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Isolation and characterization of chemical constituents from *Dalbergia sissoo* Roxb. Stem

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Abstract
Dalbergia sissoo is an important medicinal plant and commonly known as sisu, shisham, tahli, jag at different parts of world and belongs to family *Fabaceae. D. sissoo* plant parts contain a large number of chemical constituents; leaves of plant contain trisaccharides, oligosaccharides, phenols and the flower of shisham contains tectorigenin biochanin. Stem-bark contains flavonoids, dalbergichromene cinnamylphenols, 4-phenylchromene. Root-bark contains chalcone, isoflavone biochanin A, flavone, retenoid, dehydroamorphigenin. Methanolic extract of *D. sissoo* stem was mixed with silica gel (60-120 mesh) and subjected to column chromatography to carry out isolation of the compounds from stem of *D. sissoo*. Chromatographic separation was carried out over silica gel (60-120 mesh) column and eluted with the solvents of increasing polarity. The column chromatography afforded three compounds suchas D-1(Tritriacontane), D-2 (Dalbergichromene) and D-3 (1,6-Dihydro-1,6-dihydroxy-4-methoxydalbergione).

Keywords: *Dalbergia sissoo, Fabaceae,* Tritriacontane, Dalbergichromene and 1,6-Dihydro-1,6-dihydroxy-4-methoxydalbergione

Introduction
Natural plant products are easily degradable, less persistent in the environment and eco-friendly in nature. Natural products obtained from various plant materials including terrestrial plants play an important role in chemistry of drugs [1]. They provide amazing source of new drugs leading to development of new chemical entities for further drug development. The scope, importance and methodology of this field have been out lined by various workers [2, 3]. From the human civilization, medicinal plants have been the part and parcel of human society to combat disease. *D. sissoo* is an important medicinal plant and commonly known as sisu, shisham, tahli, jag at different parts of world and belongs to family *Fabaceae.* It is native to the Indian subcontinent and southern Iran. It is found in India, Pakistan, Burma, Sri Lanka and Mauritius. The genus *Dalbergia* consists of 300 species out of which 25 species occur in India and the most famous species from these are the rosewoods [4]. *D. sissoo* is a medium to large size tree of about 25 meters height with grey yellow trunk, leaves are leathery and 2-3 meters in diameter, pinnately compound. They are broad, ovate, acuminate, glabrescent, petiolate with finepointed tip [5]. Flowers of Shisham whitish to pink in colour, give fragrant and sessile, they are 5-8 mm long racemes, 2.5 - 3.7cm long in short axillary panicles and its crown in oval shape and pods are oblong, flat, thin, strap like 4-8cm long and cm wide with 1-4 seeds. Seeds are 4-5 mm size and kidney shaped, thin and flat, light brown. They have long taproot system and numerous surface roots which produce suckers. The sapwood is white to pale brown in colour. The flowering period of plant is March-May. *D. sissoo* plant parts contain a large number of chemical constituents; leaves of plant contain trisaccharides, oligosaccharides, phenols, neoflavenes [6, 7, 8]. The flower of shisham contains tectorigenin biochanin. Stem-bark contains flavnoids, dalbergichromene cinnamylphenols, 4-phenylchromene [9, 10, 11]. Root-bark contains chalcone (2, 3-dimethoxy-4′-γ, γ-dimethylallyloxy-2′-hydroxychalcone), isoflavone (7-γ, γ-dimethylallyloxy-5-hydroxy-4′-methoxyisoflavone), biochanin A, flavone, 7-hydroxy-6-methoxyflavone, retenoid, dehydroamorphigenin [12].

Materials and Methods
The *D. sissoo* stem were collected from CCS, Haryana Agricultural University grounds, Hisar.

Chemicals: The economically accessible chemicals from Sigma Aldrich, Qualigens, Merk and Ranbaxy, of high virtue, were utilized for different exploratory methodology.

Preparation of extracts of *D. sissoo*

Stem of *D. sissoo* were manually separated, washed with water to remove mud, undesirable materials and shadow dried for 15 to 20 days. Samples of plant materials were prepared by chopping, grinding and then transferred to labeled ziploc bags to store at room temperature in dark.

The shadow dried chopped/grinded pieces of plant parts of *D. sissoo* were taken into round bottom flask (5 lit.) and extracted with hot methanol through refluxing for eight hours. The solvent was removed to get extractives. The procedure was repeated thrice. The extractives were concentrated over water bath under reduced pressure to obtain the dark coloured viscous mass labeled as methanolic extracts and kept in refrigerator for column chromatography.

Extraction and isolation of compounds from *D. sissoo* stem

The shadow dried chopped pieces of stem of *D. sissoo* (2.5 kg) were taken into round bottom flask (5 lit.) and thoroughly percolated with hexane, kept overnight at room temperature to remove excess of chlorophyll. Extraction of stem was carried out next day with hot methanol through refluxing for eight hours. The procedure was repeated thrice. The solvent was removed on a rotary evaporator to get the extractives. The extractives were concentrated over water bath under reduced pressure to obtain the methanolic extract of stem of *D. sissoo.* Methanolic extract was mixed with silica gel (60-120 mesh) and subjected to column chromatography to carry out isolation of the compounds from stem of *D. sissoo*. Chromatographic separation was carried out over silica gel (60-120 mesh) column and eluted with the solvents of increasing polarity. The eluotropic series with increasing polarity comprising of hexane, benzene, ethyl acetate, methanol and their mixtures were used. Fractions of 500 ml were collected and excess of solvent was distilled over hot water bath. Each elute obtained from column was monitored by thin layer chromatography (TLC) on silica gel-G plates. The chromatograms were developed in iodine chamber. Similar fractions were combined and purified to get the respective compound. The column chromatography afforded three compounds labeled as D-1 to D-3.

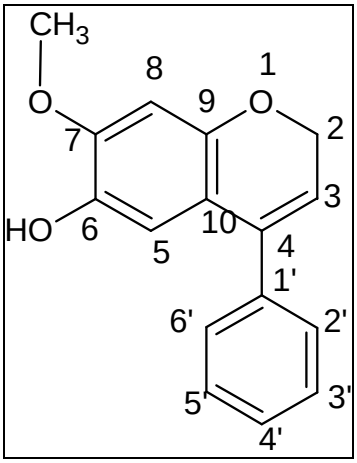
The solvent run in column chromatography

Sr. No.	Solvents	Volume
D-1	Hexane	72x500 ml
	Benzene: Hexane (1: 19)	60x500 ml
	Benzene: Hexane (1: 14)	58x500 ml
	Benzene: Hexane (1: 9)	45x500 ml
D-2	Benzene: Hexane (1: 1)	70x500 ml
	Benzene	50x500 ml
	Ethyl acetate: benzene (1:19)	82x500 ml
D-3	Ethyl acetate: Benzene (1: 9)	62x500 ml

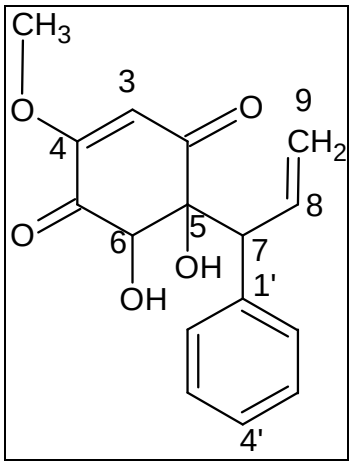
Results

The compounds were isolated by column chromatography. While packing the column, hexane was used as solvent.

The compound D-1 (Tritriacontane) was obtained as colourless solid on elution with hexane and recrystallized from chloroform, m.p. 69-71 °C. Its R_f value was found to be 0.45 in benzene: hexane (3:7) solvent. Its molecular formula $C_{33}H_{68}$ was deduced from molecular ion peak m/z 464 [M^+] by LCMS. IR values (cm^{-1}): 2848, 1378, 1261, 1097, 1023, 866, 802,722. UV-Visible-λ_{max}^{MeOH} (nm): there is no unsaturation in compound D-1.^{1}H NMR (δ, $CDCl_3$) 0.88 (6H, t, J=8.0Hz, 2xCH_3), 1.13-1.40 (62H, br, 31xCH_2). LCMS (m/z, rel. int.) 464 (M^+, 4.79), 436 (11.98), 414 (11.98), 412 (34.73), 352 (100), 174 (17.36). The compound D-2 (Dalbergichromene) was obtained as pale yellow powder on elution with benzene: hexane (1:1) and recrystallized from benzene, m.p. 97-98 °C. Its R_f value was found to be 0.25 in benzene solvent. Its molecular formula $C_{16}H_{14}O_3$ was deduced from molecular ion peak m/z 254 [M^+] by LCMS analysis. IR values are (cm^{-1}): 3590, 1620, 815, 810. UV-Visible-λ_{max}^{MeOH} (nm): 325, 285. ^{1}H NMR (δ, $CDCl_3$) 3.76 (3H, s, C_7 -OCH_3), 4.76 (2H, d, J=4.0Hz C_2-H), 5.21 (1H, s, C_6 -OH), 5.69 (1H, t, J=4.0Hz, C_3-H), 6.51 (1H, s, C_5-H), 6.62 (1H, s, C_8-H), 7.24-7.31 (5H, m, Ar-H). LCMS (m/z, rel. int.) 254 (100), 241 (29), 213 (77), 209 (54), 193 (18), 181 (35), 165 (12), 153 (5). The compound D-3 (1,6-Dihydro-1,6-dihydroxy-4-methoxydalbergione) was obtained on elution with ethyl acetate: benzene (1:9) as yellowish viscous mass. Its R_f value was found to be 0.56 in ethyl acetate-benzene (1:1) solvent. It responded to ammonia test and gave yellow colouration when ammonia solution of this compound on added with concentrated sulphuric acid which disappear after sometimes. Its molecular formula $C_{16}H_{16}O_5$ was deduced from molecular ion peak m/z 289 [M^+] by LCMS analysis. IR values are (cm^{-1}): 3417, 1710, 1670, 1610, 1230, 1007. UV-Visible-λ_{max}^{MeOH} (nm): 347, 256. ^{1}H NMR (δ, $CDCl_3$) 3.75 (3H, s, C_4 -OCH_3), 3.76 (1H, s, C_6-H), 4.54 (1H, d, J=7Hz, C_7-H), 5.04 (1H, d, J=8.0Hz, C_{9b}-H), 5.27 (1H, d, J=8.0Hz, C_{9a}-H), 5.74 (1H, s, -C_3-H), 5.94 (1H, t, J=8.5Hz, C_8-H), 7.22 – 7.30 (5H, m, Ar-H). LCMS (m/z, rel. int.) 289(14), 257(12), 253(100), 225(17), 221(14), 207(4), 197(3), 169(3), 91(4).



CH_3-$(CH_2)_{31}$-CH_3



(D-1) (D-2) (D-2)

Conclusion

Dalbergia sissoo is an important medicinal plant and belongs to family *Fabaceae.* The plant have different medicinal uses in treatment of diseases like syphilis, stomach problems, dysentery, skin diseases, inhibitory, anti-inflammatory, analgesic and antipyretic activities, larvicidal and growth inhibitor. The compound D-1 was obtained as colourless solid on elution with hexane The compound D-2 was obtained as pale yellow powder on elution with benzene: hexane (1:1) and recrystallized from benzene, m.p. 97-98 °C. The compound D-3 was obtained on elution with ethyl acetate: benzene (1:9) as yellowish viscous mass.

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