WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 766-778. Research Article ISSN 2277– 7105

COMPARATIVE PHYTOCHEMICAL STUDY OF HEARTWOOD VERSUS SMALL BRANCHES OF *DALBERGIA SISSOO* LINN USING HPTLC-UV DETECTION METHOD

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Article Received on
12 Jan 2014,

Revised on 07 Feb 2015,
Accepted on 03 Mar 2015

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ABSTRACT

Dalbergia sissoo Linn is Indian rosewood which is a deciduous forest tree. It is natively found in Indian subcontinent. It is called as Shisham which is best known premier timber tree. It is also used as fuel wood with its multiple product uses and agro-forestry application, it is consider as best timber wood tree. *D. sissoo* is a widely growing plant which is used traditionally as anti-inflammatory, antipyretic, analgesic, anti-oxidant, anti-diabetic and antimicrobial agent. Several phytoconstituents have been isolated and identified from different parts of the plant belonging to the category of alkaloids, glycosides, flavanols, tannins, saponins, sterols and terpenoids. Our results revealed that the chromatographic fingerprint combined with similarity measurement could efficiently identify and distinguish *D. sissoo* heartwood and small branches. The phytochemical fingerprint profiling of heartwood and small branches of *D. sissoo* were found similar as an official part of *D. sissoo* plant i.e. heartwood, therefore small branches may be used in place of heartwood and vice-versa after comparison and confirmation of same pharmacological activities. The method can also be used for identification of different *D. sissoo* species and adulterants.

KEYWORDS: *Dalbergia sissoo*., HPTLC–UV detection, phytochemical fingerprint profiling analysis.

Abbreviations: HPTLC–UV, high performance thin layer chromatography-ultra violet detection; R_f, retention factor; **min**., minutes; **Ht.Wd.,** heart wood., **Sm. Br.**, small branches;

INTRODUCTION

Dalbergia sissoo Linn [Fig. 1] belongs to family Fabaceae commonly known as Shisham. The genus, *Dalbergia,* consists of 300 species and about 25 species occur in India. Many species of *Dalbergia* are important timber trees and appreciated mainly for their decorative and often fragrant wood which contains aromatic oils.[1] The most famous species under the genus *Dalbergia* are named rosewoods because of the smell, but several other valuable woods are yielded by the genus.[2] The generic name *Dalbergia* honors the Swedish brothers, Nils and Carl Dalberg, who lived in the 18th century. The former was a botanist and the latter explored Surinam.[3] This tree has many reputed medicinal properties and has been used culturally for a variety of diseases. *D. sissoo* is found from tropical to subtropical climates in natural and planted forests, widely distributed in Pakistan, India, Afghanistan, Persia, Iraq, Kenya and Tanzania. It grows along the hilly area, beds of river banks which have rich in alluvium soil.[4] This is a best known internationally for timber wood. *D. sissoo* is a medium to large-sized deciduous tree. It grows as elevation of 3000ft. Its trees are from 30 m to 80 m height and 2-3m in diameter under favorable conditions. Bark is thin, grey, longitudinally furrowed with exfoliating in narrow strips. The plant develops a long tap root from an early age and numerous lateral ramifying roots. The leaves are imparipinate; leaflets 3-5, alternate, 2.5-3.6 cm in diameter, broad ovate, acuminate, and glabrescent, petiolules 3-5 mm long. It has leathery leaves which are up to 15 cm long. Flowers are 5-8 mm long, pale white to dull yellow, racemes 2.5-3.7 cm long in short auxiliary panicles. Pods are 5-7.5 cm x 8-13 mm, narrowed at the base, indehiscent, glabrous, with 1-4 seeds. Seeds are 6-8 x 4-5 mm, kidney shaped, thin and flat, light brown. The fruit is dry and hard. On average account heartwood is golden to dark brown and the sapwood is white to brown which is durable, tough and resistant. The sapwood is white to pale brown in colour and the heartwood is golden to dark brown in color.[5,6] A number of phytochemical like tannins, steroid, terpenoids, saponins, flavonoids, alkaloid including isoflavonoids, neoflavonoids, O-Prenylated flavonoids glycosides cinnamyl phenols quinones, furans, oligosaccharide, trisaccharides and other miscellaneous compounds have been isolated from various parts of the *D. sissoo*.[7-11] Major compounds found in *D. sissoo* plant are isoflavones irisolidone, biochanin-A, muningin, tectorigenin, prunetin, genestein, sissotrin and prunetin-4-O galactoside. One flavone named as nor- artocarpotin, F3-amyrin, F3- sitosterol and stigmasterol were isolated and identified from the green branches of aerial parts of *D. sissoo.* Leaves contain sissotrin, isoflavone-O-glycoside -, caviunin 7-O-[β-d-apiofuranosyl-(1→6)-β-d-glucopyranoside] and a itaconic

acid derivative named as (E)-4-methoxy-2-(3,4-dihydroxybenzylidene)-4-oxobutanoic acid (along with series of isoflavones and flavonols with their glucosides and a lignan glucoside were isolated from the ethanolic extract of *D. sissoo* leaves . Flowers contain biochenin A, tectorigenin, 7, 4-dimethyl tectorigenin and 7-O-methyltectorigenin. Green pods contain meso-inisitol, 7-O-methyltectorigenin and its 4´-rhamnoglucoside Caviunin 7-O-gentiobioside.[12] Mature pods contain isocaviumin, tectorigenin, dalbergin, biochamin A and 7-hydroxy-4-methyl coumarin, 7-O-glucosides of tectorigenin, caviunin and tannins. Stem bark contains dalberginone, dalbergin, methyldalbergin, a 4-phenylchromene, neoflavenes-dalbergichromene[13] and isotectorigenin. Heartwood contains chalcones (isoliquiritigenin), isosalipurposide, amino acids (glycin, alanine, threonine, isolucine, phenylalanine) dalbergin, nordalbergenones,[14] Neoflavenes-dalbergichromene[13] 3, 5-dihydorxy-trans-stilbene, biochanin A and an allylphenol of latifolin type - dalbergiphenol. Heartwood also contains fixed oil, containing myristic, palmitic, stearic, Arachidic, Linoleic, oleic acid and essential oil, containing two sesquiterpene derivatives bisabolene and nerolidol. 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-methoxflavone, Rotenoid, Dehydroamorphigenin . From the roots, neoflavonoids such as (S)-4-methoxy dalbergione, (R)-latifolin & dalbergin and also cardiac gycosides, anthraquinones and saponins were isolated. From the tree trunk a flavnoide named as Naringenin was isolated.[15,16] When the methanol extract of the heartwood part was partitioned using separating-funnel into ethyl acetate, then this separated ethyl acetate fraction from the heartwood of *D. sissoo* was analyzed by using column chromatography and 20 organic components were isolated. The major compounds identified through GC-MS of this fraction, were 1,2-benzenedicarboxylic acid dibutyl ester (13.68%) and 5-nirto-2,4 (1H,3H)-pyrimidine dione (7.94%).[16] These major compound showed antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus* (as a Gram-positive strains), *Serratia marcescens* and *Proteus mirabilis* (as a Gram-negative strains), 3-hydroxycarbonyl-2,5-diethylpyrrolidine (7.83%) and formic acid, 1-methylethyl ester (7.38%).[17] The ethanol extract of *D. sissoo* leaves reported to have isoflavones glycoside named dalsissooside and one itaconic derivative named sissooic acid in addition with fourteen known compounds -genstein ,biochanin A , pratensein , biochanin 7-O-glucoside ,biochenin A 7-O-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside, biochenin A 7-O-[β-D-apiofuranosyl-(1→5)-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside , genistein 8-C-β-D-glucopyranoside , caviunin , caviunin7-O-β-D- glucopyranoside , kaempferol 3-O-β-D-glucopyranoside , kampferol-3-O-

rutinoside, quercetin 3-O-β-D-glucopyranoside, quercetin 3-O-rutinoside, and syringaresinol-4″-O-β-D-monoglucoside were isolated.[18] From alcoholic extract of the mature pods of *D. sissoo*, a new isoflavanone glucoside ($C_{31}H_{38}O_{18}$, m.p. 210°C- 214°C) identified as 7-Gentiobioside of 5,7–dihydroxy 6,2,4,5- tetra methoxy flavonons and novel isocaviunin (5-7–dihydroxy – 8,2´,4,5´– tetra methoxy isoflavone,(m.p. 193 -194ºC isocavianin–7-O-glucoside (isocaviudin), along with tectorigenin–7-O-glucoside (tectoridin) and caviunin–7–O-glucoside were isolated. On chemical analysis of alcoholic extract of heart wood, the presence of oleanolic acid (0.2%), isoliquiritigenin (2′, 4′, 4-tri hydroxy chalcone) (0.1%) and liquiritigen (7,4′-dihydroxy 35 flavonone), β-sitosterol (0.1%), latifolin (0.15%) from the sap wood and biochanin-A, kaempferol (0.08%) from the tender leaves of *D. sissoo* were found. From methylene chloride extract of heart wood of *D. sissoo,* latifolin was isolated and found to exhibit the inhibition of β-amyloid synthesis. The ethanol extract of the bark of *D. sissoo* was tested and it was found out that the total phenolic was 58.06 gallic acid equivalents (GAE) mg/g of extract and tannin content was varied from 218.34 to 61.75 mg catechin equivalent (CE)/g of extract.[19]

The heartwood of this plant is extremely durable and is resistant to dry-wood termites. Thus wood is used mainly for house construction, e.g. for door and window shutters and frames, flooring and panelling, and also for cabinet making, vehicle bodies, boat building, handles, implements such as shoe lasts, turnery, carving, cabinets, decorative veneer, marine and aircraft grade plywood ,ornamental turnery engraving, tool handles and sporting goods. It is excellent for high-class bentwood furniture, walking-sticks, umbrella handles and other bentwood articles. It is highly valued as firewood and for charcoal production. Pulp from the wood is suitable for papermaking. *D. sissoo* is used as a shade tree in agroforestry systems in India and neighboring countries, for afforestation of eroded soils, and as a soil improver that fixes nitrogen and provides mulch. It is also planted as a windbreak, shelter belt, as an ornamental and roadside tree. The foliage and young pods are useful as fodder, although it has been reported that fresh leaves may cause digestive disorders in livestock during the dry season. A non-drying oil which is suitable as a lubricant for heavy machinery can be obtained from the heartwood. Its root wood is used for tobacco pipes. Some women use the sap from the leaves to lighten their skin color and to get rid of dark patches on their skin. It has been found that rosewood oil can stimulate new cell growth, and regenerate tissues, so it could prevent too many wrinkles. The oil is also good for acne and is used in some perfumes.[20]

In addition to uses in day today life *D. sissoo* plant consists of a large number of reputed medicinal properties.[21] *D. sissoo* has been used mainly as aphrodisiac, abortifacient, expectorant, anthelmintic and antipyretic. It is also used in conditions like ulcers, leucoderma, dyspepsia, syphilis, skin diseases, blood diseases, stomach problems and dysentery, nausea, eye and nose disorders. Different parts such as roots, bark, wood, leaves and seeds are being used as remedy in many diseases from ancient time. It is used not only in Ayurveda but also in Unani medicines. In Ayurveda the bark and wood were described as bitter, hot and acrid used as aphrodisiac, abortifacient, expectorant, antihelmintic, antipyretic, anti-inflammatory,[22] diseases of the blood, anal disorders burning sensations skin ailments and refrigerant. The wood is used for diseases of eye, nose and also used in scabies and syphilis. *D. sissoo* plant's bark has antioxidants and thus can used for inflammatory disorders. The whole plant has long been employed in ancient Unani preparations. In Unani medicines the heartwood is used for blood disorders, eye and nose disorders, scabies, scalding urine, stomach problems, and syphilis. The alterative heartwood is used in India for boils, eruptions, leprosy and nausea. A decoction of the bark is used for cleaning wounds.[23-25] Additionally, the bark and wood are reported to stimulate appetite and act as an aphrodisiac. Tannins extracted from the bark are used for a number of medicinal purposes. The bark from the root and the stem is an antidiarrhetic and the smoke of burning roots is inhaled to treat headaches and bronchitis. The roots contain tectoridin, which is used medicinally. The roots provide an astringent used to treat inflammations and infections. The roots can also be used to treat abdominal pain, hernia, gonorrhoea, and in abortion**.** Oil obtained from the seeds is used to cure skin diseases. Sissoo oil is used to treat blue itching, burning on the skin, and scabies. *D. sissoo* oil also showed strong repellant activity against mosquitoes like Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus, and is also resistant to some wood boring insects. Its aerial parts showed significant bronchodilator and estrogen like activities.[13] Leaf juice is used for eye ailments. The alcoholic extract of *D. sissoo* leaves produces significant anti-inflammatory effects in different models of inflammation, without having any side effects on the gastric mucosa.[25] Rural people in India and Nepal use *D. sissoo* leaves to treat animals suffering from non-specific diarrhea. Leaf extract has been used to treat sore throats, heart problems, dysentery, syphilis, and gonorrhea. The extract of *D. sissoo* leaves showed antioxidant activity and is almost two times higher than other commonly used antioxidants like Selenium and vitamin E. The extract of *D. sissoo* leaves contains a large amount of flavanoids. *D. sissoo* leaves extract can be used with poise to treat colorectal cancer along

with other usual treatments with chemotherapeutic agents boiled leaf filtrate is also used to wash hair for removing dandruff and for long hair.[26]

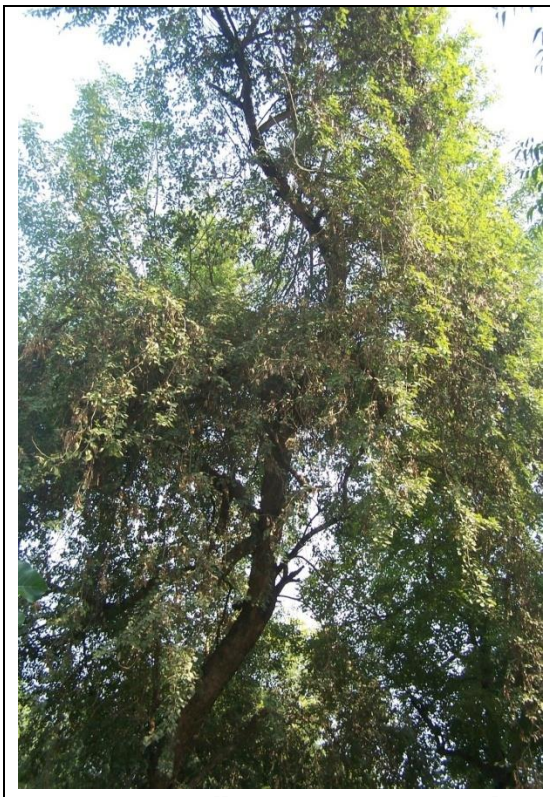


Figure 1: *Dalbergia sissoo* Linn Plant

Figure 2: Small Branches

Figure 3: Heartwood

TAXONOMICAL CLASSIFICATION[15]

Domain	Eukaryota
Kingdom	Plantae
Division	Magnoliophyta
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Subfamily	Faboideae
Tribe	Dalbergieae
Genus	*Dalbergia*
Species	*D. sissoo*
Binomial name	*Dalbergia sissoo*

MATERIALS AND METHODS

Plant Materials and Chemicals

Plant materials i.e Small branches of stem (Fig.2) and heartwood (Fig.3) and of *D. sissoo* were collected in December 2013 and authenticated by Dr. R. K. Tiwari, Research Officer,

Pharmacognosy, National Veterinary Research Institute & Hospital, Lucknow. All chemicals (AR grade) and TLC plates were purchased from E. Merck Pvt. Ltd. (Mumbai, India).

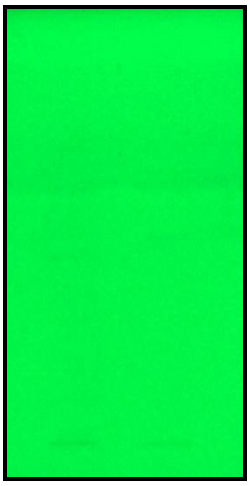
Sample preparation

The plant parts were dried under a gentle stream of air in the laboratory till no loss in weight (temperature 30± 20C and relative humidity 50 ± 5%) and powdered in an electric grinder.

Conventional extraction of heartwood and small branches of stem of *D. sissoo* were performed at room temperature (28o ± 3oC) with a variety of solvents ranging from non-polar to polar ones, i.e. *n*-hexane, ethyl acetate and ethanol. Dried and powdered parts of *D. sissoo* (10 g each) were extracted three times (3 × 50 mL) for 18 h of each extraction with each of the above-mentioned solvents separately. Each extract was filtered by using Whatman filter paper no. 1 and the solvents were removed under vacuum at 50°C, separately and concentrated up to 10 mL to get the sample solution of 100 mg mL-1. 5 µL of each sample was applied separately to TLC plate for the development of fingerprints.

HPTLC-UV detection Method

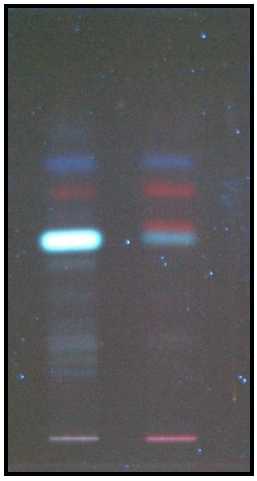
High Performance Thin Layer Chromatography was performed on 10 cm × 10 cm TLC plates pre-coated with 0.25 µm thin layers of silica gel 60 F254 (E. Merck). Both samples (heartwood and small branches) were applied on the plates as bands 10 mm wide by use of a Linomat-IV applicator (CAMAG, Switzerland) fitted with a 100 µL syringe (Hamilton, Switzerland). The application positions X and Y were both 10 mm, to avoid edge effects. Linear ascending development to a distance of 80 mm with *Toluene : Ethyl acetate (9 : 1)(/v/v)* and as mobile phase for both *n*-hexane extract was performed in a twin-trough glass chamber (20 cm × 10 cm) previously saturated with vapors of mobile phase for 20 min. The plates were dried in air and visualized under λ 254 nm and λ 366 nm for ultra violet detection and taken the fingerprints as evident in Figures 4 – 5. Further, the same TLC plate was derivatized with anisaldehyde-sulphuric acid reagent and visualized in white light obtained fingerprints were as evident in Figures 6 using CAMAG Reprostar and Win CATs software (V 1.4.2; CAMAG). HPTLC of ethyl acetate extract and alcoholic extract of both drugs were performed with same procedure in the mobile phase of *Toluene : Ethyl acetate (8:2)(v/v)* and *Toluene : Ethyl acetate (6:4) (v/v)* and then visualized in λ 254 nm, λ 366 nm and white light using CAMAG Reprostar and WinCATs software as shown in Figure 7-12.



1 2

254 nm

Figure 4



1 2

366 nm

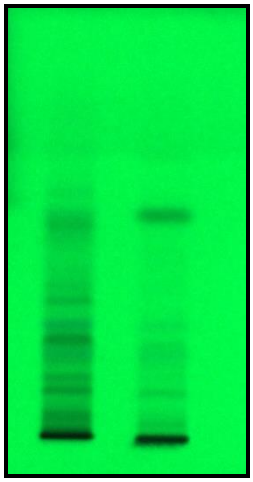
Figure 5

1 2

After derivatization with anisaldehyde sulphuric acid reagent

Figure 6

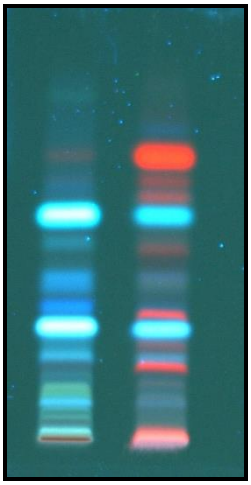
Figure 4-6: TLC fingerprint of *n*- hexane extract of *Dalbergia sissoo* (1= Ht.Wd; 2= Sm. Br.)



1 2

254 nm

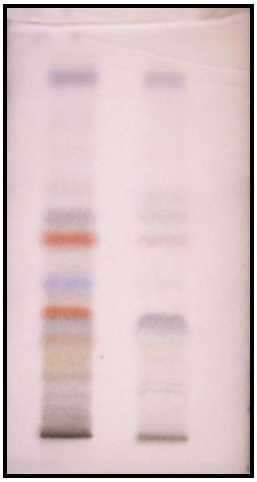
Figure 7



1 2

366 nm

Figure 8

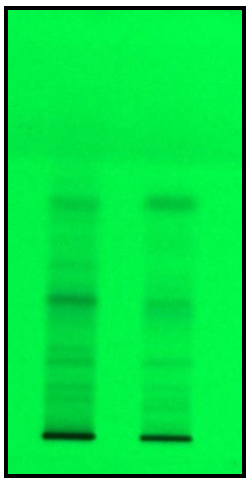


1 2

After derivatization with Anisaldehyde sulphuric acid reagent

Figures 9

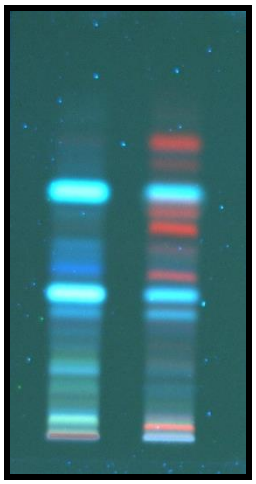
Figure 7-9: TLC fingerprint of ethyl acetate extract of *Dalbergia sissoo* (1= Ht.Wd; 2= Sm. Br.)



1 2

254 nm

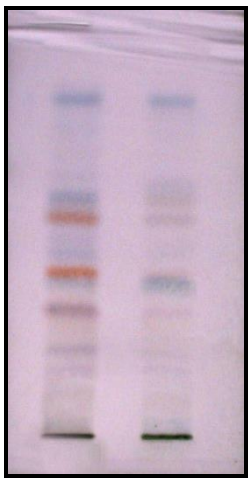
Figure 10



1 2

366 nm

Figure 11



1 2

After derivatization with Anisaldehyde sulphuric acid reagent

Figure 12

Figure 10-12: TLC fingerprint of ethanol extract of *Dalbergia sissoo* (1= Ht.Wd; 2= Sm. Br.)

Table 1: R_f value of phytochemicals present in *n*-hexane, ethyl acetate and ethanol extract of *Dalbergia sissoo* (Ht. Wd. and Sm. Br.) at different wave-lengths.

Wave-length	*n*- Hexane extract		Ethyl acetate extract		Ethanol extract	
	Heartwood	Small branches	Heartwood	Small branches	Heartwood	Small branches
254 nm	No band	No band	0.08, 0.15, 0.18, 0.28, 0.30, 0.37, 0.41,0.59, 0.67	0.08, 0.15, 0.26, 0.30, 0.62	0.13, 0.16, 0.22,0.27,0.40,0.51,0.68	0.11, 0.16, 0.22, 0.40, 0.68
366 nm	0.28,0.47, 0.54,0.67, 0.75	0.54,0.58, 0.67,0.75	0.11, 0.15, 0.24,0.30, 0.36, 0.40, 0.44,0.52, 0.59, 0.66, 0.74,0.89	0.10, 0.20,0.23, 0.30, 0.34, 0.44, 0.50,0.59 0.64,0.68, 0.74,0.81	0.05,0.14, 0.18, 0.21, 0.24,0.28,0.37, 0.44, 0.50, 0.63	0.06, 0.18, 0.28, 0.37, 0.42, 0.49, 0.54, 0.59, 0.63, 0.70, 0.76
Visible light after derivatization	0.16,0.23, 0.28,0.39, 0.53,0.87	0.28,0.87	0.13,0.16 0.25,0.30, 0.33, 0.40, 0.50, 0.56, 0.90	0.13, 0.25,0.30, 0.50, 0.56, 0.90	0.20,0.24,0.31,0.35,0.41,0.44,0.57,0.64,0.87	0.20,0.24,0.31,0.35,0.41,0.44,0.57,0.62, 0.87

RESULTS AND DISCUSSION

No such study was found in literature for comparative phytochemical study of heartwood versus small branches of *D. sissoo* Linn by using High Performance Thin Layer Chromatographic-Ultra Violet detection Method. Comparative study of TLC fingerprints of heartwood and small branches of *D. sissoo* revealed that many similarities in phytochemical fingerprints were found and evident in Table-1 and Fig. 4-12.

Phytochemical fingerprints of *n*-hexane extract of heartwood and small branches showed no band under UV detection at 254 nm. Under 366 nm UV detection, heartwood and small branches showed five and four bands respectively, out of which, three bands at R_f 0.54 (light blue), 0.67 (red) and 0.75 (blue) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, six and two bands were visible in heartwood and small branches respectively, out of which two bands at R_f 0.28 (blue) and 0.87 (blue) were found similar as represented in Table 1 and Fig. 4-6.

Phytochemical fingerprints of ethyl acetate extract of heartwood and small branches (stem) showed, nine and five bands respectively, out of which, three band at R_f 0.08, 0.15, 0.30 (all are black) were found similar under UV detection at 254 nm. Under 366 nm, heartwood and small branches showed thirteen and twelve bands respectively, out of which, five bands at R_f 0.11 (blue), 0.30 (blue), 0.40 (blue), 0.44 (light blue), 0.74 (red) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, nine and six bands were visible in heartwood and small branches respectively, out of which six bands at R_f 0.13 (blue), 0.25 (orange), 0.30 (blue), 0.50 (orange), 0.56 (violet), 0.90 (violet) were similar as showed in Table 1 and Fig. 5-8.

Phytochemical fingerprints of ethanol extract of heartwood and small branches (stem) showed total four bands in both the parts and all four bands at R_f 0.16, 0.22, 0.40, 0.68 (All are black) were found similar under UV detection at 254 nm. Under 366 nm, heartwood and small branches showed ten and twelve respectively, out of which, four bands at R_f 0.18 (red), 0.28 (blue), 0.37 (light blue) and 0.63 (light blue) were found similar. After TLC plate derivatized with Anisaldehyde sulphuric acid reagent and visualized under white light, both parts showed ten bands, out of which nine bands at R_f 0.20 (blue), 0.24 (blue), 0.31 (yellow), 0.35 (purple), 0.40 (greenish-blue), 0.44 (orange), 0.57 (orange), 0.87 (blue) were found similar in both parts (Ht. Wd and Sm. Br.) as evident in Table 1 and Fig.10-12.

CONCLUSION

The phytochemical fingerprint profiling of heartwood and small branches of *D. sissoo* were found similar as an official part of *D. sissoo* plant i.e. heartwood, therefore small branches may be used in place of heartwood and vice-versa after comparison and confirmation of same pharmacological activities. TLC phytochemical fingerprint profiling of *n*-hexane, ethyl acetate, ethanolic extracts of heartwood and small branches of *D. sissoo* have been given an idea about the presence of various phytochemicals in their reported parts. The TLC spots provided valuable clue regarding presence or absence of various phytochemicals or metabolites of the plants.

ACKNOWLEDGMENTS

Authors are thankful to Director General, Central Council for Research in Ayurvedic Sciences, New Delhi to provide the financial support under 2nd IMR-PEMC scheme for this research work. Authors are grateful to Dr. R. K. Tiwari, NVRI&H Lucknow for providing the genuine plant materials.

Authors have no conflict of interest

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