



STUDIES ON THE COMPOSITION AND STANDARDIZATION OF “BAKUCHI OIL”, AN AYURVEDIC MEDICINAL OIL PREPARED FROM *PSORALEA CORYLIFOLIA* L. USED IN THE TREATMENT OF VITILIGO

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ABSTRACT

“Bakuchi oil” is Ayurvedic medicinal oil prepared from the dried fruits of *Psoralea corylifolia* L. (Fabaceae) and Sesame oil is used widely in the treatment of vitiligo in Sri Lanka. The standardization of the oil is important for its safe and effective clinical use. A method was developed to quantitatively extract psoralen, the major photoactive compound in the oil and quantify it by TLC fluorodensitometry. The precision of the method was 4.3% CV (n = 6). A TLC fingerprint of the oil highlighting six fluorescent secondary metabolites of *Psoralea corylifolia* (psoralen, isopsoralen, corylin, psoralidin, dehydroisopsoralidin and isobavachalcone) was also developed. Of these dehydroisopsoralidin is being reported for the first time from a natural source. Analysis of the oil indicates that Bakuchiol, a major secondary metabolite of *Psoralea corylifolia* is not incorporated into the medicinal oil.

Key words: *Psoralea corylifolia*, Bakuchi oil, Psoralen, Standardization, Dehydroisopsoralidin.

INTRODUCTION

Vitiligo is a disfiguring disease which is characterized by the appearance of white patches on the skin. “Bakuchi oil” is an Ayurvedic medicinal oil prepared from the dried fruits of *Psoralea corylifolia* L. (Fabaceae) and Sesame oil, used widely in the treatment of vitiligo in Sri Lanka. The treatment consists of applying the oil to the affected areas of the skin and exposing them to sunlight. The major secondary metabolite found in *Psoralea corylifolia* is the furanocoumarin, psoralen. Psoralen stimulates repigmentation by sensitizing the skin to ultra violet light. In modern medicine, 8-methoxypsoralen (methoxalen), is used in combination with ultra violet A light (PUVA) for the treatment of vitiligo and psoriasis. However, the control of phototoxicity¹ is a major concern, and the amount of the methoxalen applied and the dose of UV irradiation used needs to be carefully controlled. Thus standardization of the psoralen content of “Bakuchi oil” is important for its safe and effective use.

According to Ayurveda, the complex mixture of compounds found in an herbal drug is more effective and less toxic than pure compounds isolated from these drugs. The presence of coumarins, coumestans, flavonoids, chalcones and terpenoids in the fruit of *Psoralea corylifolia* have been reported²⁻¹⁰. Thus “Bakuchi oil” may be expected to also incorporate secondary metabolites of *Psoralea corylifolia* other than psoralen. In this study, some of the major secondary metabolites incorporated in “Bakuchi oil” were identified and a chromatographic finger print was developed for the standardization of the oil. A TLC-FD densitometric method for the quantification of psoralen was also developed.

MATERIALS AND METHODS

Plant materials

Dried fruits of *Psoralea corylifolia* were purchased from a local drug store and their authenticity was confirmed by comparing them with the herbarium specimen (No. 248) at the Bandaranaike Memorial Ayurveda Research Institute, Navinna, Sri Lanka.

Bakuchi oil

A sample of Bakuchi oil (BMARI-1) prepared at the Bandaranaike Memorial Ayurveda Research Institute was used as the reference sample.

General procedures

¹H NMR and ¹³C NMR spectra were measured at 600 MHz and 150 MHz respectively on a Bruker avance 600 spectrophotometer or at 300 MHz and 75 MHz respectively on a Varian 300 spectrophotometer in *d*₆-DMSO or CDCl₃ with or without tetramethylsilane as an internal standard. Chemical shifts are given in δ (ppm). IR spectra were recorded on a Thermo Nicolet AVATAR 320 FT-IR spectrometer (equipped with Ez-omic software). Ultra Violet spectra were recorded on a Thermo Spectronic HeλIOS α double beam spectrophotometer. Electron impact (EI) mass spectra were determined on a Varian Saturn 2000 mass spectrophotometer coupled with Varian 3800 GC. Electrospray Ionization Mass Spectra (ESIMS) using both flow injection analysis and liquid chromatography-diode array-mass spectrometry (HPLC-DAD-MS) were determined on an Agilent 1100 instrument with a quadrupole mass detector. GC was carried out on Agilent 689 N GC system using a DB wax column (30 m, 0.25 mm, 0.5μm) and Flame Ionization Detector. Densitometric studies were carried out on a Desaga CD 60 HPTLC densitometer using Merck silica gel 60 precoated plates. Melting points were recorded on a

Reichert Thermover hot stage melting block apparatus. All melting points recorded are uncorrected. Precoated (Merck) analytical plates with and without fluorescent indicator (silica gel 60 F₂₅₄ and silica gel 60) were used for thin layer chromatography (TLC). Preparative plates were prepared in the laboratory using Merck silica gel PF₂₅₄. Layer thickness of analytical and preparative plates was 0.25 mm and 0.5 mm respectively. Gravity column chromatography was carried out using silica (70-230 mesh) and flash column chromatography was carried out with silica gel 60 (Fluka 60738). All solvents used were either analytical grade or were general purpose reagent grade purified in the laboratory by fractional distillation.

Isolation and chemical characterization of compounds from the fruits of *Psoralea corylifolia*

Psoralen (I) and isopsoralen (II)

Powdered fruits (225.0 g) were extracted with hexane in a Soxhlet apparatus for 12 hours, and the extract was concentrated under vacuum to ~ 25 ml and kept 5-10 °C. The precipitated psoralen/isopsoralen mixture was then separated by suction filtration and the precipitate was washed with a little hexane to remove adhering coloured impurities to obtain a mixture of the two isomers as a creamy white amorphous mass (1.51 g, 0.67 %). The mixture of the two isomers (0.893 g) was chromatographed over a silica gel column, using a gradient of ethyl acetate in hexane. Isopsoralen started eluting at 5 % ethyl acetate in hexane, followed by psoralen. The elution of psoralen was completed when the concentration of ethyl acetate was 8 %. Isopsoralen (364 mg) and psoralen (402 mg) were obtained as solids by evaporation of the solvent from the combined fractions containing each compound. The compounds were purified by recrystallization from hexane (isopsoralen) or hexane/ethanol (psoralen). The purity of the compounds were checked by TLC, GC and determination of melting points.

Psoralen (I) White needles, m.p. 161-163 °C (lit.² 162-163 °C). UV λ_{\max}/nm (CHCl₃) 250, 290, 333. IR ν_{\max} (KBr) 1722 cm⁻¹. EIMS m/z 186[M⁺], 158, 130, 102, 63. ¹H NMR (CDCl₃, 300 MHz) δ 6.37 (1H, d, *J* = 9.3 Hz, H-3), 6.83 (1H, dd, *J* = 2.3 Hz, 1.0 Hz, H-3'), 7.45 (1H, m, H-8), 7.68 (1H, bs, H-5), 7.69 (1H, d, *J* = 2.3 Hz, H-2'), 7.78 (1H, d of m, *J* = 9.3 Hz, H-4). ¹³C NMR (CDCl₃, 75 Mz) δ 160.99 (C-2), 156.45 (C-7), 152.06 (C-9), 146.92 (C-2'), 144.06 (C-4), 124.89(C-6), 119.86 (C-5), 115.43 (C-10), 114.66 (C-3), 106.42 (C-3'), 99.84 (C-8).

Isopsoralen (II) White needles, m.p. 140-143 °C (lit.⁸ 143-144 °C). UV λ_{\max}/nm (CHCl₃) 250, 299, IR ν_{\max} (KBr) 1709 cm⁻¹, EIMS m/z 186[M⁺], 158, 130, 102, 49. ¹H NMR (CDCl₃, 300 MHz) δ 6.39 (1H, d, *J* = 9.6 Hz, H-3), 7.13 (1H, dd, *J* = 2.3, 1.0 Hz, H-3'), 7.37 (1H, d, *J* = 8.4 Hz, H-5), 7.43 (1H, dd, *J* = 8.4 Hz, 1.0 Hz, H-6), 7.69 (1H, d, *J* = 2.3 Hz, H-2'), 7.80 (1H, d, *J* = 9.6 Hz, H-4), ¹³C NMR (CDCl₃, 75 MHz) δ : 160.80 (C-2), 157.39 (C-7), 148.50 (C-9), 145.89 (C-2'), 144.49 (C-4), 123.83 (C-5), 116.94 (C-8), 114.17 (C-3), 113.53 (C-10), 108.80 (C-6), 104.12 (C-3').

Bakuchiol (VI)

Powdered fruits (225.0 g) were extracted with hexane in a Soxhlet apparatus and the solvent was evaporated under

reduced pressure to yield a semisolid (21.15 g). The semi solid (11.5 g) was repeatedly stirred with 80 % MeOH (10 x 75 ml) for 12 hours. The 80% MeOH soluble fraction of the hexane extract was partitioned with hexane. Evaporation of the solvent from the hexane fraction under reduced pressure resulted in a sticky residue (2.05 g). The residue (0.630 g) was subjected to column chromatography over silica gel. Elution was carried out with a gradient of ethyl acetate in hexane. The fraction eluting with 4% ethyl acetate in hexane yielded bakuchiol with a red pigment as an impurity. The red pigment was removed by subjecting the sample to preparative TLC (toluene: ethyl acetate: acetic acid, 10: 1: 0.1, triple development), and pure bakuchiol (39 mg) which appeared as a dark blue spot under excitation at 254 nm on TLC, was obtained as a colourless oily compound.

Bakuchiol Colourless oil, ESIMS m/z 255[M-H]⁻, 257[M+H]⁺. ¹H NMR (*d*₆-DMSO, 600 MHz) δ 1.20(3H, s, H-16), 1.50 (2H, m, H-10), 1.59 (3H, s, H-15), 1.69 (3H, s, H-14), 1.96 (2H, m, 7.8, H-11), 5.02 (1H, d, *J*=17.4 Hz, H-18a), 5.05 (1H, d, *J*=10.5 Hz, H-18b), 5.11 (1H, t, *J*=7.2 Hz, H- 12), 5.88 (1H, dd, *J*=17.4 Hz, 10.5 Hz, H-17), 6.07 (1H, d, *J*=16.2 Hz, H-8), 6.26 (1H, d, *J*=16.2 Hz, H-7), 6.78 (2H, d, *J*=8.4 Hz, H-3,5), 7.26 (2H, d, *J* = 8.4 Hz, H-2,6). ¹³C NMR(*d*₆-DMSO, 150 MHz) δ 154.58 (C-4), 145.93 (C-17), 135.85 (C-8), 131.30 (C-13), 130.89 (C-1), 127.35 (C-2,6), 126.44 (C-7), 124.78 (C-12), 115.34 (C-3,5), 111.86 (C-18), 42.50 (C-9), 41.27 (C-10), 25.69 (C-14), 23.33 (C-16), 23.21 (C-11), 17.63 (C-15).

Corylin (III), dehydroisopsoralidin (VII), psoralidin (V) and isobavachalcone (IV)

Dried whole fruits (150 g) were ground to a powder and extracted successively with hexane and methanol in a soxhlet apparatus. Evaporation of the solvent under reduced pressure from the methanol extract yielded a semisolid (30 g) of which a sample of 10 g was subjected to silica gel column chromatography using a gradient of ethyl acetate in hexane. Thirty five fractions were collected and subjected to further treatment based on their TLC analysis. Flash chromatography of the fraction eluting with 10% ethyl acetate in hexane yielded dehydroisopsoralidin (VII) (4 mg) as a yellow crystalline solid. The fraction eluting with 15 % ethyl acetate in hexane yielded the known compound corylin (III) as a white crystalline solid (21 mg). Addition of dichloromethane to the fraction eluting with 20 % ethyl acetate in hexane resulted in the deposition of psoralidin (V) as a white powder (14 mg). Preparative TLC of the supernatant yielded isobavachalcone (IV) as yellow needles (8 mg).

Dehydroisopsoralidin (VII) Pale yellowish crystalline solid, m. p. 290 °C (dec.) (lit.¹¹ 292-294 °C). ESIMS m/z 333[M-H]⁻, 335[M+H]⁺. ¹H NMR (*d*₆-DMSO, 600 MHz) δ 1.45 (6H, s), 5.91 (1H, d, *J* = 9.9 Hz), 6.63 (1H, d, *J* = 9.9 Hz), 6.96 (1H, dd, *J* = 8.4 & 2.0 Hz), 6.97 (1H, s), 7.17 (1H, d, *J* = 2.0 Hz), 7.71 (1H, d, *J* = 8.4 Hz), 7.77 (1H, s), 10.2 (1H, bs). ¹³C NMR (*d*₆-DMSO, 150 MHz) δ 157.2 , 102.9 , 159.0, 118.5, 118.7, 155.9, 104.2 , 153.9 , 105.6 , 114.5, 156.0, 98.6, 157.3, 114.1 , 120.4, 120.8 , 131.8, 77.8, 27.9. See table 1. for assignments.

Corylin (III) White crystalline solid, m. p. 242-243° C (lit.⁹ 238-239 °C). IR ν_{\max} (KBr) 1628 cm^{-1} . ESIMS m/z 319[M-H]⁻, 321[M+H]⁺. ¹H NMR (d_6 -DMSO, 600 MHz) δ 1.39 (6H, s, H-3''Me), 5.78 (1H, d, J = 9.0 Hz, H-2''), 6.43 (1H, d, J = 9.0 Hz, H-1''), 6.78 (1H, d, J = 8.1 Hz, H-5'), 6.86 (1H, d, J = 2.2 Hz, H-8), 6.93 (1H, dd, J = 8.7 & 2.2 Hz, H-6), 7.28 (1H, d, J = 2.0 Hz, H-2'), 7.29 (1H, dd, J = 8.1 & 2.0 Hz, H-6'), 7.96 (1H, d, J = 8.7 Hz, H-5), 8.34 (1H, s, H-2). ¹³C NMR(d_6 -DMSO, 600 MHz) δ 174.5 (C-4), 162.7 (C-7), 157.44 (C-9), 153.1 (C-4'), 152.1 (C-2), 131.2 (C-2''), 129.6 (C-6'), 127.2 (C-5), 126.9 (C-2'), 124.4(C-1'), 123.09 (C-3), 121.7 (C-1''), 120.5 (C-3'), 116.4 (C-10), 115.5 (C-5'), 115.2 (C-6), 102.1 (C-8), 76.24 (C-3''), 27.7 (C-3''-Me).

Psoralidin (V) White crystalline solid, IR ν_{\max} (KBr) 1720 cm^{-1} . ESIMS m/z 335[M-H]⁻, 337[M+H]⁺. ¹H NMR (d_6 -DMSO, 600 MHz) δ 1.71 (3H, s, C-3''-Me), 1.74 (3H, s, C-3''-Me), 3.32 (2H, d, J = 7.2 Hz, H-1''), 5.35 (1H, t, J = 7.2 Hz, H-2''), 6.92 (1H, s, H-8), 6.93 (1H, dd, J = 8.4 Hz & 2.0 Hz, H-5'), 7.16 (1H, d, J = 2.0 Hz, H-3'), 7.62 (1H, s, H-5), 7.68 (1H, d, J = 8.4 Hz, H-6'), 10.09 (1H, s, O-H), 10.75 (1H, s, O-H).

Isobavachalcone (IV) Bright yellow needles, m. p. 153-154 °C (lit.¹⁰ 154-156° C). IR ν_{\max} (KBr) 1630 cm^{-1} . ESIMS m/z 323[M-H]⁻, 325[M+H]⁺. ¹H NMR (d_6 -DMSO, 600 MHz) δ 1.62 (3H, s, H-4''), 1.72 (3H, s, H-5''), 3.22 (2H, d, J = 7.2 Hz, H-1''), 5.17 (1H, t, J = 7.2 Hz, H-2''), 6.46 (1H, d, J = 8.9 Hz, H-5), 6.835 (2H, d, J = 8.6 Hz, H-5', 3'), 7.718 (1H, d, J = 15.6 Hz, H-b), 7.77 (1H, d, J = 15.6 Hz, H-c), 7.749 (2H, d, J = 8.6 Hz, H-6', 2'), 8.03 (1H, d, J = 8.9 Hz).

TLC analysis of "Bakuchi oil"

"Bakuchi oil" (30 ml) was extracted with methanol (8 x 60 ml), and the solvent was evaporated under vacuum to obtain the methanol extract of the oil (4.21 g). The methanol extract (3.8 g) was partitioned between 80% methanol and hexane. The 80% methanol soluble fraction was separated out and the solvent was evaporated under reduced pressure. This extract was analyzed for the presence / absence of compounds isolated from *Psoralea corylifolia* by TLC with triple development using toluene: ethyl acetate: hexane, 1: 3: 9 as the solvent. The plates were observed at 254 nm without spraying and at 366 nm after spraying with 10% methanolic potassium hydroxide.

Determination of the concentration of psoralen in "Bakuchi oil"

Standard curve

Pure psoralen isolated from the fruits of *Psoralea corylifolia* was used to prepare the standard solutions. Stock solution was prepared by dissolving 5.00 mg of psoralen in 25.00 ml of acetone. Standard solutions containing 40, 80, 120, 160 and 200 ng/ μ l were prepared by diluting the stock solution. From each solution 5.00 μ l was applied on a pre-coated TLC plate (MERCK, silica gel 60 G) in duplicate with a Hamilton micro syringe. The plate was developed in hexane: ethyl acetate, 7 : 1 (solvent front 8.0 cm) after saturating the chamber for two hours. The plate was dried with a stream of hot air and psoralen was observed at R_f = 0.62 as a blue fluorescent spot under excitation at 366 nm. Densitometric scanning

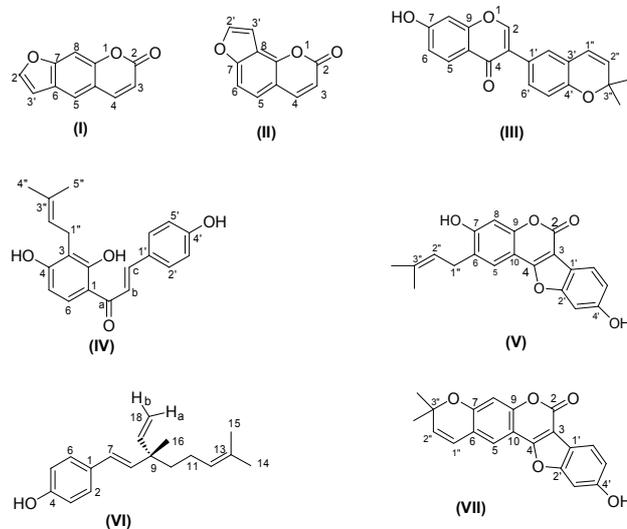
was carried out in the fluorescence mode with excitation at 240 nm and a filter of 370 nm.

Sample preparation

The reference sample of "Bakuchi oil" (4.00 ml) was dissolved and mixed well with 50 ml of acetone and 30 ml of methanol was added. The mixture was cooled at -10° - 15° C for three days for precipitation of fats. The supernatant was decanted and the precipitate was washed with cold methanol. The washings and the supernatants were combined and the solvent was evaporated completely under vacuum. The residue was dissolved in acetone and made up to 5.00 ml and 5.00 μ l of the solution was applied on the TLC plate in duplicate along with a standard solution of psoralen. The plates were developed and scanned as described for the standard curve. The psoralen concentration was determined using the standard curve.

RESULTS AND DISCUSSION

Seven compounds were isolated from the dried fruit of *Psoralea corylifolia*. Of these, psoralen (I), isopsoralen (II), corylin (III), isobavachalcone (IV), psoralidin (V) and bakuchiol (VI) were known compounds and their identities were established by analysis and comparison of their spectra with those reported in the literature.



Dehydroisopsoralidin (VII) is a new natural product whose structure was determined from the analysis of its Mass and ¹H NMR, ¹³C NMR, HSQC and HMBC spectra. Thus the mass spectrum of the compound gave pseudo molecular ions at m/z 335[M+H]⁺ and m/z 333[M-H]⁻ corresponding to a MW of 334 amu. Initial comparisons of the UV, mass and ¹H NMR data with those of the compounds reported from *Psoralea* sp. suggested the compound to be a coumestan. The ¹H NMR spectrum showed signals for 14 protons including a phenolic OH, while the ¹³C NMR gave signals for 20 carbons. Based on DEPT and HSQC NMR experiments these were assigned for two methyl carbons and 7 methine carbons with the rest being accounted for quaternary carbons. A molecular formula of C₂₀H₁₄O_x was evident from these interpretations.

The singlet at δ 1.45 (6H) in the ^1H NMR indicated the presence of a *gem*-dimethyl group. Further, the downfield proton signals observed at δ 6.97 (δ_{C} 104.2) and 7.77 (δ_{C} 118.5) for a 1,2,4,5 tetra substituted benzene ring and a *meta* coupled doublet at δ 7.17 ($J = 2.0$ Hz), a *ortho* coupled doublet at δ 7.71 ($J = 8.4$ Hz) and a double doublet at 6.96 ($J = 8.4$ & 2.0 Hz) for a 1,2,4-tri substituted benzene ring were all in agreement with a coumestan class of compound. The HMBC correlations from both CH_3 groups at C-3'' to C-2'' and C-3'' confirmed the position of the two methyl groups at C-3'' while the downfield shift of C-3'' (δ_{C} 77.8) favored the pyran oxygen attachment. Further J_2 and J_3 HMBC correlations (Figure 1) from H-1'' and H-2'' protons to C-7 and C-6 confirmed the pyran ring system while the deshielded NMR signals for C-1'' (δ_{C} 120.7) and C-2'' (δ_{C} 131.8) olefinic positions in comparison to the already known coumestan, corylidin [13] indicated this to be a dihydropyran system. HMBC correlations from H-5 to C-7, H-8 to C-6 and H-1'' to C-7, H-2'' to C-6 confirmed the connectivity of the tetra-substituted aromatic ring attachment to the dihydropyran system. Strong correlations from H-5 to deshielded carbons C-9 (δ_{C} 153.9) and C-4 (δ_{C} 159.0) indicated they were adjacent to two oxygen atoms, which accounted for the ring oxygens of the coumarin fragment and the furan in the system. A downfield proton signal at δ 10.2 accounting for a phenolic OH was placed at C-4', based on 3-bond correlations from both H-5' (dd) and H-3' (d) to C-1'. The downfield shift of C-2' (δ_{C} 156.0) and the aromatic carbon shift of C-1' (δ_{C} 114.5) were found to be characteristic for a furan / pyran substituted aromatic system.

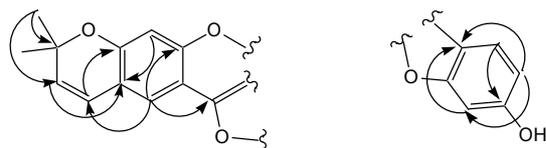


Figure 1: Some key HMBC correlations of (VII) for the coumestan fragments

From these interpretations it was possible to account for a fragment of $\text{C}_{18}\text{H}_{14}\text{O}_4$. Since an HMBC correlation was not observed from H-6' to C-3, but given that the molecular formula accounts for two more quaternary carbons, one of which has to be a carbonyl as supported by both the coumestan structure and chemical shift evidence (δ_{C} 157.2) the unaccounted carbons were assigned as C-2 and C-3. ^1H NMR and ^{13}C NMR assignments for dehydroisopsoralidin are given in Table 1.

Table 1: NMR data for dehydroisopsoralidin (VII)

Carbon	$^{13}\text{C}/\delta$	$^1\text{H}/\delta$	gHMBC
2	157.2 ^a		
3	102.9		
4	159.0		
5	118.5	7.77(s)	C-1'', C-7, C-9, C-4
6	118.7		
7	155.9		
8	104.2	6.97(s)	C-6, C-7, C-9, C-10
9	153.9		
10	105.6		
1'	114.5		
2'	156.0 ^b		
3'	98.6	7.17(d, $J = 2.0$)	C-1', C-5', C-4', C-2'
4'	157.3 ^{ab}		
5'	114.1	6.96 (dd, $J = 8.4$ & 2.0)	C-1', C-3'
6'	120.4	7.71 (d, $J = 8.4$)	C-4', C-2'
1''	120.8	6.63 (d, $J = 9.9$)	C-3'', C-7, C-5
2''	131.8	5.91(d, $J = 9.9$)	C-3'', C-1'', C-6
3''	77.8		
3''-Me	27.9	1.45(s)	C-3''-Me, C-3'', C-2''

^aChemical shift values can be interchanged between these two carbons.

^bChemical shift values can be interchanged between these two carbons

On the basis of the above spectroscopic evidence this compound was established as dehydroisopsoralidin (VII). Dehydroisopsoralidin has been synthesized from psoralidin (V) and was first reported¹¹ in 1977. The compound is being reported here for the first time as a natural product and its complete NMR assignments are also being reported for the first time. Some of the ^1H NMR assignments in the earlier report need to be corrected according to those given in table 1, which are based on an analysis of the 2D NMR data for the compound.

“Bakuchi oil” was analyzed for the presence or absence of these seven compounds by TLC on silica gel. All the compounds except bakuchiol could be identified as being present in the oil. The direct methanol extract of the oil was not suitable for tlc, due to dissolved fatty matter. The 80% aqueous methanol soluble fraction of the methanol extract obtained by partitioning the methanol extract between 80% aqueous methanol and hexane, however gave a clean TLC profile with good separation of compounds when developed three times in toluene: ethyl acetate: hexane, 1: 3: 9, (Figure 2). This profile is a useful finger print of “Bakuchi oil

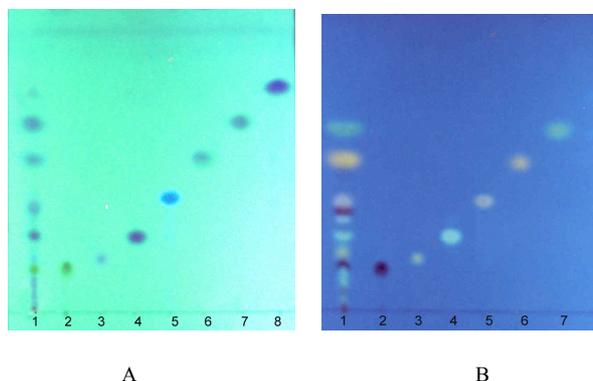


Figure 2: TLC finger print of "Bakuchi oil"

Track 1: 80% methanol soluble fraction of the methanol extract, 2: (IV), 3: (V), 4: (III), 5: (VII), 6: (I), 7: (II), 8: (VI).

A: detection under 366 nm after spraying with methanolic KOH.

B: detection under 254 nm.

The non detection of bakuchiol (VI) in 'Bakuchi oil' was surprising as it is a major component of the fruit. The faint spot observed in the finger print at the R_f value for bakuchiol (track 1 Figure 2. B) has been shown by control experiments to arise from sesame oil. The oil sample which was left after extraction with methanol was saponified and analysed by TLC. It did not show the presence of bakuchiol. TLC analysis of the hexane fraction of the methanol extract also did not show the presence of bakuchiol. These results indicate that bakuchiol is not incorporated into "Bakuchi oil". The absence of bakuchiol in "Bakuchi oil" was confirmed by gas chromatographic analysis of the steam distillate of the oil. A possible explanation of this observation is that bakuchiol in the fruit is lost by steam distillation during the process of manufacture.

Chromatographic finger prints combined with quantification of selected active compounds provide the best method to standardize complex herbal drugs as it gives information on both the identity and potency of the drug. The major photoactive compound in *Psoralea corylifolia* is psoralen, and a thin layer chromatography densitometric method with fluorescence detection (TLC-FD) was developed to quantify psoralen in "Bakuchi oil". The TLC profile used to fingerprint the oil is not suitable for quantitative estimation of psoralen as a certain amount of the compound is lost by dissolution in hexane during the partitioning process. A method was developed to quantitatively extract psoralen from "Bakuchi oil" based on adjusting the polarity and hydrogen bonding capacity of a solvent mixture to permit freezing out of the fatty material leaving the other components in solution. Thus, adding 30 ml of methanol to 4.00 ml of "Bakuchi oil" dissolved in 50 ml of acetone, and cooling the mixture at -10^0 to -15^0 C, for three days resulted in the near total precipitation of fats leaving behind psoralen in solution. Examination of the precipitate by TLC indicated that it did not contain psoralen in detectable amounts. Thus, the extract of psoralen so obtained was used for densitometric studies. Although the chromatographic behavior of psoralen and isopsoralen are similar, they could be well separated from each other on silica TLC, using hexane : ethyl acetate (7:1) as the developing solvent. Specificity and sensitivity in quantifying psoralen on the TLC plate was achieved by using the fluorescence mode with excitation at 240 nm and a filter at 370 nm.

The standard curve was drawn using purified psoralen isolated from the fruit. It was linear ($Y = 7.2844X + 1933.3040$) with a correlation coefficient of 0.9931 over the range 200 ng/spot to 1000 ng/spot. The psoralen concentration of the reference sample of "Bakuchi oil" was determined to be 0.083 mg/ml. The precision of the method was determined by six replicate analyses of the same sample of oil, and the coefficient of variation was found to be 4.3%. A sample of oil with an approximately

30 % addition of psoralen gave a recovery of 103%. Thus the method developed is of acceptable precision and accuracy for the purpose of quality control and standardization of "Bakuchi oil" with respect to its psoralen content. The psoralen content of a different batch of "Bakuchi oil" prepared at the BMARI (BMARI II) and three other samples of "Bakuchi oil" from different manufacturers was analyzed using the standard curve, and the results are given in table 2.

These results show that there is a wide range of concentrations (0.038 mg/ml to 0.226 mg/ml) of psoralen in the oils tested. Furthermore, the widely different results obtained for the two BMARI oil samples (BMARI-I and BMARI-II) prepared using the same process, indicates the need for better process control in the preparation of the oil. The level of psoralen found in "Bakuchi oil" is less than that found in the topical applications used in modern medicine (0.2 – 1%). Synergistic interactions amongst the different photoactive compounds in "Bakuchi oil" may be important for its activity.

Table 2: Psoralen concentration in different samples of "Bakuchi oil"

"Bakuchi oil" sample	Psoralen concentration (mg/ml)
BMARI - I (reference)	0.083
BMARI - II	0.226
Ayurvedic Drugs Corporation	0.087
Ayurvedic hospital – Kurunegala	0.038
Ayurvedic hospital - Beliatta	0.103

REFERENCES

1. Averbek D. Recent advances in psoralen phototoxicity mechanisms. Photochem. Photobiol. 1989; 50(6): 859-882.
2. Jiangning G, Xinchu W, Hou W, Quinhua L, Kaishun B. Antioxidants from Chinese medicinal herb – *Psoralea corylifolia* L. Food Chem., 2005;91: 287-292.
3. Gupta, S. Jha BN, Gupta GK, Gupta BK, Dhar KL. Coumestans from the seeds of *Psoralea corylifolia*. Phytochem., 1990; 29(7): 2371-2373.
4. Wang D, Famei L, Jiang Z. Osteoblastic proliferation stimulating activity of *Psoralea corylifolia* extracts and two of its flavonoids. Planta Med., 2001; 67(8): 748-749.
5. Yin S, Fan CQ, Wang Y, Dong L, Yue JM. Antibacterial prenylflavone derivatives from *Psoralea corylifolia*, and their structure activity relationship study. Bioorg. Med. Chem., 2004; 12(16): 4387-4392.
6. Sun JN, Woo SH, Cassady JM, Snapka RM. DNA polymerase and topoisomerase II inhibitors from *Psoralea corylifolia*. J.Nat.Prod., 1998; 61: 362-366.
7. Gupta GK, Dhar KL, Atal CK. Corylin : A new isoflavone from seeds of *Psoralea corylifolia*. Phytochem. 1978;17(1): 164.
8. Bajwa BS, Khanna PL, Seshadri TR. Components of different parts of seeds (fruits) of *Psoralea corylifolia*. Ind. J. Chem., 1974; 12 (1): 15-19.
9. Jain AC, Gupta GK, Rao PR. Isolation and constitution of corylin: A new isoflavone from the fruits of *Psoralea corylifolia*. Ind. J. Chem., 1974; 12: 659-660.
10. Bhalla VK, Nayak UR, Sukh Dev. Some new flavonoids from *Psoralea corylifolia*. Tet. Lett. 1968;9(20): 2401-2406.
11. Gupta BK, Dhar KL, Atal CK. Cyclodehydrogenation of psoralidin with dichlorodicyanobenzoquinone. Ind. J. Chem., 1977; 15B: 657-658.