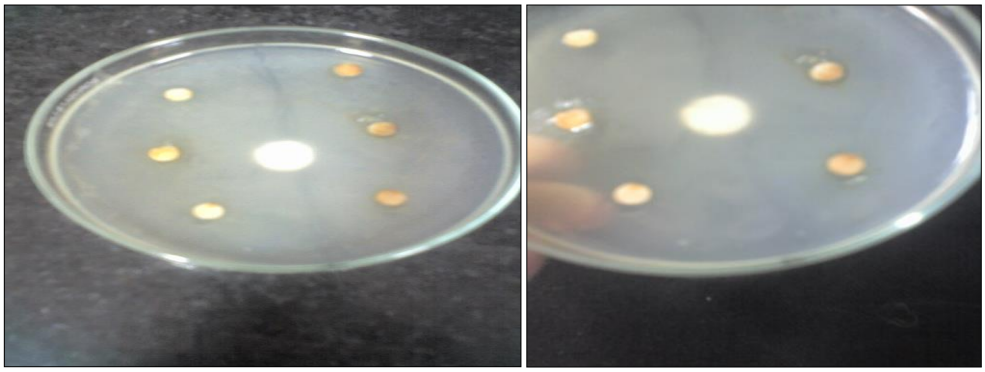


Fig 4: Antimicrobial activity of fractions 3 & 4 of *Psoralea corylifolia* Against *Bacillus subtilis*

7. Conclusion

To isolate and identify the bioactive principles of medicinal plant- *Psoralea corylifolia* using alternative methods of isolation. The compounds were isolated and investigated with the help of spectroscopic studies. Moreover, the biological activity of different components obtained from

column was studied against two bacterial strains, *Eschersia coli* and *Bacillus subtilis.*

The present work gives a direction for future investigators to carry out research on the extracts to separate some new compounds that will prove a milestone for the treatment of Leucoderma.

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