Isolation and Characterisation of naphthoquinones from the traditional medicinal plant – *Lithospermum viridiflorum* Roxb

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ABSTRACT

Four naphthoquinones, namely - deoxy shikonin, acetyl shikonin, β, β-dimethylacrylshikonin and isobutyl shikonin have been isolated from the hexane extract of the root-bark of the traditional medicinal plant *Lithospermum viridiflorum*. The structures of the compounds were characterized by using Mass, Proton and 13C-NMR respectively.

Keywords: *Lithospermum viridiflorum*, Boraginaceae, shikonin, Medicinal plants

INTRODUCTION

The family *Boraginaceae* is another rich source of naphthoquinones, especially shikonins. *Lithospermum viridiflorum* consists of ovate leaves, blossoms during the rains and is distributed throughout the Bengal and Northeast region of India, and it is used in Ayurveda [1-4]. The bioactivity of the root-bark of *L. viridiflorum* is largely due to the shikonins, present in it. As part of our work to obtain information on the phytochemistry of medicinal plants[5-10], examination of *Lithospermum viridiflorum* was taken up.

MATERIALS AND METHODS

General experimental procedure: All reagents were purchased from Sigma-Aldrich. TLC was monitored with silica gel-precoated aluminum sheets (Type 60 F254, Merck, Darmstadt, Germany) and the spots were visualized in the ultraviolet light chamber, iodine chamber, 5% MeOH-H₂SO₄ mixture. Elemental analyses were carried out on an automatic Flash EA 1112 Series, CHN Analyzer (Perkin elmer, series II 2400). ¹H NMR and ¹³C NMR spectra were determined on a Bruker-300 NMR spectrometer and chemical shifts were expressed as part per million against TMS as internal reference. Mass spectra were recorded on Agilent 1200 (Liquid Chromatography), Agilent 6320 (Quadrupole Mass Analyzer) spectrophotometer.

Plant material: 2kg of *Lithospermum viridiflorum* was collected from tropical evergreen forests of Nagaland, India. The plant was authenticated by Dr. Jayendran, Department of Botany, Government Arts College, Ootacamund,
India. A voucher specimen (JLV1809) was deposited in Government Arts College, Ootacamund, India. The collected plant material (root-bark) was shade dried and coarsely powdered for extraction.

**Extraction and isolation:**
Finely powdered *Lithospermum viridiflorum* aerial parts (2 kg) were percolated with hexane (10 L, three times) for 24 hrs. The resulting extract was then concentrated under vacuum to obtain a residue (50 g). The residue was chromatographed over a silica column (70-325 mesh) and eluted with Hexane/Ethylacetate combinations [1-100%]. The column elution was monitored by TLC and fractions were pooled based on similar TLC profiles. Initial fractions eluted in chloroform alone yielded sterols and waxes. Fractions 2-10 (eluted with 100% hexane) afforded deoxyshikonin 1 and β,β-dimethylacrylshikonin 2. Fractions 11-19 yielded acetyl shikonin 3 alone. Further elution with 5% Ethylacetate in hexane yielded, isobutyl shikonin 4. The compounds were carefully separated, evaporated to dryness and characterized for structural elucidation.

**RESULTS AND DISCUSSION**
Figure 1 clearly represents the structures of the molecules isolated from the root-bark of *L. viridiflorum*. Those isolated compounds have been characterized by using $^1$H and $^{13}$C NMR which is discussed below.

**Deoxyshikonin (1):** dark red powder; mp: 93-94°C. $^1$H NMR (400 MHz, CDCl$_3$, δ) 12.62 (s, 1H, –OH), 12.46 (s, 1H, –OH), 7.19 (d, J = 1.3 Hz, 2H), 6.85 (d, J = 1.3 Hz, 1H), 5.19 (t, J = 7.1 Hz, 1H), 2.65 (t, J = 7.6 Hz, 2H), 2.31 (q, J = 7.6 Hz, 2H), 1.69 (s, 3H), 1.58 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$, δ) 182.2, 182.1, 181.9, 162.2, 152.5, 136.6, 135.7, 132.0, 131.9, 122.4, 111.9, 29.8, 26.6, 25.7, 17.9.

**β,β-dimethylacrylshikonin (2):** dark red powder; mp: 112-113°C; ESI-MS m/z = 369[M-1]; $^1$H NMR(400 MHz, CDCl$_3$, δ) 12.59 (s, 1H, –OH), 12.43 (s, 1H, –OH), 7.18 (s, 2H), 6.97 (s, 1H), 6.01 (ddd, J = 1.3, 4.6, 7.1 Hz, 1H), 5.77 (t, J = 7.1 Hz, 1H), 2.62 (m, 1H), 2.48 (m, 1H), 2.15 (s, 3H), 1.93 (s, 3H), 1.70 (s, 3H), 1.57 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$, δ) 179.1, 166.9, 166.4, 159.0, 149.1, 135.9, 132.7, 132.5, 131.7, 118.1, 115.4, 112.0, 111.7, 68.7, 33.0, 27.7, 25.8, 20.5, 18.04.

**Acetylshikonin (3):** red powder; mp: 105-106°C; ESI-MS m/z = 329[M-1]; $^1$H NMR (400 MHz, CDCl$_3$, δ) 12.56 (s, 1H, –OH), 12.40 (s, 1H, –OH), 7.16 (d, J = 1.2 Hz, 1H), 6.0 (ddd, J = 1.2, 4.6, 7.2 Hz, 1H), 5.11 (t, J
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\[ \text{\(1^3\text{C NMR (100 MHz, CDCl}_3, \delta \)} \]

178.3, 176.8, 169.8, 167.5, 167.0, 148.3, 136.2, 132.9, 132.8, 131.5, 117.7, 111.9, 111.6, 69.6, 32.9, 25.8, 21.0, 18.0.

**Isobutylshikonin (4):** dark red powder; mp: 89-90°C; ESI-MS m/z = 357[M-1]; \(^1\text{H NMR (400 MHz, CDCl}_3, \delta \)} d 12.58 (s, 1H, –OH), 12.42 (s, 1H, –OH), 7.18 (s, 2H), 6.96 (d, J = 1.3 Hz, 1H), 6.01 (ddd, J = 1.3, 4.6, 7.4 Hz, 1H), 5.11 (t, J = 7.1 Hz, 1H), 2.62 (m, 2H), 2.47 (m, 1H), 1.70 (s, 3H), 1.57 (s, 3H), 1.21 (d, J = 5.0 Hz, 3H), \(^1\text{C NMR (100 MHz, CDCl}_3, \delta \)} 178.3, 176.8, 175.7, 167.3, 166.8, 148.5, 136.0, 132.8, 132.7, 131.3, 117.8, 111.8, 111.6, 69.0, 34.0, 32.9, 25.7, 18.9, 18.4, 17.9.

Characterization of compounds 1-4 was performed by extensive NMR studies and EI-MS. In the \(^1\text{H-NMR spectra of 1-4, characteristic phenolic OH protons(peri-hydroxyl) were seen as broad singlets in the range of \( \delta \) 12.40-12.65. H\textsubscript{6} and H\textsubscript{7} protons of 1-4 are seen as singlets in the range \( \delta \) 7.16 and 7.30 ppm. H\textsubscript{3} protons of compounds 1-4 arise between \( \delta \) 6.65 and 6.98 ppm. In the 1H-NMR spectra of 1-4, the peaks belonging to side chains were similar to each other. Olefinic protons of 4-methyl-pent-3-en-1-yl groups in 1-4 give triplet-like signals between \( \delta \) 5.11 ppm and \( \delta \) 5.14 ppm. Characteristic methyl singlets of 4-methylpent-3-en-1-yl groups are seen at \( \delta \) 1.67-1.70 ppm and \( \delta \) 1.56-1.59 ppm. In addition, 13C-NMR and mass spectra confirmed the structures.

**CONCLUSION**

In conclude the present work, Four shikonins, namely - deoxy shikonin, acetyl shikonin, \( \beta \), \( \beta \)-dimethylacrylshikonin and isobutyl shikonin have been isolated from the hexane extract of the root-bark of the traditional medicinal plant *Lithospermum viridiflorum*. This kind of studies is useful in the future for the investigation of bioactive secondary metabolites present in the rare medicinal plants.

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**REFERENCES**