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IDENTIFICATION, PURIFICATION AND CHARACTERIZATION OF COMPOUND ISOLATED FROM *PSORALEA CORYLIFOLIA* USING A NOVEL APPROACH

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ABSTRACT

Psoralea corylifolia Linn. (Leguminosae) is one of the important pharmaceutical potent plant possesses various activities like antibacterial, anticancer, antioxidant, antifungal, antidepressant and antitumor, therefore used in different medicinal formulations worldwide. This plant has given important lead in drug research due to its scientific finding, resulting in the discovery of various novel molecules. The objective of the present study was to develop a flash chromatography method for isolation and purification of psoralen biomolecule from *Psoralea corylifolia* Linn. seeds extract and its further identification and characterization using different spectral analytical techniques. Absorption maxima of psoralen at 247 nm was confirmed using UV spectrophotometer. TLC and HPLC quantification of psoralen were done before and after isolation from seeds extract of *Psoralea corylifolia* Linn. Flash chromatography method was used for purification and isolation. About 250 gm of raw material yielded 15.63 gm of crude hexane extract. Further using flash chromatography about 68 mg of white needle-shaped crystalline powder of psoralen of over 98% purity, determined by high performance liquid chromatography (HPLC) was obtained. The identification and characterization of psoralen performed using FTIR, ^{1}H NMR and ^{13}C NMR spectral data.

Key words : *Psoralea corylifolia*, Flash Chromatography, High Performance Liquid Chromatography (HPLC).

Introduction

Psoralea corylifolia Linn. is a traditional medicinal herb, belongs to Leguminosae family, commonly known as "Bakuchi" and 'Bu Gu Zhi' in Indian traditional medicine and in traditional Chinese medicine respectively (Patel, 2023; Alam *et al.*, 2018). *Psoralea corylifolia* Linn., has diversified uses in agriculture, ayurvedic as well as allopathic system of medicines with thousands of years of clinical application. Most of the therapeutically active components are present in its seeds or fruits are coumarins (psoralidin, psoralen, isopsoralidin, isopsoralen, angelicin and corylifolin), flavonoids namely neobavaisoflavone, isobavachalcone, bavachalcone, bavachinin, bavachin, corylin, corylifol, corylifolin and 6-prenylnaringenin and meroterpenes (3-hydroxybakuchiol and bakuchiol). Its essential oil is rich in limonene, α-elemene, γ-elemene, β-caryophylenoxide, 4-terpineol, linalool, geranylacetate (Koul *et al.*, 2019; Bertoli *et al.*, 2004). *Psoralea corylifolia* found throughout India in Himalayas, Uttarakhand, adjoining area of Uttar Pradesh, semi-arid regions of Punjab and Rajasthan, Bundelkhand, Bengal and Bombay, Deccan and Karnataka (Vidua *et al.*, 2015). The extracts and active components of *Psoralea corylifolia* Linn. demonstrated multiple biological activities, including anticancer, estrogenic, antitumor, anti-oxidant, anti-microbial, anti-depressant, anti-inflammatory and osteoblastic and hepatoprotective activities (Zhang *et. al.*, 2016; Pandey *et al.*, 2013). The principal bioactive compound of bakuchi is psoralen, which is a tricyclic coumarin like aromatic compound, having

molecular structure 7H-Furo [3, 2-g] benzopyran-7-one (Pathak *et al*., 1959). *Psoralea corylifolia* Linn. biomolecule psoralen (coumarins) widely used as quality control components in various herbal formulations. Psoralen which on exposure to sun forms melanin in depigmented skin, found to intercalate into DNA covalently, where on exposure to long wave UV light form mono- and di- adducts and thus are used for the treatment of leucoderma a hypo pigmented lesions of the skin and vitiligo an autoimmune disease (Sui *et al*., 2020). The isolation and purification of Psoralen and related compounds from *Psoralea corylifolia* Linn., reported methods are tedious and usually require highly sophisticated instruments which are not cost effective. Due to the important biological properties and broad applications of psoralen, we have attempted to develop an efficient method to isolate and purify the pure compound from the extract of *Psoralea corylifolia* Linn. seeds. The subsequent preparative isolation of compounds of interest used here is flash chromatography (flash purification). It enables rapid separation, reliable and expeditious approach for isolation of pure compounds from complex natural product mixtures, as it is hybrid of gravity column chromatography and preparative liquid chromatography, in this solvent coupled with a pressurized gas like nitrogen in order to assist in the separation process (Roge *et al*., 2011; Girijal *et al*., 2018). The advantage of flash chromatography method over other crude purification techniques is that it has ability to separate broad variety of pure compounds more efficiently, offering rapid and finest results (Kustrin *et al*., 2022). This study focus on flash chromatography method development to separate and isolate high purity bioactive compound psoralea from *Psoralea corylifolia* Linn. extract in the most cost-efficient way and its further characterization via FTIR, NMR spectroscopy, guided by thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). In addition, this study gives a comprehensive overview of psoralen pharmacological potential, which will be helpful in further research.

Materials and Methods

Bakuchi plant seeds : The seeds of *Psoralea corylifolia* Linn. were collected from local vendor, Haridwar. Authenticity of raw seeds were confirmed by Patanjali Research Foundation Herbarium (PRHF), Haridwar. The seeds were air dried in shadow, reduced to coarse powder with grinder and stored in airtight zipper bag till further use.

Chemicals: All solvent used in were of reagent grade and analytical grade purchased from Merck Ltd, Mumbai, India. Reference standards of psoralen (purity ≥99.0%) was purchased from Sigma Aldrich, Bangalore, India.

Extraction of crude Psoralen : Air dried powder (250 gm) of *Psoralea corylifolia* Linn. seeds was extracted successively by soxhletion (3 times, each for 3 hours, 50 ± 5^0C), using n-hexane. The extraction solvents used were removed under vacuum pressure using rotary evaporator. The extracts were collected weighed 15.62 g and their yield percentage were recorded and was thereafter, stored under cool temperature until further analysed.

Instrumentation

Determination of absorption maxima

UV spectrophotometer : The measurement of the UV spectra and absorbance readings was conducted using the Shimadzu UV- 1800 model, UV-Visible Double Beamed Spectrophotometer with bandwidth 1 nm, wavelength accuracy 0.1 nm and quartz cuvettes with 1 cm path length, over the specific λmax at 200-400 nm. The glassware used during the process were treated with a chromic acid and sulphuric acid solution, further rinsed thoroughly with double distilled water and dried in the hot air oven. Accurately weighed 10mg of psoralen, transferred to 100 ml volumetric flask. Volume makeup to 100 ml by adding methanol in order to get the concentration of 100 µg/ml. The resultant solution (100 µg/ml) were scanned on UV spectrophotometer so as to observe the absorption maxima of psoralen.

Identification and Quantification

TLC (Thin layer chromatography) : Method for qualitative determination of psoralen in solvent extracted *Psoralea corylifolia* Linn. seeds extract and isolated compound were determined. The advantage of using thin-layer chromatography (TLC) method as large number of samples can be simultaneously analyzed using small volume of mobile phase thus lowering analysis time and cost per analysis. Precoated (Merck) analytical plates with fluorescent indicator (silica gel 60 F_{254}, aluminum sheets) were used for thin layer chromatography (TLC). Glass capillary tubes were used for spotting in duplicate. One mg psoralen RS in 10 ml volumetric flask. Add 5ml methanol dissolve and makeup the solution. For sample preparation 1 gm crude extract was dissolved in methanol in 25 ml volumetric flask. The plate was developed in developing chamber, pre-saturated for 2 hours with mobile phase hexane: ethyl acetate, (7:3 v/v) (solvent front 6.0 cm). The plates were observed at wavelength 254 nm and 366 nm. Retention factor (R_f value) for reference standard and sample were calculated.

HPLC (High performance liquid chromatography)

Psoralen was simultaneously determined in *Psoralea corylifolia* Linn. seeds extract using HPLC system (Shimadzu *i*-series LC 2030), equipped with a quaternary pump, a variable wavelength UV-VIS detector, RP-18 column (250 mm × 4.6 mm i.d., particle size 5µ). The mobile phase ratio A: Mixture of 0.146 g KH_2PO_4 / 0.5 ml OPA in 1000 ml miili Q water, mobile phase B: 100 % Acetonitrile (v/v). Gradient phase used is shown in Figure 1. The flow rate was 1.5 ml/ min, psoralen was detected at wavelength of 247 nm at room temperature 25°C. The retention time of psoralen reference standard was 13.397 min under the above conditions. Data were collected and processed using computing integrator chromatography workstation (Labsolution software).

Standard preparation: 100 mg reference standard of psoralen was dissolved in 100 ml methanol, sonicated for 15 min. Further 10 ml was taken and diluted with 100 ml methanol.

Sample preparation: One gm extract sample was dissolved in methanol in 100 ml volumetric flask. Further 10 ml was taken and diluted with 100 ml methanol and filtered through 0.45 µm millipore filter prior to HPLC analysis.

Flash Chromatography

Preparative isolation and purification: *Psoralea corylifolia* Linn. seeds extract containing psoralen was purified using Flash (Buchi, C- 850 Flash Prep), integrated with a variable wavelength UV-VIS detector and ELSD, Pressure range flash mode: 0-50 bar and prep mode: 0-300 bar. Flash cartridges (60-120) mesh) column. The solvent used were hexane: ethyl acetate (v/v) in gradient mode depicted in Figure 2. Flow rate was at 25 mL/min. Variable wavelength were used in range λ_1 – 210, λ_2 – 247, λ_3 –310, λ_4 – 420 at room temperature 25°C.

Sample preparation for loading : Ten gm hexane extracted *Psoralea corylifolia* seeds extract was added into 30 gm silica gel (60-120 mesh size), mixed properly by gradually adding small amount of hexane. Then dried in oven at 50-60°C till solvent evaporates completely. Further packed under vacuum in flash empty solid loader.

Characterization

FTIR (Frontier Transform Infrared spectroscopy): The characterization of isolated compound was carried out using FTIR (Shimadzu IR). The testing was carried out by using KBr pellet method. KBr was dried in the oven at 40°C and it was then mixed with the sample. The sample was compressed using a pellet press. The sample was placed in IR sample holder. A background scan was taken followed by the IR scan of the sample in range of 500- 4000 cm^{-1}

NMR (Nuclear magnetic resonance, ^{1}H and ^{13}C) : ^{1}H NMR and ^{13}C NMR spectra of preparative isolated compound were measured at 500 MHz and 125 MHz respectively on a Bruker avance NEO 500 spectrophotometer, CDC13 with or without tetramethylsilane as an internal standard. Chemical shifts are given in δ (ppm).

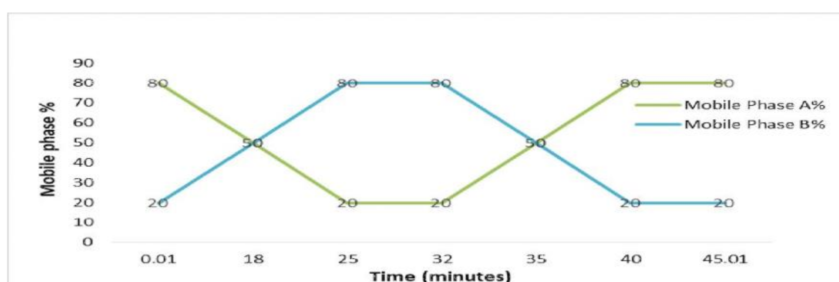

Figure 1 : Gradient mobile phase method for Hplc.

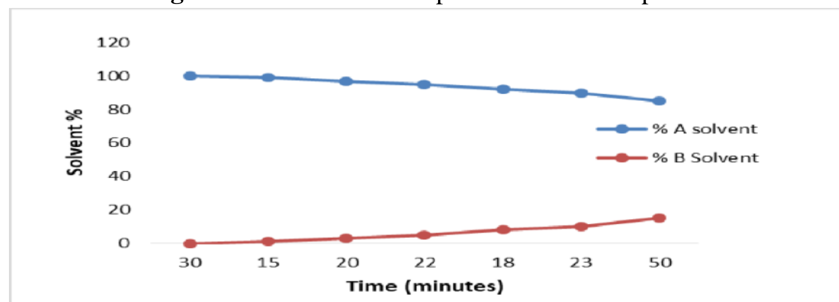

Figure 2 : Gradient mobile phase method for flash chromatography.

Results and Discussion

The identification and characterization of the compounds usually involves a combination of different spectral optimized techniques including UV, FTIR, ^{1}H and ^{13}C NMR. Under the TLC chromatographic conditions, the retention factor (R_f value) for reference standard, crude extract and isolated compound were found to be 0.72 ± 0.02, which indicates the presence of psoralen. The characteristic retention time (Rt) chromatograms of the psoralen standard and crude extract of seed of *Psoralea corylifolia Linn.* are shown in Figures 3 and 4, respectively, in which the Rt of psoralen reference standard was obtained at 14.397 and Rt of psoralen in crude extract of *Psoralea corylifolia* Linn. seeds were obtained at 14.395. Further, shown in Figure 4, quantitative HPLC analysis of the crude extract from *Psoralea corylifolia* Linn

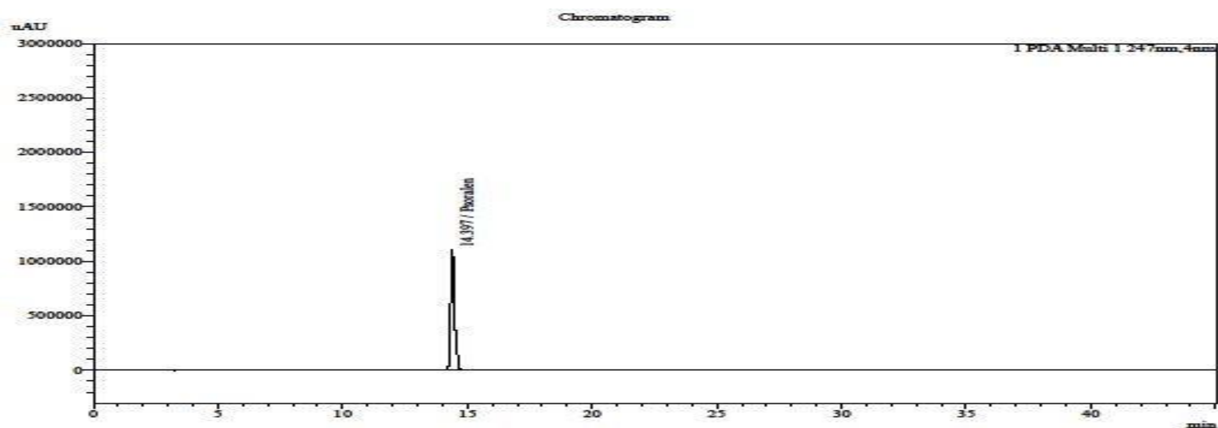

Figure 3: Hplc chromatogram of psoralen reference standard.

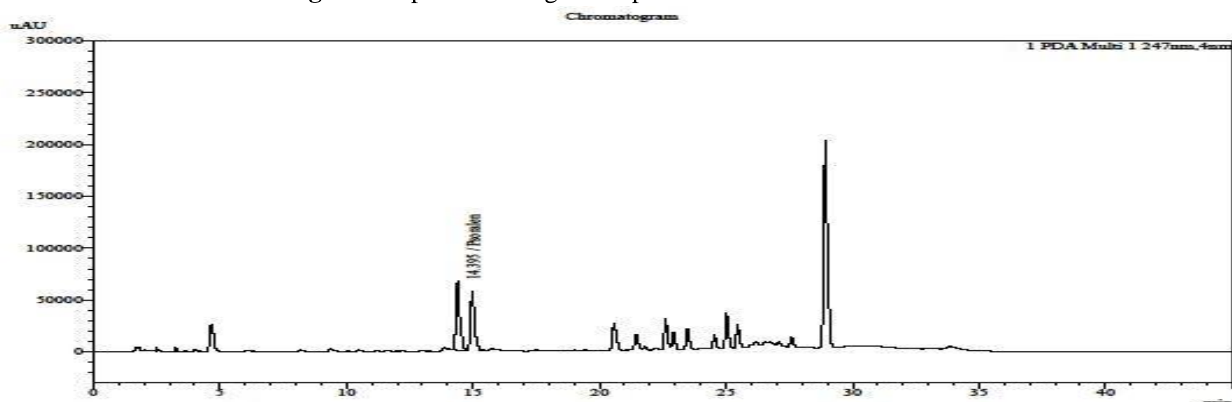

Figure 4: Hplc chromatogram of psoralen present in *Psoralea corylifolia* seeds crude extract.

seeds contained psoralen a major compound, purity based on HPLC peak area percentage in crude extract was reported 49.3%.

Until now, no studies have been reported of psoralen isolation by flash chromatography. Past literature data has reported isolation of psoralen by column chromatography (Rajput *et al*., 2008; Lu *et al*., 2014). Crude *Psoralea corylifolia* extract containing psoralen was loaded on flash chromatography and first eluent hexane (100 %) and ethyl acetate (0 %) v/v after 30 min elute fraction consisted of yellow brown colour liquid characterized as Bakuchiol (50 mg). while the fraction eluted with hexane and ethyl acetate (95:05 v/v) at 1 hour 27 min consisted of white needle-shaped crystalline powder (68 gm) which was characterized as psoralen (>98%) by comparing with reference standard using TLC and HPLC, shown in Figure 5.

The isolated psoralen solution 100 µg/ml were scanned on UV spectrophotometer so as to observe the absorption maxima. The UV spectrum exhibited absorption maxima at λmax 245 nm and 300 nm, consistent with the psoralen chromophore, shown in Figure 6.Further, structural identification and characterization of purified psoralen carried out by FTIR, ^{1}H NMR and ^{13}C NMR.

Identification and Characterization of isolated compound using FTIR

Fourier Transform Infrared Spectroscopy (FTIR) serves as a fingerprint technique, helps characterize the functional groups present in isolated compound, depicted absorption bands in Figure 7. The unique pattern of peaks allow identification and confirm presence of psoralen (Rajput *et al*., 2008). FTIR analysis is important technique for understanding the chemical properties and potential medicinal uses of psoralen.

Aromatic C-H Stretch: Peaks at approximately 3155 and 3060 cm^{-1} indicate the presence of aromatic C-H bonds. C=O Stretch (Lactone): A strong peak around 1712 cm^{-1} signifies the carbonyl (C=O) group in unsaturated lactone ring. C=C Ring Stretch: Absorption at approximately 1634 cm^{-1} is attributed to the C=C bonds within the fused rings. C-O Stretch: Absorption around 1131 cm^{-1} is characteristic of C-O stretching vibrations. C-O-C Ether Group: The peak at about 1092 cm^{-1} corresponds to the C-O-C ether linkage. Out-of-plane C-H Bend: A peak at approximately 896, 823 and 717 cm^{-1} is associated with the bending of the C-H bonds. The above data clearly indicates that the isolated

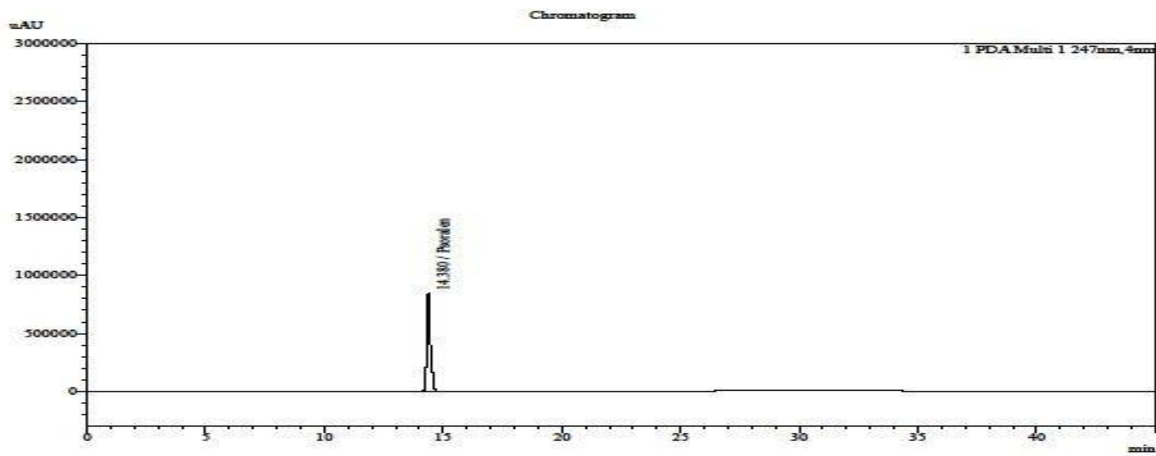

Figure 5: Hplc chromatogram of flash isolated psoralen.

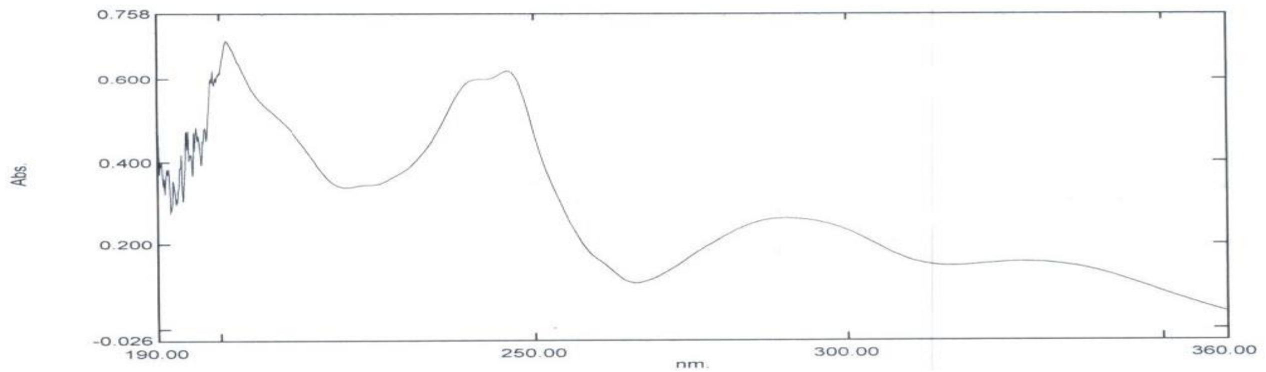

Figure 6: UV spectrum of psoralen.

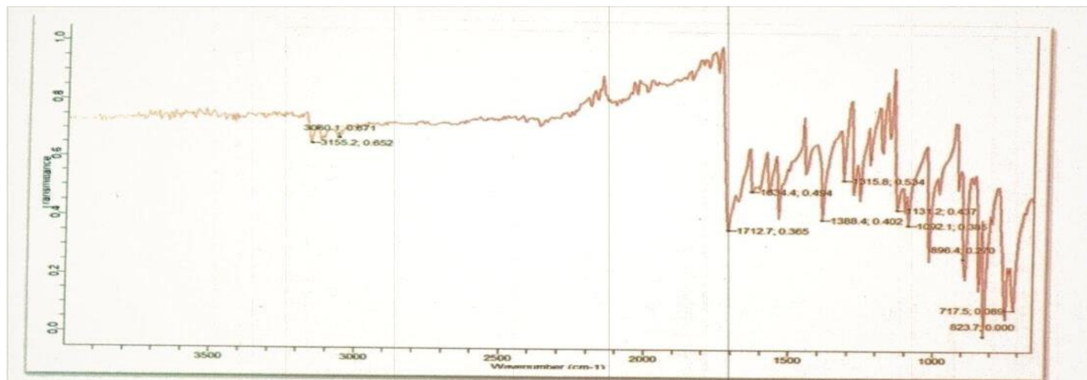

Figure 7: FTIR of isolated compound (psoralen).

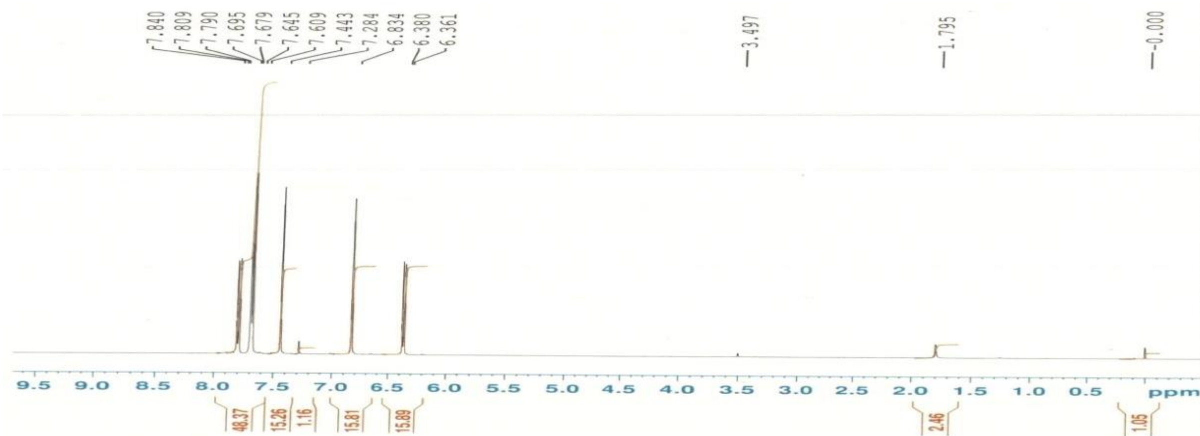

Figure 8: ^{1}H NMR of isolated compound (psoralen).

compound is psoralen as it contains carbonyl groups and lactone bonding arrangements in a ring fused to a coumarin.

Identification and characterization of isolated compound by ^{1}H NMR and ^{13}C NMR: The flash isolated white needles crystal with MeOH; mp 162-166°C was characterized by NMR spectroscopy. The ^{1}H NMR (CDCl3, 400 MHz) spectrum (Figure 8) showed characteristic signals (500 MHz, CDCl3) ä, 6.36 (^{1}H, d, J= 9.6 Hz, H- 3), 6.81 (^{1}H, d, J = 2.4 Hz, H-11), 7.46 (^{1}H, s,H-8), 7.66 (^{1}H, s, H-5), 7.67 (^{1}H, d, J = 2.4 Hz, H-12), 7.79 (^{1}H, d, J = 9.6 Hz, H-4); EIMS m/z (relative intensity) 186 (100, [M]+), 158, 130, 102.

The psoralen ^{13} C-NMR, (100.5MHz, CDCl3) spectrum exhibited characteristic signals at ä (ppm):161.0 (2-C),114.6 (3-C),144.0 (4-C),119.8 (5-C),124.8 (6-C),156.4 (7-C),99.8 (8-C),152.0 (9-C), 115.4 (10-C),146.9 (2'-C),106.3 (3'-C), confirming the psoralen structure.

Conclusion

The optimized flash chromatography was efficiently used for the preparative isolation and purification of marker compound, psoralen from *Psoralea corylifolia* Linn. seeds. High-purity (>98%) psoralen was obtained from crude extract in one step separation that can be used for further scientific studies. The separation and yields of pure psoralen obtained by this method are reproducible. Further, identified and comprehensive characterized as psoralen via FTIR and NMR spectral data was done. The separation and yields of pure psoralen obtained by this method are reproducible. This streamlined flash chromatography approach enable isolation of biomolecule psoralen which have remarkable medicinal benefits include anti-inflammatory, antimicrobial and anticancer properties. Therefore, invariably on high demand in pharmaceutical, cosmetic, herbal and other related industries.

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