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Phytochemical and pharmacological profiling of *Dalbergia sissoo* Roxb. Stem

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Abstract
Dalbergia sissoo is very important medicinal plant possessing several pharmacologically potent chemicals. It belongs to the family Fabaceae. Woody bark contains various chemicals that make it antihelmintic, antipyretic and analgesic. It is reported to possess various phytochemicals such as norartocarpotin, stigmasterol, neoflavonoids, flavnoids, dalbergichromene cinnamylphenols, 4-phenylchromene, dalbergichromene, 4-phenyl chromene, dalbergichromene, chalcones, isosalipurposide, amino acids, fatty acids, dalbergin and dalbergenone. The biological activity of *D. sissoo* is because of compounds such as flavones, isoflavones, quinines and coumarins. 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 two compounds i.e. Dalbergin, 2,5-Dihyroxydalbergiquinol.

Keywords: *Dalbergia sissoo*, 4-phenyl chromene, 2,5-Dihyroxydalbergiquinol

Introduction

Medicinal plants are rich in such phytochemicals which upon isolation or in crude form can serve as potent drugs. The pharmacological significance of these phytochemicals is due to their less or negligible side effects as compared to allopathic drugs used for cure of various ailments. Besides it, low cost and easy availability enhances the widespread use of these medicinal plants in traditional system of medicine for primary health care.

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 [1]. Different medicinal uses of *D. sissoo* is like blood diseases, syphilis, dysentery, ulcers, skin diseases, larvicidal, growth inhibitor, anti-inflammatory, analgesic and antipyretic activities [2]. Moreover it is also reported to be a stimulant used in folk medicines and remedies. Ayurvedic system of medicines prescribes the leafy juice for eye ailments, the woody bark paste as antihelmintic, antipyretic & analgesic [3]. The crop protectant activity of the powdered dry leaf, the insecticidal activity of the bark extract against adult mosquitoes, and the antimicrobial activity of both the leaf and bark have also been reported [4-7]. Antimicrobial activity of plant kills the growth of microorganisms such as bacteria, fungi, and protozoan. The ethanolic extract of the fruits of *D. sissoo* exhibited molluscicide effect against eggs of the freshwater snail *Biomphalaria pfeifferi* [8].

D. sissoo plant parts contain a large number of chemical constituents; leaves of plant contain trisaccharides, oligosaccharides, phenols, neoflavenes [9-11]. Stem-bark contains flavnoids, dalbergichromene cinnamylphenols, 4-phenylchromene [12-14]. 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 [15]. The heartwood of plant contains 4-phenylchromene, dalbergichromene, chalcones, isosalipurposide, amino acids, fatty acids, dalbergin and dalbergenone [16-19]. Phytochemical isolation have significant potential of identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins etc. Therefore, the present study was carried out to evaluate the phytochemical patterns of *D. sissoo* by extracting and isolating two compounds from it.

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 two compounds labeled as D-1 to D-2.

The solvent run in column chromatography

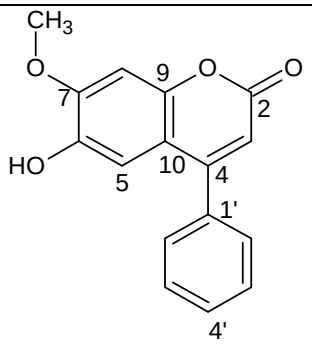
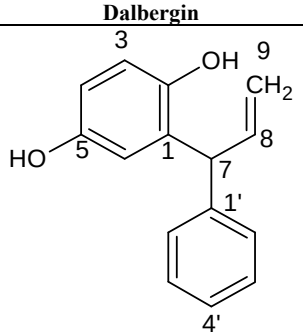
Sr. No.	Solvents	Volume
	Hexane	72x500 ml
	Benzene: Hexane (1: 19)	60x500 ml
	Benzene: Hexane (1: 14)	58x500 ml
	Benzene: Hexane (1: 9)	45x500 ml
	Benzene: Hexane (1: 1)	70x500 ml
	Benzene	50x500 ml
	Ethyl acetate: benzene (1:19)	82x500 ml
	Ethyl acetate: Benzene (1: 9)	62x500 ml
	Ethyl acetate: Benzene (1: 3)	62x500 ml
D-1	Ethyl acetate: Benzene (1: 1)	52x500 ml
	Ethyl acetate	70x500 ml
D-2	Methanol: Ethyl acetate (1: 49)	60x500 ml
	Methanol: Ethyl acetate (1: 29)	40x500 ml
	Methanol: Ethyl acetate (1: 19)	35x500 ml

Results

The compounds were isolated by column chromatography. While packing the column, hexane was used as solvent: The compound D-1 was obtained as colourless solid on elution with ethyl acetate: benzene (1:1) and recrystallized from ethyl acetate, m.p. 208-209 °C. Its R_f value was found to be 0.72 in ethyl acetate solvent. Its molecular formula $C_{16}H_{12}O_4$ was deduced from molecular ion peak m/z 269 [M^+] by LCMS analysis. It did not give any colour with alcoholic ferric chloride but responded to Shinoda's test and gave reddish colour with magnesium in hydrochloric acid. It proved the presence of 4-phenyl coumarins. **IR** ν_{max}^{KBr} (cm^{-1}): 3240, 1670, 820, 805. **UV-Visible** λ_{max}^{MeOH} (nm): 237, 259, 302, 356. **^{1}H NMR (δ, $CDCl_3$)** 3.99 (3H, s, C_7 -OCH_3), 5.61(1H, s, C_6 -OH), 6.12 (1H, s, C_3-H), 6.81(1H, s, C_8-H), 6.92 (1H, s, C_5-H), 7.48 (5H, s, Ar-H). **LCMS (m/z, rel. int.)** 269 (100), 241 (29), 213 (77), 209 (54), 193 (18), 181 (35), 165 (12), 153 (5).The compound D-1was obtained as dark brown oil on elution with methanol: ethyl acetate (1:49). Its R_f value was found to be 0.52 in methanol: ethyl acetate (1:19). Its molecular formula $C_{15}H_{14}O_2$ was deduced from peak m/z 226 [M^+] by LCMS analysis. It responded to ammonia test. **IR** ν_{max}^{KBr} (cm^{-1}): 3365, 1629, 1596 1375, 1280, 1006. **UV-Visible** λ_{max}^{MeOH} (nm): 308, 296. **^{1}H NMR (δ, $CDCl_3$)** 4.90 (1H, d, J=7.0Hz, C_7-H), 4.92 (1H, d, J=8.0Hz, C_{9b}-H), 5.18 (1H, d, J=8.0, 1.5Hz, C_{9a}-H), 6.19 (1H, t, C_8-H), 6.49 (1H, d, J=8.5Hz, C_4-H), 6.53 (1H, s, C_6-H), 6.57 (1H, d, J=8.0Hz, C_3-H), 7.15-7.23 (5H, m, Ar-H). **LCMS (m/z, rel. int.)** 226(100), 207(4), 197(3), 169(3), 91(4).

Compounds isolated from *D. sissoo* stem

Compd. Code	Elution	Structure	Melting Point (ºC)
D-1	Ethyl acetate: benzene (1: 1)	**D**	**208-209**

		 **Dalbergin**	
D-2	Methanol: ethyl acetate (1:49)	 **2,5-Dihyroxydalbergiquinol**	**Oil**

Conclusion

The compound D-1 was obtained as colourless solid on elution with ethyl acetate: benzene (1:1) and recrystallized from ethyl acetate, m.p. 208-209 °C. Its R_f value was found to be 0.72 in ethyl acetate solvent. Its molecular formula $C_{16}H_{12}O_4$ was deduced from peak m/z 269 [M^+] by LCMS analysis. It did not give any colour with alcoholic ferric chloride but responded to shinoda's test and gave reddish colour with magnesium in hydrochloric acid. It proved the presence of 4-phenyl coumarins. The compound D-2 was obtained as dark brown oil on elution with methanol: ethyl acetate (1:49). Its R_f value was found to be 0.52 in methanol: ethyl acetate (1:19). Its molecular formula $C_{15}H_{14}O_2$ was deduced from peak m/z 226 [M^+] by LCMS analysis. It responded to ammonia test.

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