

Chemical Examination and Biological Studies on the Seeds of *Psoralea Corylifolia* Linn.

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Abstract

The aim of the present investigation was to isolate chemical constituents and study of its biological activity from the seeds of acetone extract of *Psoralea corylifolia* Linn. The bio-assay guided isolation of acetone extract of seeds yielded five known compounds, γ -cadinene (1), bakuchiol (2), psoralen (3), isopsoralen (4) and psoralidin (5). The structures of these compounds were elucidated by physical and spectral data (UV, IR, ¹H, ¹³C NMR and mass). The compound, γ -cadinene (1) is first report from this plant. Different extracts, fractions and compounds from the seeds were investigated for antimicrobial property. The methanolic, acetone and hexane extracts and isolated compound, bakuchiol (2) of *Psoralea corylifolia* were tested for antimicrobial studies against three gram positive bacteria and showed positive results. The compound bakuchiol (2) showed an excellent antibacterial activity than its crude extract.

Key words: *Psoralea corylifolia*, bakuchiol, psoralidin, antibacterial studies, tetracycline.

Introduction

Plants are being used as source of medicine since ages. Many medicinal plants are nature's gift to human beings to make disease free healthy life. More than 80% of the world population are in poor and less developed countries depends on traditional plant based medicines for their primary health care needs. India is one of the diverse countries in the world where the medicinal plant sector is a part of time-honored tradition that is respected even today¹. India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk medicines or indirectly as pharmaceutical preparation of modern medicine². Over the past five decades focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional system. Medicinal plants are major source and biodynamic compounds of therapeutic value³. Interestingly, *Psoralea corylifolia* is an endangered plant and medicinally important plant and found in tropical and subtropical region of the world. Its medicinal usage is reported in Indian pharmaceutical codex, Chinese, British and the American pharmacopoeias and in different traditional system of medicines such as Ayurveda, Unani and Siddha¹.

Psoralea corylifolia Linn., is belongs to Fabaceae family. It is an erect annual, 30-180 cm high, found almost throughout India. It is reported to be grown on some extent in Rajasthan and the eastern districts of Punjab, adjoining Uttar Pradesh

for its seeds. The fruits of *P. corylifolia* consist of a sticky oily pericarp, a hard seed coat and kernel. The seeds are used in indigenous medicine as laxative, aphrodisiac, anthelmintic, diuretic and diaphoretic in febrile conditions. The seeds have been specially recommended in the treatment for leucoderma, leprosy, psoriasis and inflammatory diseases of the skin⁴. The seed extracts inhibits the growth of *Staphylococcus citreus*, *S. aureus* and *S. albus* including strains resistant to penicillin's. The seeds posses anthelmintic activity against earth worms, psoralen being the active principle. The essential oil shows a selective activity against the skin *Streptococci* and used in the treatment of skin affections. The seeds are used locally in the preparation of certain types of medicated oils and incense preparations. The root is useful in the caries of teeth⁴. Previous studies reported the presence of several new and known compounds like, furanocoumarins⁵, prenyl flavonoids⁶, aromatic terpenoids and chromenes⁷.

In our continuous interest on the isolation and characterization of bioactive compounds from the indigenous medicinal plants for personal care applications⁸⁻¹⁸, we have undertaken chemical examination of the seeds of *Psoralea corylifolia* Linn. In this article we report the isolation and characterization of five known compounds. The crude extracts, its fractions and isolated compound were analyzed for antimicrobial activity. The crude extracts of acetone and methanol showed less activity than hexane fraction and compound 2 showed potent antimicrobial activity against oral care organisms.

Material and Methods

General: Melting points reported are uncorrected. The 400 MHz NMR spectra were recorded on a Bruker AMX 400 in CDCl₃ or *d*₆-DMSO with TMS an internal standard. The ¹³C NMR spectra were recorded at 100 MHz in CDCl₃ and *d*₆-DMSO. IR spectra were recorded on a Shimadzu IR prestige 21; UV spectra were recorded on Shimadzu UV spectrophotometer; GC-MS were on a Jeol SX 102/DA 6000 mass spectrometer. TLC was performed on pre-coated silica gel 60 F254 plates (Merck) and the spots were visualized by exposure to iodine vapor or spraying with 5% sulphuric acid in methanol followed by heating the plate at 110°C for 5 min.

Plant material: The seeds of *P. corylifolia* were obtained from bazaar and was authenticated by Dr. P. Santhan, botanist, M/s. Durva Herbal Centre, Chennai. A voucher specimen was deposited in M/s. CavinKare Research Centre, Chennai, India.

Extraction and Isolation: Air-dried seeds of *P. corylifolia* (670g) were crushed and coarsely powdered. The powdered material was successively extracted with acetone followed by methanol to get corresponding extracts 126 g and 30.4 g, respectively. The crude acetone extract suspended in acetone and water (2:8), partitioned with hexane and chloroform to get corresponding fractions 51g and 16.5 g, respectively. The aqueous fraction was concentrated to get 55g of the extract. All three fractions were submitted for biological studies and hexane fraction was found to potent than other fractions.

After TLC analysis, the dark brown residue from hexane (51.0 g) was subjected to silica gel vacuum liquid chromatography, eluted with hexane, hexane: ethyl acetate (98:1, 98:2, 95:5, 90:10, 75:25). The homogeneous fractions were combined based on TLC and divided into six major fractions A (1.6 g), B (25.3 g), C (5.5 g), D (2.5g), E (3.7g), F (9.0 g). Fraction A was further purified by silica gel column using hexane and obtained compound 1 as colorless liquid (275mg). Fraction B was found to be mixture of two compounds and was purified by silica gel column using hexane, hexane: chloroform (8:2, 1:1), obtained again mixture of two compounds. Part of the fraction B (2.0g) was purified by silver nitrate impregnated column using hexane as eluent to obtain compound 2 (1.1g) Fraction C was showed one major and several minor spots along with lipid on TLC. It was purified by another small column using hexane: ethyl acetate mixture, followed by crystallization with ethyl acetate to get compound 3 (1.13g) and compound 4 (800mg). Fraction D was found to be solid nature in the

flask and was crystallized with hexane: chloroform to obtain compound 5 (0.91g). Fraction E and F were found to be lipids and coloring pigments and did not yield any compound.

Results and Discussion

Compound 1 (γ-Cadinene)¹⁹: Colorless liquid; UV (CHCl₃, γ_{max} in nm): 215; IR (KBr, ν_{max} in cm⁻¹): 2850, 1620, 1400, 980, ¹H NMR (CDCl₃): δ 0.98 (6H, d, *J*=5.6Hz, 24-H), 1.65 (3H, s), 2.0- 2.35 (7H, m), 4.88 (1H, s), 4.95 (1H, s), 5.30 (1H, br t), ¹³C NMR (CDCl₃): δ 16.2, 22.5, 28.2, 29.2, 29.9, 34.6, 39.9, 40.3, 49.2, 53.5, 111.5, 124.4, 135.4, 154.6. GC-MS (m/z): 204 [M⁺], 161, 146.

Compound 2 (Bakuchiol)²⁰: Light brown colored oil; UV (CHCl₃, γ_{max} in nm): 265; IR (KBr, ν_{max} in cm⁻¹): 3360, 2850, 1620, 1400, 980; ¹H NMR (400 MHz, CDCl₃): δ 1.21 (3H, s, H-16), 1.51 (2H, m, H-10), 1.60 (3H, s, H-15), 1.69 (3H, s, H-14), 1.98 (2H, m, H-11), 5.04 (2H, m, H-18), 5.12 (1H, br t, H-18), 5.90 (1H, dd, *J*=17.3, 10.8 Hz, H-17), 6.07 (1H, d, *J*=16.0 Hz, H-8), 6.27 (1H, d, *J*= 16.0 Hz, H-7), 6.78 (2H, d, *J*=8.0 Hz, H-3,5), 7.26 (2H, d, *J*=8.0Hz, H-2,4). ¹³C NMR (100 MHz, CDCl₃): δ 17.6 (C-15), 23.1 (C-16), 23.3 (C-11), 25.6 (C-14), 41.2 (C-10), 42.4 (C-9), 111.8 (C-18), 115.3 (C-3 and 5), 124.7 (C-12), 126.4 (C-7), 127.3 (C-2 and 6), 130.7 (C-1), 131.2 (C-13), 135.8 (C-8), 145.9 (C-17), 154.4 (C-4). GC-MS (m/z): 256 [M⁺], 241, 213, 186, 173, 158, 145, 107.

Compound 3 (Psoralen)²⁰: Colorless needles, mp 162-63°C; UV (CHCl₃, γ_{max} in nm): 245, 290, 327; IR (KBr, ν_{max} in cm⁻¹): 1720, 1629, 1568, 980; ¹H NMR (400 MHz, CDCl₃): δ 6.38 (1H, d, *J*=9.6 Hz, H-3), 6.84 (1H, d, *J*=1.9Hz, H-11), 7.47 (1H, s, H-8), 7.68 (1H, s, H-5), 7.70 (1H, d, *J*=1.9Hz, H-12), 7.80 (1H, d, *J*=9.6 Hz, H-4). ¹³C NMR (100 MHz, CDCl₃): δ 99.7 (C-8), 106.3 (C-11), 114.6 (C-3), 115.3 (C-10), 119.7 (C-5), 124.8 (C-6), 143.9 (C-4), 146.8 (C-12), 152.0 (C-9), 156.3 (C-7), 160.8 (C-2). GC-MS (m/z): 186[M⁺], 158, 130, 102.

Compound 4 (Isopsoralen)²⁰: Colorless needles, mp 137-38°C; UV (CHCl₃, γ_{max} in nm): 246, 296; IR (KBr, ν_{max} in cm⁻¹): 1721, 1629, 1568, 980; ¹H NMR (400 MHz, CDCl₃): δ 6.39 (1H, d, *J* = 9.6 Hz, H-3), 7.13 (1H, d, *J* = 1.97Hz, H-11), 7.37 (1H, d, *J* = 8.5Hz, H-5), 7.43 (1H, d, *J* = 8.5Hz, H-6), 7.70 (1H, d, *J* =1.97Hz, H-12), 7.81 (1H, d, *J* = 9.6 Hz, H-4). ¹³C NMR (100 MHz, CDCl₃): δ 104.0 (C11), 108.7 (C-6), 113.4 (C-3), 114.0 (C-10), 116.8 (C-8), 123.7(C-7), 144.4 (C-4), 145.8 (C-12), 148.3 (C-9), 157.2 (C-7), 160.7 (C-2). GC-MS (m/z): 186 [M⁺], 158, 130, 102.

Table-1
Comparison of MIC values of various organism

Sl. No.	Extract / Fraction / Compound	Tested organisms MIC value (µg/mL)		
		<i>Streptococcus mutans</i> - MTCC 497	<i>Actinomyces viscosus</i> - ATCC 15987	<i>Streptococcus sanguis</i> - ATCC 10556
1	Methanolic extract	1250	NA	NA
2	Acetone extract	>1250	NA	NA
3	Hexane fraction	19.53	NA	NA
4	Bakuchiol	9.76 - 19.5	9.76 – 19.5	9.76 – 19.5
5	Tetracyclin	0.48	1.95	1.95

* NA = Not analyzed

Compound 5 (Psoralidin)²¹: Pale yellow crystals, mp: 286-88°C; UV (CHCl₃, γ_{max} in nm): 244, 305, 347; IR (KBr, ν_{max} in cm⁻¹): 3440, 3349, 1720, 1629, 1568, 980; ¹H NMR (400 MHz, d₆-DMSO): δ1.69 (3H, s), 1.73 (3H, s), 3.28 (2H, d, J = 8.0 Hz), 5.32 (1H, d, J = 8.0 Hz), 6.83 (1H, s), 6.92 (1H, d, J = 8.0Hz), 7.14 (1H, s), 7.54 (1H, s), 7.65 (1H, d, J = 8.0 Hz), 10.00 (1H, br s), 10.68 (1H, br s). ¹³C NMR (100 MHz, d₆-DMSO): δ18.0, 25.9, 27.9, 99.0, 102.3, 102.7, 104.1, 114.2, 115.0, 120.9, 122.1, 126.8, 132.8, 153.2, 156.3, 157.3, 158.0, 159.3, 159.8. GC-MS (m/z): 336[M⁺], 281.

Antibacterial studies: Antibacterial activity was evaluated by turbidometric method on oral microorganisms especially the effects on adherent Streptococci sp., and *Actinomyces viscosus*. The experimental methodology has been well documented and published²². The current test cultures were obtained from MTCC and ATCC. It was found that the methanolic and acetone extract, hexane fractions and bakuchiol (2) showed significant antimicrobial activity against Oral care bacteria. MIC value of bakuchiol was found to be 9.76-19.5 µg/mL while the MIC value of the control, tetracyclin was found to be 0.48-1.95µg/mL [table 1].

Conclusion

Out of five isolated compounds, γ-cadinene is the first report from the plant and the remaining four compounds, bakuchiol, psoralen, isopsoralen and psoraledin have already been reported from this plant. Bakuchiol (2) is an alternative to synthetic compounds.

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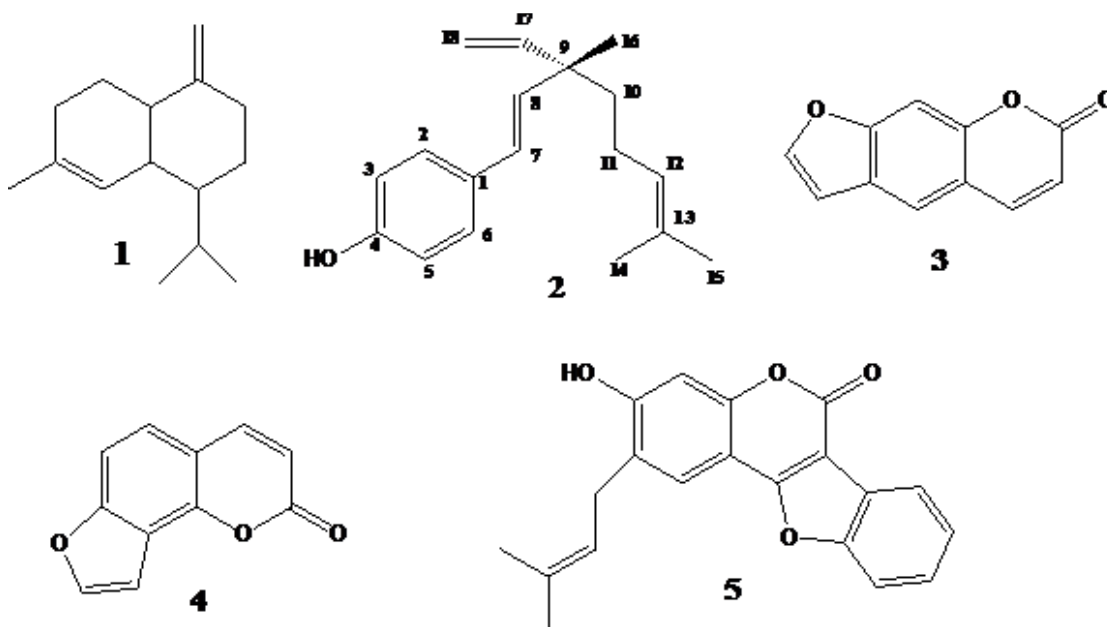
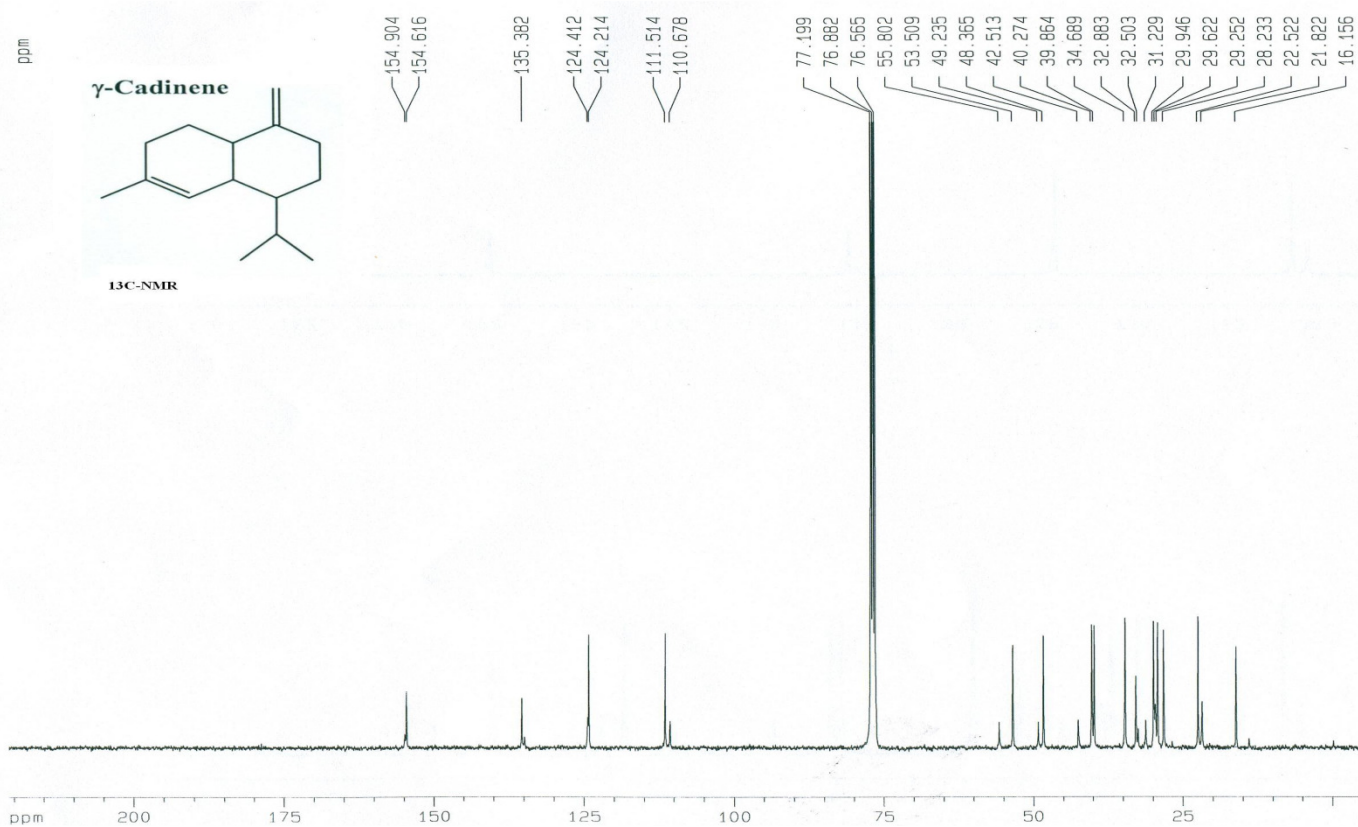
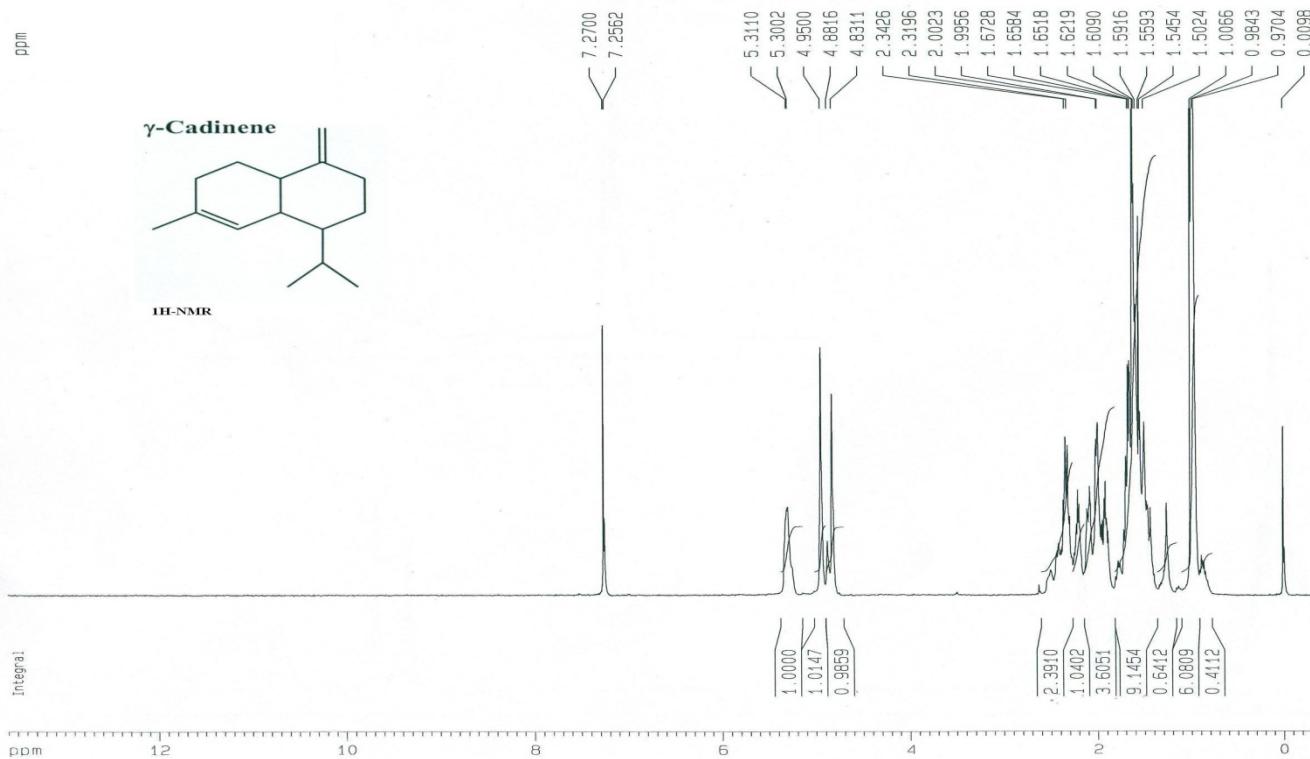
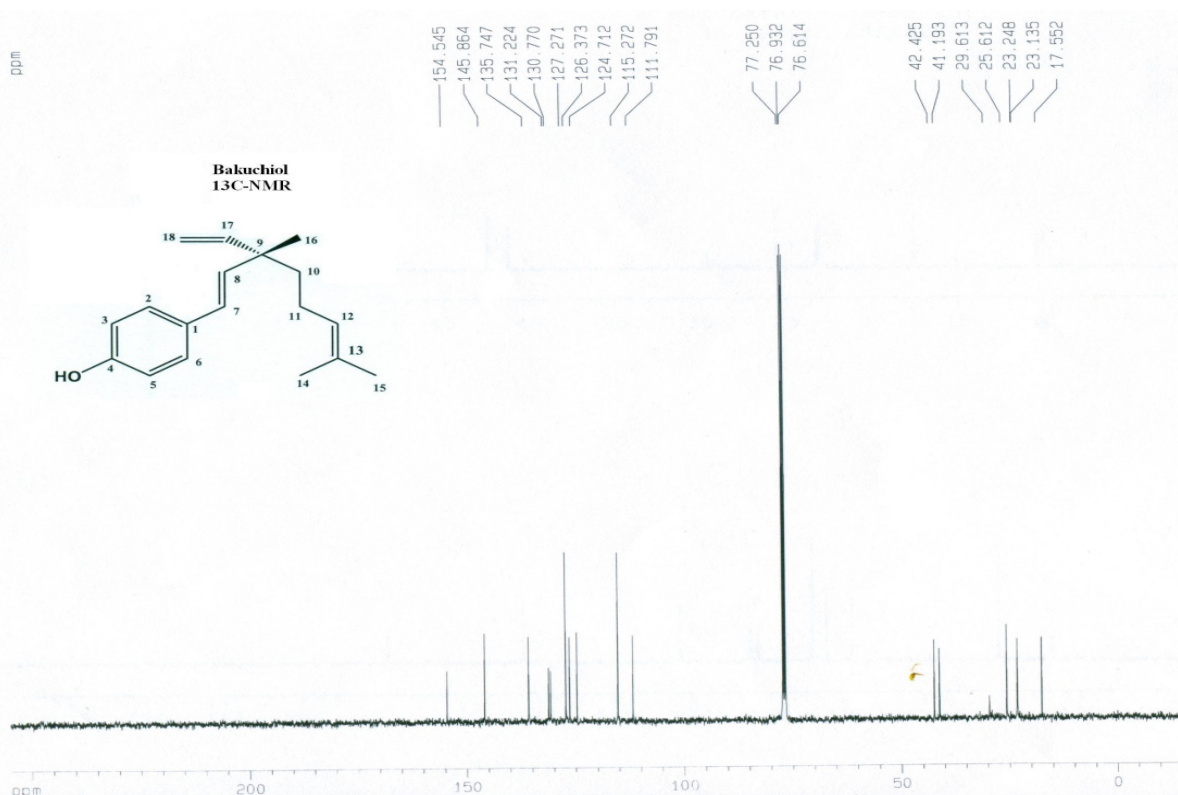
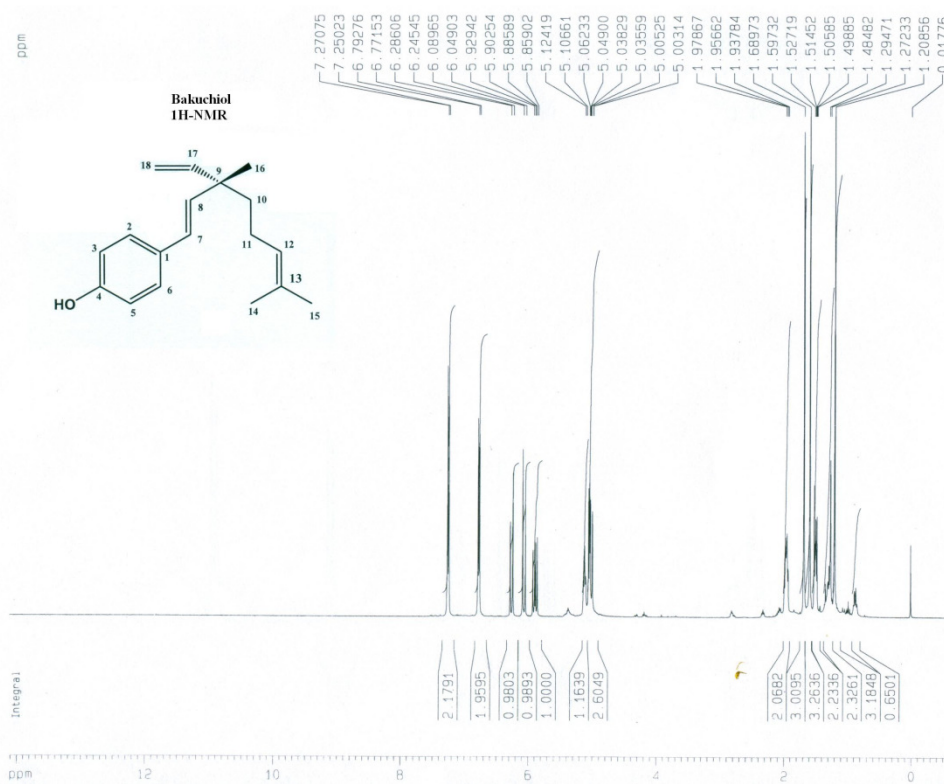
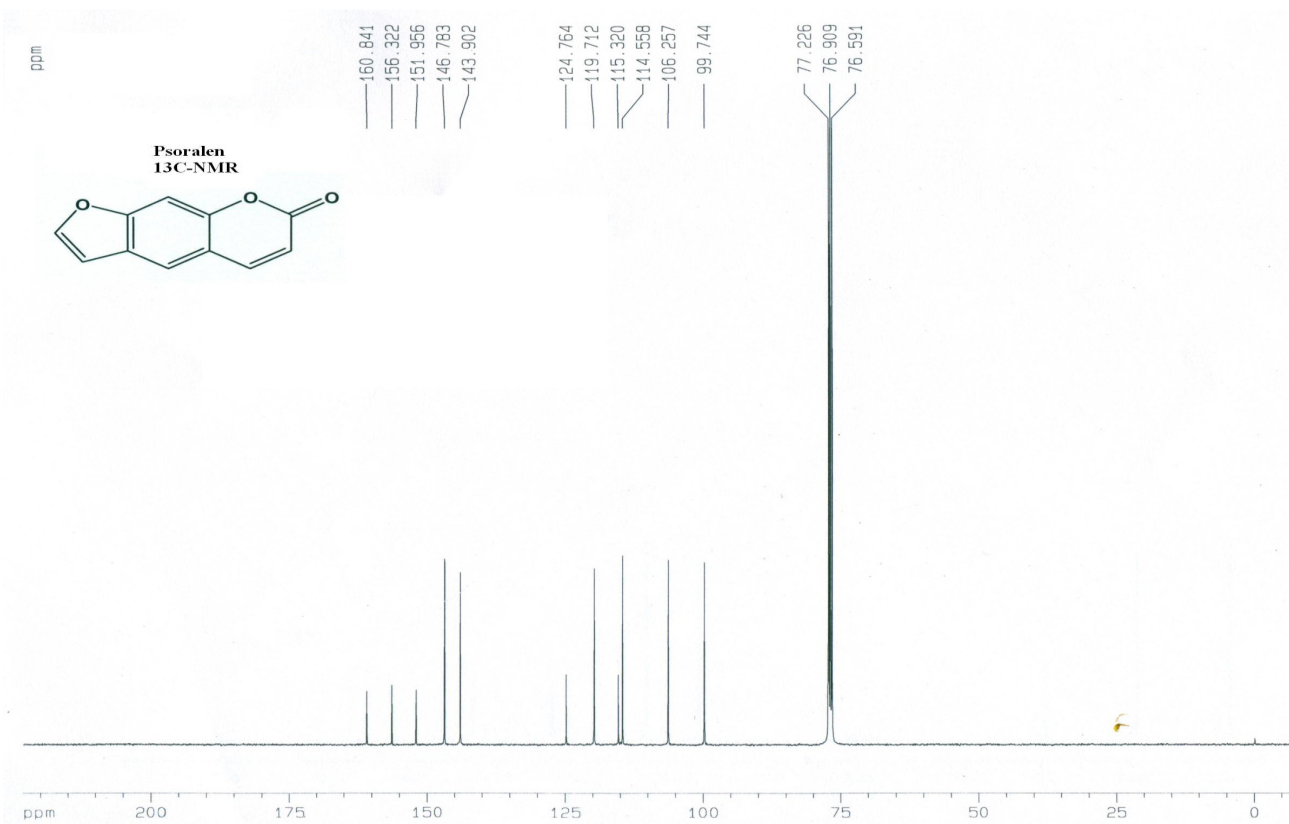
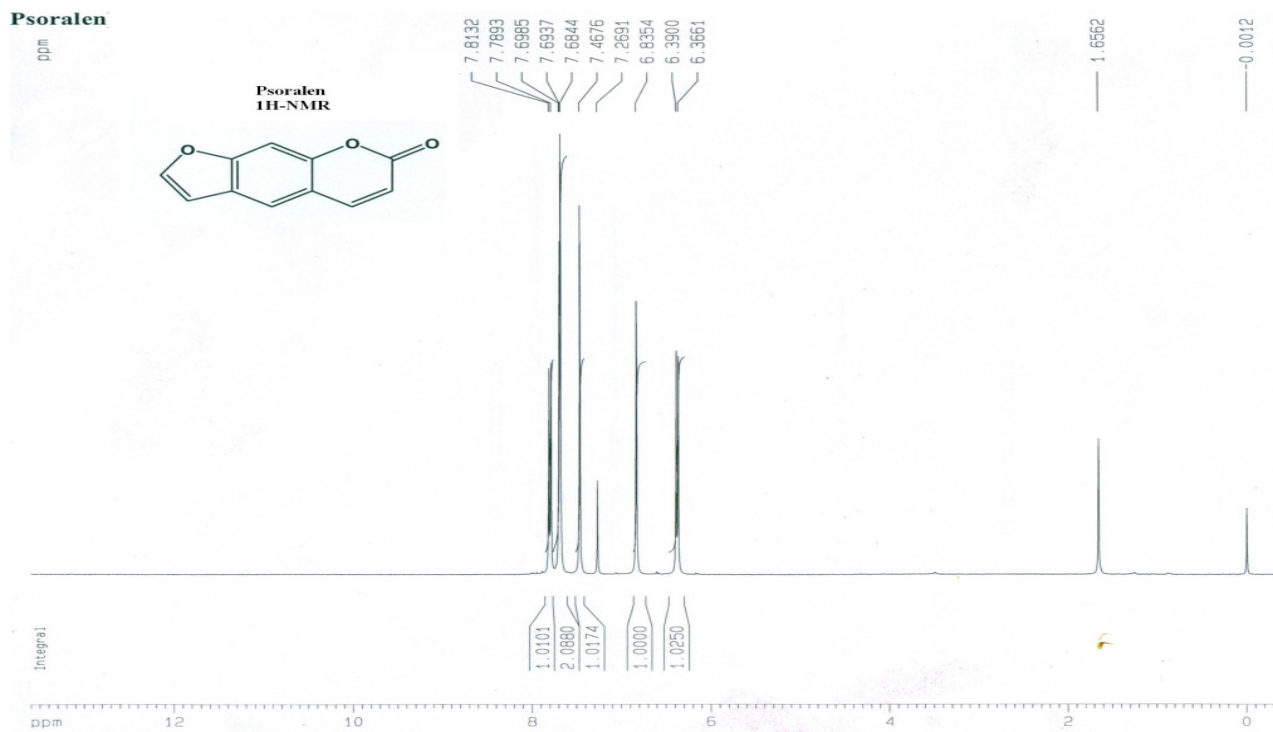
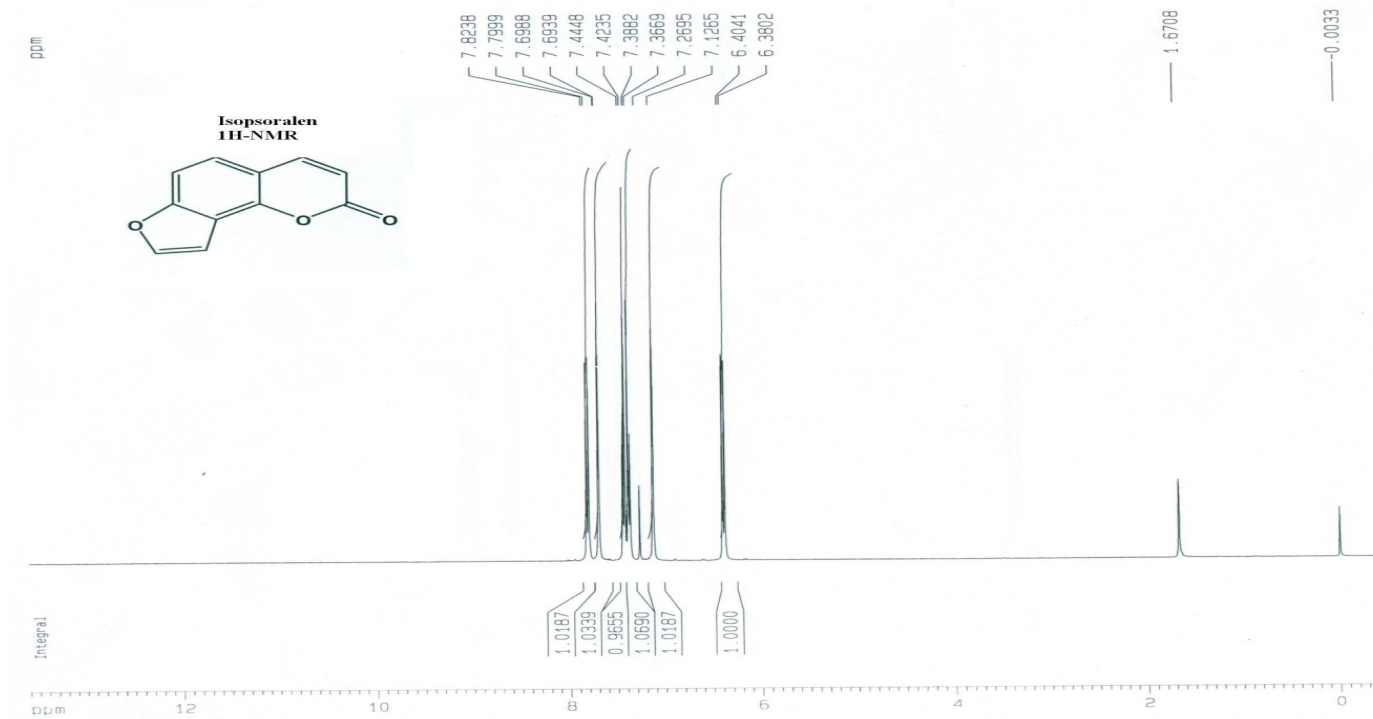


Figure-1
Compounds from *Psoralea corylifolia*









Isopsoralen

