Pharmacognosy Reviews, 2024; 18(36):137-150.
www.phcogrev.com

Review Article

Dalbergia sissoo Roxb. ex-DC. - A Monograph

Tangirala Sudhakar Johnson[1,*] , Sujit Nair[1], Anselm de'Souza[1], Lal Hingorani[2,*]

[1]Phytoveda Pvt. Ltd., 1104, Universal Majestic, P. L. Lokhande Marg, Govandi, Mumbai, Maharashtra, INDIA.
[2]31, Park West, West Premises Co-operative Society, Union Park, Pali Hill Road, Khar, Mumbai, Maharashtra, INDIA.

ABSTRACT

Dalbergia sissoo (Indian rosewood) has long been recognized for its remarkable medicinal properties; it offers various therapeutic benefits including its role in treating osteoporosis, microbial infections, skin disorders, diabetes, ulcer and spermatogenesis. *D. sissoo* is also known for its valued timber and leaves as fodder. While its bark has an anthelmintic property, leaves are used as expectorant and wood as an antipyretic and abortifacient. *D. sissoo* is a rich source of phenolics, alkaloids, flavonoids, saponins, phytosterols, tannins and carbohydrates and proteins. Recently, several groups have isolated caviunin glucosides from leaf extract known to possess osteogenic activity. The objective of the present monograph is to provide taxonomical information, traditional and pharmacological uses, methods of simultaneous separation and quantification of bioanalytical marker compounds, safety, toxicity and efficacy of caviunin glucosides. Pharmacognosy, conditions for methods of simultaneous separation and quantification by HPTLC and UHPLC were provided. Review of published resources and authors' own work on safety, toxicity and efficacy has been presented. Description of crude drug, pharmacognosy of bark, heartwood and leaf presented in detail. Separate HPTLC finger printing and UHPLC analysis revealed clear separation of bioactive compounds. Safety, toxicity studies revealed that the extract is safe and well tolerated. Ethanolic extract of leaf has potential osteogenesis property. The details presented in monograph can lead to further studies on beneficial properties of *D. sissoo* leaf extracts, especially bone regeneration and bone health. Caviunin glucosides from leaf, heartwood can be a potential dietary supplement to treat osteoporosis.

Keywords: Caviunin glucoside, Dietary Supplement, Indian rosewood, Osteogenesis, Safety, Toxicity.

Correspondence:
Dr. T. Sudhakar Johnson, Ph. D
Technical Head and Regulations, Phytoveda Pvt. Ltd., #1104, Universal Majestic, P. L. Lokhande Marg, Govandi, Mumbai-400043, Maharashtra, INDIA.
Email: johnson@viridisbiopharma.com

Dr. Lal Hingorani, Ph. D
31, Park West, West Premises Co-operative Society, Union Park, Pali Hill Road, Khar, Mumbai-400052, Maharashtra, INDIA.
Email: lalhingorani@gmail.com

Received: 22-11-2024 ;
Revised: 23-11-2024 ;
Accepted: 25-11-2024

INTRODUCTION

Dalbergia sissoo is a fast-growing multi-purpose tree cultivated for its precious heartwood (sheesam, Indian rosewood). Heartwood, bark and leaf are plant parts of economic importance. The tree is available plenty in wild. Heartwood is hard, strong, durable, elastic and resists decay and hence used as quality timber. Its leaves are useful as fodder. Bark, wood and leaves have many traditional uses. Although the bark has anthelmintic properties, the leaves are used as an expectorant and the wood as an antipyretic and abortifacient. In Nepal and India, extracts of *D. sissoo* leaves are also used to treat gonorrhea, syphilis, dysentery, sore throat and heart problems.[1] *D. sissoo* is native to India and Pakistan growing naturally at an altitude ranging from 1000 m to 1500 m in sub-Himalayan region. This is long-lived tree, deciduous in nature, with a spreading canopy and thick branches. The natural habitat spans a vast region in the Indian subcontinent that extends from Indo-Gangetic plains to Assam except in the regions where climate is too wet or too hot. The species cannot tolerate too cold conditions. *D. sissoo* attains, when fully grown, 30 m height and a 2.4 m girth. Young branches are pubescent. The bark is grey in colour and is thick and rough in texture (Figure 1). Bark has longitudinal fissures, shallow and broad.[2] The compound, imparipinnate and alternate leaves have a rachis that is 3.5-8 cm long and a pulvinus leaf base (Figure 2). There are three to five leaflets that are broadly ovate, cuspidate at the apex; pubescent when young, and turns glabrous at the time of maturity. The inflorescence is an axillary panicle (three to seven cm long) with larger terminal leaflets. The flowers are small, white to yellowish-white with a strong aroma, sessile and bisexual (Figure 3).

Recently, several groups[3-6] reported potential osteogenic activity in *D. sissoo* leaf ethanolic extract and demonstrated its efficacy in human studies. Seven different bioactive compounds in *D. sissoo* including Biochanin A, Biochanin 7-O-glucoside, Biochanin-A-7-O [β-D-apiofuranosyl-(1-6)-β-D-glycopyranoside), Biochanin A 7-O-[β-Dapiofuranosyl-(1-5)-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside, Caviunin 7-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside (CAFG), Genistein and Pratensein were isolated. Dixit *et al.*[12] established

DOI: 10.5530/phrev.20242024

structures using ^{1}H and ^{13}C NMR, COSY, HSQC, HMBC and MS data including IR. Khedgikar *et al.*[7,8] evaluated *D. sissoo* butanolic leaf extract containing CAFG along with other compounds on ovariectomized rates and found that these compounds stimulate new bone formation and was found to be beneficial in fracture healing in rat. After establishing chemical characterization, Dixit *et al.*[3] went on to demonstrate the efficacy of standardized extracts of *D. sissoo* leaves in a single-arm, pilot study in patients with long fractures (n=16). The leaf extract was safe, well tolerated and found to have the potential to treat bone fractures. Later Kothari *et al.*[4] reported chemical characterization of *D. sissoo* leaf extract by UHPLC with PDA and UHPLC-MS/MS. The same group demonstrated that an important component of leaf extract- CAFG has potential to heal bone fractures and bone strengthening. A simple, rapid analytical method for simultaneous isolation and quantification of seven isoflavone glycosides, including CAFG by UHPLC and HPTLC was developed by Saste *et al.*[6] which in addition can be used in quality control method.

While a limited number of publications on monograph are available, reports on safety, toxicity and efficacy, chemical methods of validation of analytical markers in this species is lacking. In the present report we provide an elaborate data on phytochemical constituents, analytical methods for markers separation and quantification, the safety, toxicity and efficacy based on independent in-house research as well as published research.

CITES status

In the CITES COP17 meeting in Johannesburg in 2016, *Dalbergia* genus, including *Dalbergia sissoo*, was included in Appendix II due to concerns about an illegal trade driven by demand for Dalbergia wood in export markets. However, in a more recent CITES COP19 meeting, *D. sissoo* has been delisted from CITES Appendix II, allowing its free trade and export from November 2022. *D. sissoo* is cultivated under afforestation programs and also available aplenty in the wild. It has fast growth rate and capacity to become naturalized outside its native areas, and is identified as invasive plant species in some regions of the world. Owing to harvestable high-quality timber, risk of invasiveness is considered low in many developing countries. Propagation of *D. sissoo* is primarily done through seed propagation and stem cuttings. It comes under 'Least Concern' group of IUCN Red List of Threatened Species.[9]

Taxonomy

Botanical name: *Dalbergia sissoo* Roxb. ex DC.

Synonym: *Amerimnon sissoo* (Roxb. ex DC.) Kuntze

Family: Fabaceae

Common names

English: Sissoo, Indian rosewood

Ayurvedic: Shimshapaa, Krishna shimshapaa, Picchilaa.

Unani: Seesham.

Diversity and distribution

D. sissoo is mainly recognized for its valuable timber and fuel wood. Traditionally, however, every part of the tree has been utilized in folklore in treating various health conditions. It grows plentifully, forming either pure or mixed forests on new alluvial deposits, such as sand and boulders, found in riverbeds. *D. sissoo* serves as a primary colonizer on these fresh alluvial soils along riverbanks. In India, *D. sissoo* trees are found in most parts of the country including Kerala, Karnataka, Tamil Nadu, Manipur, J and K, Arunachal Pradesh, West Bengal, Rajasthan, Haryana, Uttar Predesh, Maharashtra, Madhya Pradesh, Bihar, Orissa, Gujarat.

MATERIALS AND METHODS

Chemicals

Solvents methanol, butanol, ethyl alcohol, glacial acetic acid, acetonitrile and water are of HPLC grade. Reference standards Genistein, Pratensein, Biochanin-A are procured from Sigma-Aldrich, Mumbai, India and Chemfaces, China. Reference standards Biochanin-A-7-O-[β-Dapiofuranosyl-(1-5)-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside, CAFG, Biochanin-7-O-glucoside and Biochanin-A-7-O [β-D-apiofuranosyl-(1-6)- β-D-glycopyranoside were developed at the authors' lab.

Leaf Microscopy

Fresh leaves were collected from *D. sissoo*. For microscopy, thin and uniform hand sections were made and stained with diluted safranin, mounted temporarily in glycerine on a slide with a cover slip, sealed with wax and labeled. The cross sections were made using new stainless-steel blade or sharp knife, keeping the plant material in the pith. They were observed under compound microscope at suitable magnifications and photographed.

Plant Material

D. sissoo fresh leaves were purchased from a vendor. Shade dried leaves were used in subsequent analysis.

Raw material specifications

The specifications for raw material testing were developed as per USP standards. Leaf raw material specifications were provided in Table 1.

HPTLC analysis

One gm of powdered leaf was mixed with 10 mL of methanol, vortexed for 1-2 min, followed by 10 min sonication pulse. The homogenised mixture was pelleted down at 5000 rpm by centrifuging for 5 min. The subsequent supernatant was filtered through membrane filter (0.2 μm) before using for HPTLC

Figure 1: *D. sissoo* Bark.

Figure 2: Plant twig with leaves.

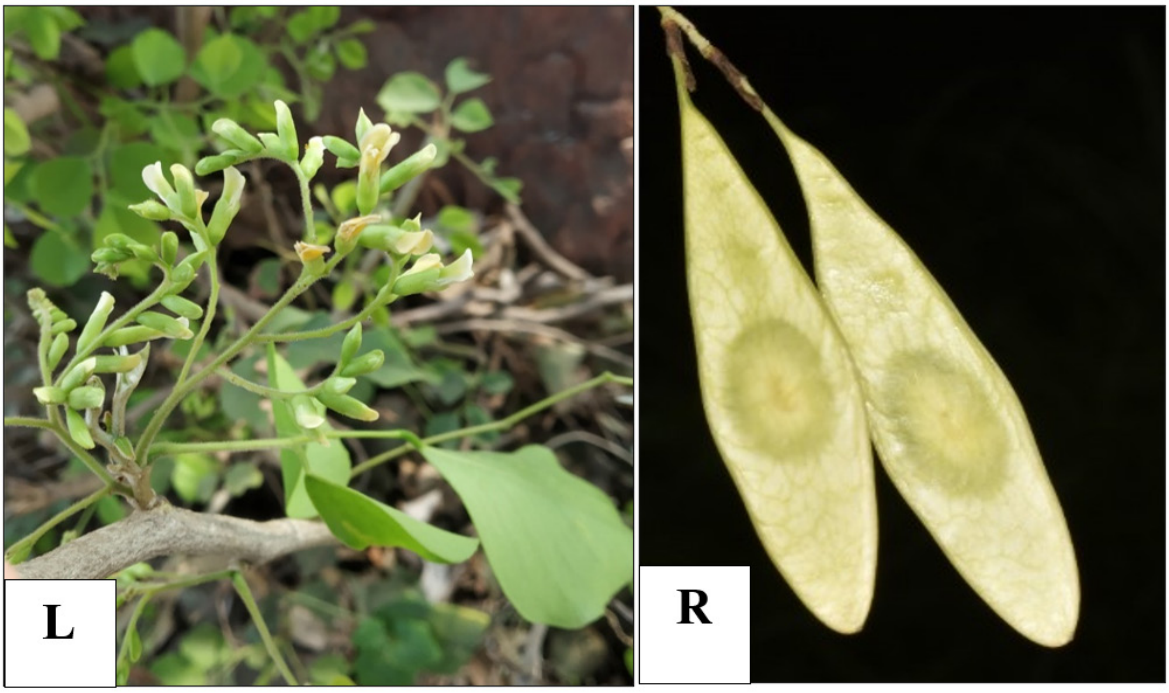

Figure 3: Inflorescence (L) and Legume (R).

analysis (CAMAG) with Linomat 5 automatic sample applicator. As described in Saste *et al.*[6] TLC scanner III was used to analyse. Mobile phase used was ethyl acetate: toluene: methanol: formic acid in the ratio of 7:2:2.2:0.5 (v/v/v/v). The developed TLC plates were dried and viewed in TLC visualizer 2 at 254 nm and 366 nm. Digital images of data were acquired using the visualizer's digital camera.

UHPLC analysis

HPLC was carried out on Shimadzu N-series (XS AI-PDA UHPLC-PDA). The conditions for chromatographic separation, mobile phase, solvent gradient program was followed as per Saste *et al.*[6] The HPLC run time was 14 min, mobile phase at 0.25 µL/min flow rate was set with injection volume 2.0 µL; eluents were detected at 254 nm.

RESULTS AND DISCUSSION

Description of crude drug

API described crude drug as dried stem bark (Shimsapa) in its pharmacopoeia (API, Part-I, Vol. III). Similarly, Khare[10] described stem bark and heart wood as crude drugs whereas Khare[11] described leaves, wood and bark having therapeutic action. Several researchers including the authors' group reported

Table 1: Raw material specification (dried leaf).

Sl. No.	Description	Specifications
1	Foreign matter	NMT 2.0%
2	Total ash	NMT 8.0%
3	Loss on drying	NMT 10.0%
4	Acid insoluble ash	NMT 2.0%
5	Alcohol soluble extractive	NLT 3.0%
6	Water soluble extractive	NLT 20.0%
7	Analytical markers (leaf)Total bitter	NLT 2.0%
8	Total polysaccharide	NLT 10.0%
9	CAFG	NLT 0.15%

isolation of novel compounds from leaves (Figure 4A and B) that have osteogenic activity. Few reports have been published on importance of hydroalcoholic extract of leaf towards its bone health and fracture healing (osteogenic) properties.[3-5,7,8,12,13] In this report, we have provided the details of above studies under various sections such as chemistry, safety, toxicity and efficacy data. Specifications of leaf raw material was provided in Table 1.

Traditional Uses

D. sissoo is a good source of phenolics, alkaloids, phytosterols, flavonoids, saponins, tannins and carbohydrates and proteins with well-established medicinal properties both in traditional medicine and folklore.

Ethnopharmacology

D. sissoo has been shown to possess established medicinal properties to treat a range of indications such as microbial infections, diabetes, ulcers, spermatogenesis issues, skin disorders, diarrhea, and osteoporosis. Comprehensive details on the ethnopharmacological properties of *D. sissoo* have been documented.[14] The ethanol extract *of D. sissoo* leaf exhibits notable antipyretic and mild analgesic properties. In experimental models, this extract significantly reduced inflammation without adversely affecting the gastrointestinal mucosa. In rural regions of Nepal and India, *D. sissoo* leaves are used to treat animals suffering from generalized diarrhoea. Additionally, in Jordan, the leaf extract has been employed to treat dysentery, heart conditions, gonorrhea and syphilis. *D. sissoo* leaf extract's antioxidant activity is nearly twice as potent as that of commonly used selenium and vitamin E. The extract from the leaves is also efficient in improving blood quality. It is effective as an anthelmintic. It also can be used to treat nasal and eye conditions. Analysis of the leaf extract revealed that compounds biochanin A, genistein, biochanin 7-O-glucoside, pratensein and CAFG exhibited osteogenic activity.

Pharmacological Uses

The ethanol extract of *D. sissoo* bark exhibited anti-inflammatory[15] and nociceptive[16] effects, attributed to its flavonoids. Kaur *et al.*[17] demonstrated that bark chloroform extract possessed marked anti-oxidant activity, whereas methanolic extract possessed moderate activity. Leaf extract displayed anti-inflammatory activity in chronic, acute and sub-acute models.[18] The reader is directed to consult a recent publication by Bharath *et al.*[19] for detailed information on pharmacological uses. Some important publications providing a wide range of pharmacological activities showed that n-butanol, petroleum ether, ethyl acetate and ethanolic leaf extracts had antidiabetic activity in alloxan-induced diabetic rats.[20,21] Further, Pund *et al.*[22] showed antidiabetic activity of ethanolic bark extracts while seed extracts had peripheral analgesic and moderate antipyretic activity.[23] In evaluating the cytotoxicity and melanogenic activity of the ethyl acetate fractions from *D. sissoo* bark, it was found that the extract fractions stimulate B16F10 melanogenesis at very low concentrations and can be used in the treatment of hypopigmentation diseases such as vitiligo.[24] The neuroprotective effects of daily oral doses of 300 and 600 mg/kg of ethanol extracts from *D. sissoo* leaves were evaluated using the Passive Avoidance (PA) method. The results suggest that *D. sissoo* leaves hold potential as a treatment for various neurological disorders.[25] Phytochemical screening and nephro-protective activity of Ethanolic Leaf extract of *D. sissoo* (ELDS) has been conducted by Saxena *et al.*[26] Ethanolic extract of *D. sissoo* leaf at concentration (100 and 200 mg/kg) was tested for nephroprotective activity which suggested improved renal function to gentamycin-induced nephrotoxicity.[26]

Osteogenic properties of *D. sissoo* leaf extract

The osteogenic property of *D. sissoo* leaf extracts has been the subject of several published reports. A novel isoflavone glucoside, CAFG, (E)-4-methoxy-2- (3, 4-dihydroxybenzylidene)-4-oxobutanoicacid and a number of flavonols and isoflavones with their glucosides, and a lignan glucoside with a potential osteogenic activity has been isolated. These compounds were

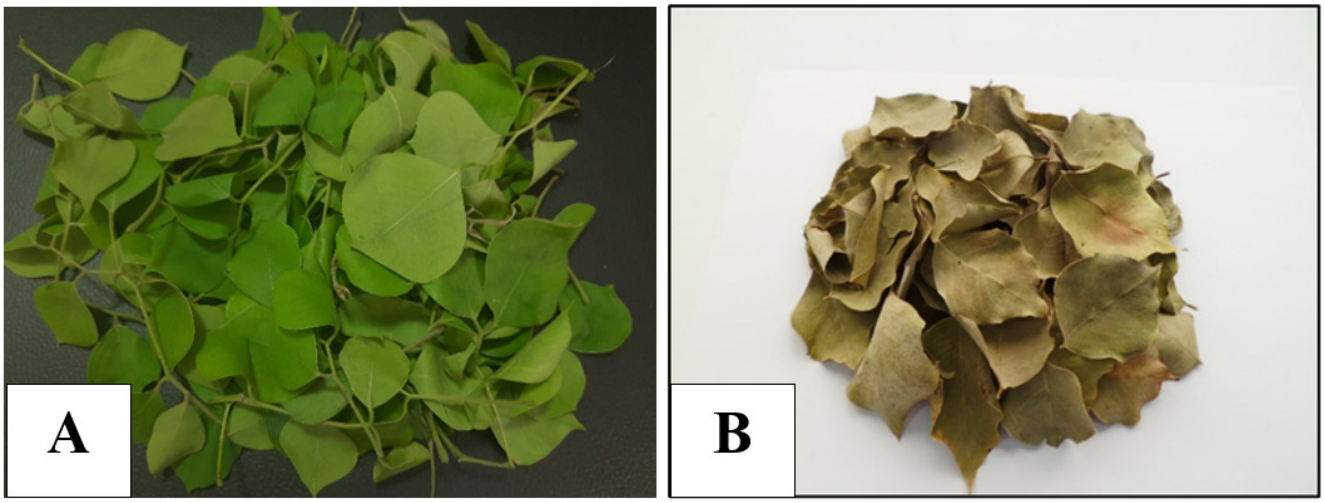

Figure 4: Fresh leaves (A) and dried leaves (B).

reported to exhibit osteogenic property in primary calvarial osteoblast cells.[12]

In addition to leaf extract, neoflavonoids were isolated from *D. sissoo* heartwood which also showed promising anti-osteoporotic activity *in vitro*. A novel compound, dalsissooal was identified from the extract. In addition to dalsissooal, known compounds dalbergin, cearoin, dalbergiphenol, 4-methoxy dalbergion, methyl dalbergin, dalbergichromene and latinone were identified. ALP activity and mineralization in calvarial osteoblast cells indicated enhanced proliferation in several of the neoflavonoids mentioned above.[27] In primary calvarial osteoblast cells, the novel isoflavone glucoside, CAFG, and four previously identified compounds—biochanin A, genistein, biochanin 7-O-glucoside and pratensein—isolated from *D. sissoo*'s leaves shown osteogenic activity.[29] The compounds exhibited elevated levels of ALP activity and mineralization, indicating possible osteogenic activity. The above compounds were evaluated on calvarial osteoblast cells at concentrations from 1 pM to 1 μM to screen both ALP activity and stimulatory effect on osteoblast formation mediated by the Estrogen Receptor (ER). It was found that the differentiation of osteoblast did not depend on the ER. With the exception of biochanin A's negligible reaction, CAFG, pratensein, genistein and biochanin 7-O-glucoside were unable to prevent the synthesis of ALP. Osteoblast development was suppressed by biochanin A at a dose of 10 nM when ICI-182780 (antiestrogen) was present.

In osteoblast cultures, five compounds reported in the study caused the development of mineralized nodules; out of the five compounds, CAFG had the highest potency.[13] Further assessment of CAFG's effectiveness and safety was conducted.

CAFG from leaf as a potential nutraceutical/health supplement for the management of osteoporosis and bone fracture healing

D. sissoo leaf and pod extracts displayed anti-resorptive and bone forming properties. CAFG, isolated from *D. sissoo*, showed high activity and was proposed as an osteoporosis therapeutic alternative to anabolic treatment. The suggested route of action is that it stimulates the Wnt/β-catenin pathway and Bone Morphogenetic Protein 2 (BMP2). In comparison to genistein, CAFG supplementation enhanced the trabecular microarchitecture of the long bones, raised the biomechanical strength metrics of the vertebra and femur followed by decreased markers of bone turnover. When osteopenic ovariectomized mice were given oral CAFG, their bone marrow's osteoprogenitor cells increased and their femur's osteogenic gene expression increased, resulting in the creation of new bone without uterine hyperplasia. By blocking skeletal osteoclastogenic genes, CAFG increased the mRNA expression of osteoprotegerin in bone and prevented osteoclast activation. In the mouse femur, CAFG also accelerated chondrogenesis and had a stimulating effect on the repair of cortical bone following drill-hole damage at the tissue, cell, and DNA levels. Cellularly, osteoblast survival, proliferation, and differentiation were all enhanced by CAFG. Signal transduction inhibitors in osteoblasts revealed the participation of p-38 MAP kinase pathway stimulated by BMP2 to initiate Wnt/β-catenin signaling to reduce phosphorylation of GSK3-β and subsequent nuclear accumulation of β-catenin. By modulating MSC reactivity to BMP2, CAFG promoted osteoblast development through the Wnt/β-catenin pathway. When compared to phytoestrogen genistein, CAFG at dose of 1 mg/kg bw/day in ovariectomized mice (human dose approximately 0.081 mg/kg) improved bone production, decreased bone resorption and bone turnover. Considering the positive outcomes mentioned above, CAFG may be projected as a possible nutraceutical for the treatment of postmenopausal osteoporosis and fractures.[28]

Dalbergiphenol from heartwood can enhance osteoblastic activity

The impact of another neoflavonoid, dalbergiphenol (DGP), extracted from heartwood, on bone loss was evaluated in ovariectomized mice.[29] After ovariectomies, adult BALB/c mice

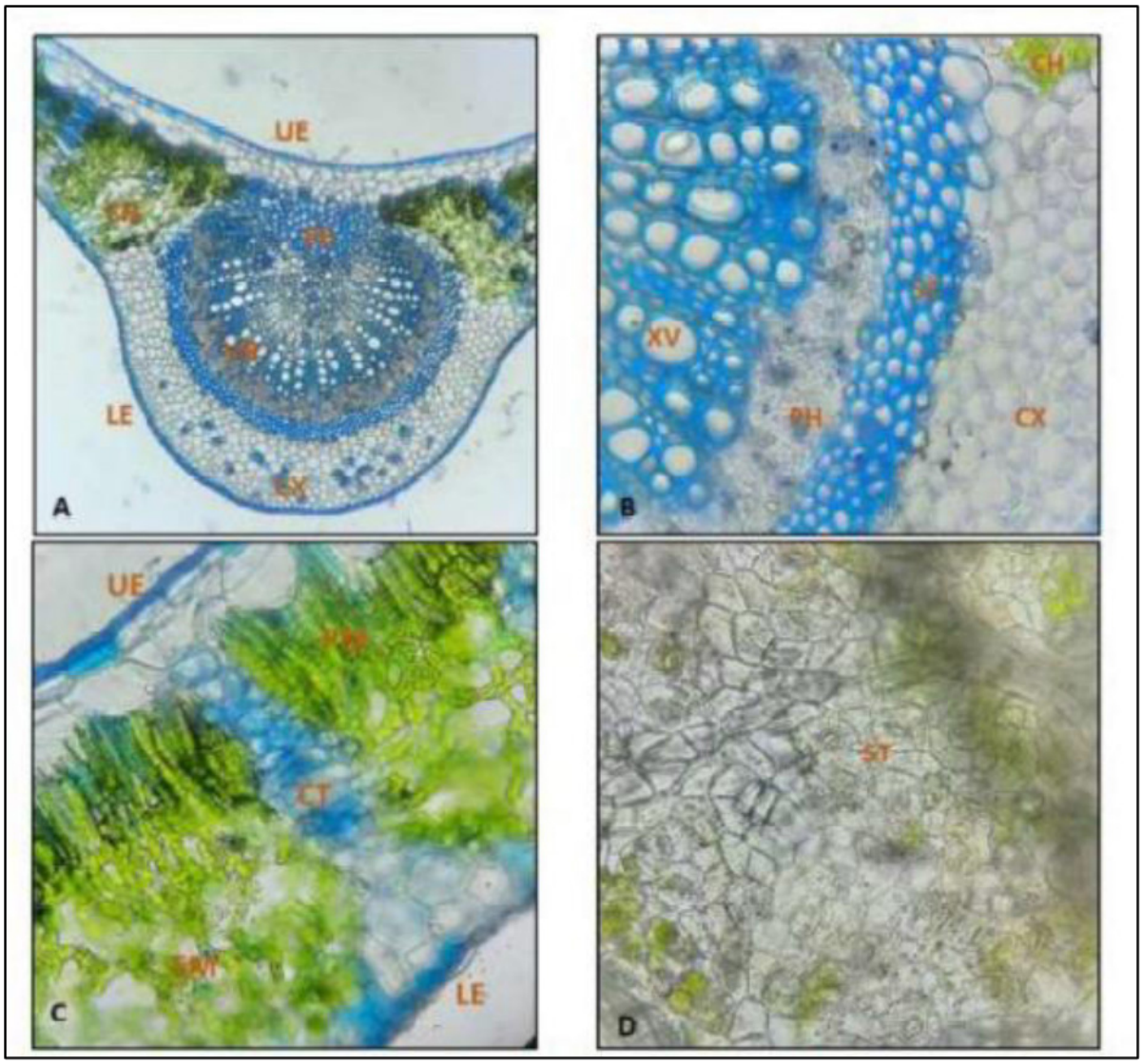

Figure 5: Microscopic structures of *D. sissoo* leaflet, A) outline through midrib (10 x), B) conducting tissues inside pericycle (40 x), C) Leaf lamina (40 x), D) Epidermal peel (40 x).

were administered DGP or 17β-estradiol (E2) orally for six weeks at two different doses of 1 and 5 mg/kg/d. Uterine estrogenicity, bone microarchitecture, biomechanical strength, production of new bone, and the skeletal expression of osteogenic and resorptive gene markers were among the endpoints evaluated. Significant increases in body weight and decreases in femoral and vertebral trabecular bone volume were seen in both the sham group and the Ovariectomy (OVX) control; these effects were lessened by DGP or E2 therapy. Similar to E2 treatment, DGP treatment increased new bone formation rates and bone biomechanical strength in ovariectomized mice. On the other hand, increased uterine weight and estrogenicity were observed with E2 treatment, but not with DGP treatment. Reduced mRNA expression of tartrate-resistant acid phosphatase and the ratio of osteoprotegerin to receptor activator of nuclear factor-κB ligand was observed in the femur of ovariectomized mice treated with DGP, whereas increased mRNA expression of transcription factor 2, osterix, and collagen type I was observed. As a result, the authors concluded that DGP therapy, possibly by enhancing osteoblastic and reducing osteoclastic activity, can effectively reverse the OVX-induced increase in bone loss and decrease in bone strength.[24]

Leaf and pod extracts have potential osteoprotective benefits

In ovariectomized rats, a model for postmenopausal osteopenia, the effects of a Butanol-Soluble Standardized Fraction (BSSF) derived from the leaves and pods of *D. sissoo* were investigated. The trabecular microarchitecture of long bones, biomechanical strength parameters in the vertebra and femur, and the expression of osteoclastogenic genes were all improved by BSSF treatment in comparison to ovariectomized rats treated with a vehicle. Additionally, bone turnover markers (osteocalcin and type I collagen) were decreased. Furthermore, in the femur, BSSF

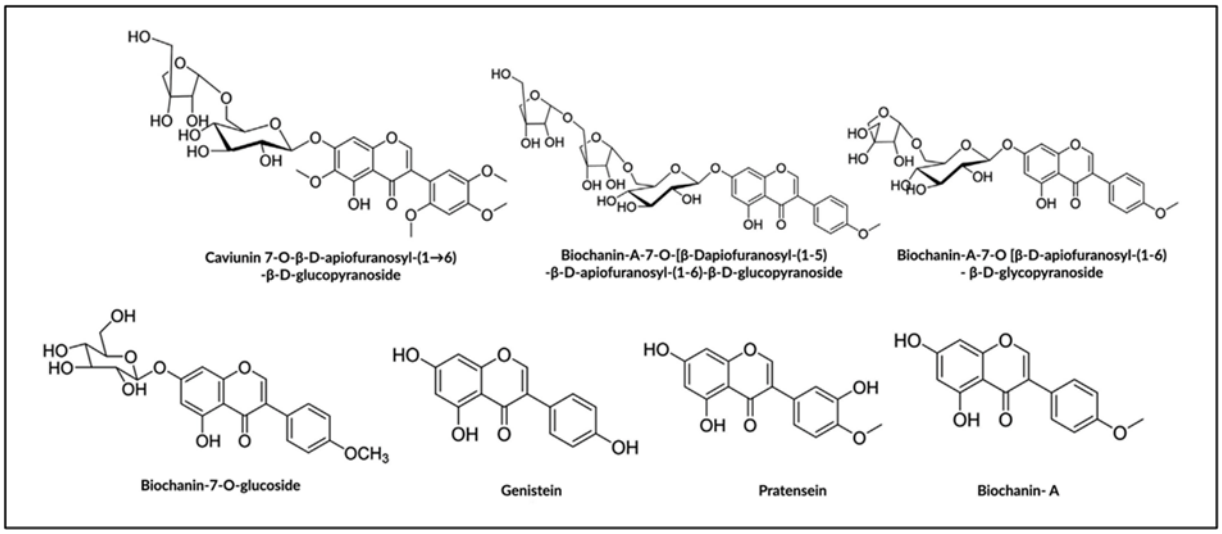

Figure 6: Active biochemicals isolated from *D. sissoo* leaf.

enhanced the expression of osteogenic genes and the production of new bone. It was discovered that the osteoprotective properties of BSSF were similar to those of 17β-estradiol.[7]

Pharmacognosy of Bark, Heartwood and leaf Microscopy

Bark

Organoleptic characters: Smooth thin small fragments, astringent in taste, light brown in colour and indistinct odour.[30]

Macroscopy: Grey or light brown, young parts, grey downy, inner side light brown, later turning to dark brown. Thickness: 0.1 to 3-5 cm. Bark reticulately longitudinally furrowed, exfoliating in narrow irregular woody strips and scales, fibrous. Odour: Indistinct or non-specific.

Microscopy: The transverse section of the bark reveals 6 to 25 or more rows of rectangular, thin-walled cork cells arranged radially, with a few outer layers exfoliating. The secondary cortex is wide and consists of round or oval, thin-walled parenchymatous cells scattered throughout. Some cortical cells contain prismatic crystals of calcium oxalate. The secondary phloem is very wide, comprising typical elements of thin-walled cells and tangential strips of phloem fibers. Collapsed, thin-walled parenchymatous cells are present in tangential strips throughout the secondary phloem. Most phloem fibers and parenchyma cells contain calcium oxalate crystals. The phloem rays are short and range from uni- to tri-seriate, featuring radially elongated thin-walled parenchymatous cells.

Powder microscopy: Powder is light brown in colour; the presence of trichomes, cork cells, xylem fibres, tracheids and starch grains were noticed. It further shows thin-walled parenchymatous cells, phloem fibres, fragments of cork cells and prismatic cells of calcium oxalate.

Heartwood

Macroscopy: Dried longitudinally broken piece of heartwood is 5-7 cm in length and 2-3 cm in width, 0.8-1 cm in thickness; Heartwood shows concentric bands of annular rings; break hard; colour dark brown; Taste slightly astringent; odour slightly aromatic.[31]

Microscopy: TS of wood is dark reddish brown in colour and shows isolated or in groups of two, oval to circular xylem vessel often showing ingrowths of tylosis; uni- to triseriate, pitted medullary rays; compactly arranged thick-walled groups of xylem fibres occupying the major area of the section and vasicentric xylem parenchyma, which frequently runs in tangential bands and are loaded with colouring matter and tannins. RLS shows medullary rays crossing pitted vessels, groups of thick-walled fibres and pitted parenchyma, while in TLS medullary rays appear as vertically running lenticular masses of 4 to 14 celled high and 1 to 3 celled wide.

Powder microscopy: Powder microscopy shows pitted medullary ray cells crossing the bordered pitted vessels and other elements in radially longitudinal cut fragments of wood and lenticular masses of medullary rays in tangentially cut pieces; thick-walled lignified fibres; fragments of dark brown colouring matter scattered or loaded in the vessels.[31]

Leaves (in-house data)

Macroscopy: Dried leaves as raw material may contain broken leaves in which pinnate venation is helpful in identification. The midrib is more apparent in the lower surface while the secondary veins are prominent on the upper surface of the leaves.

Colour: Yellowish to greyish green

Odor: Characteristic bitter

Taste: Bitter

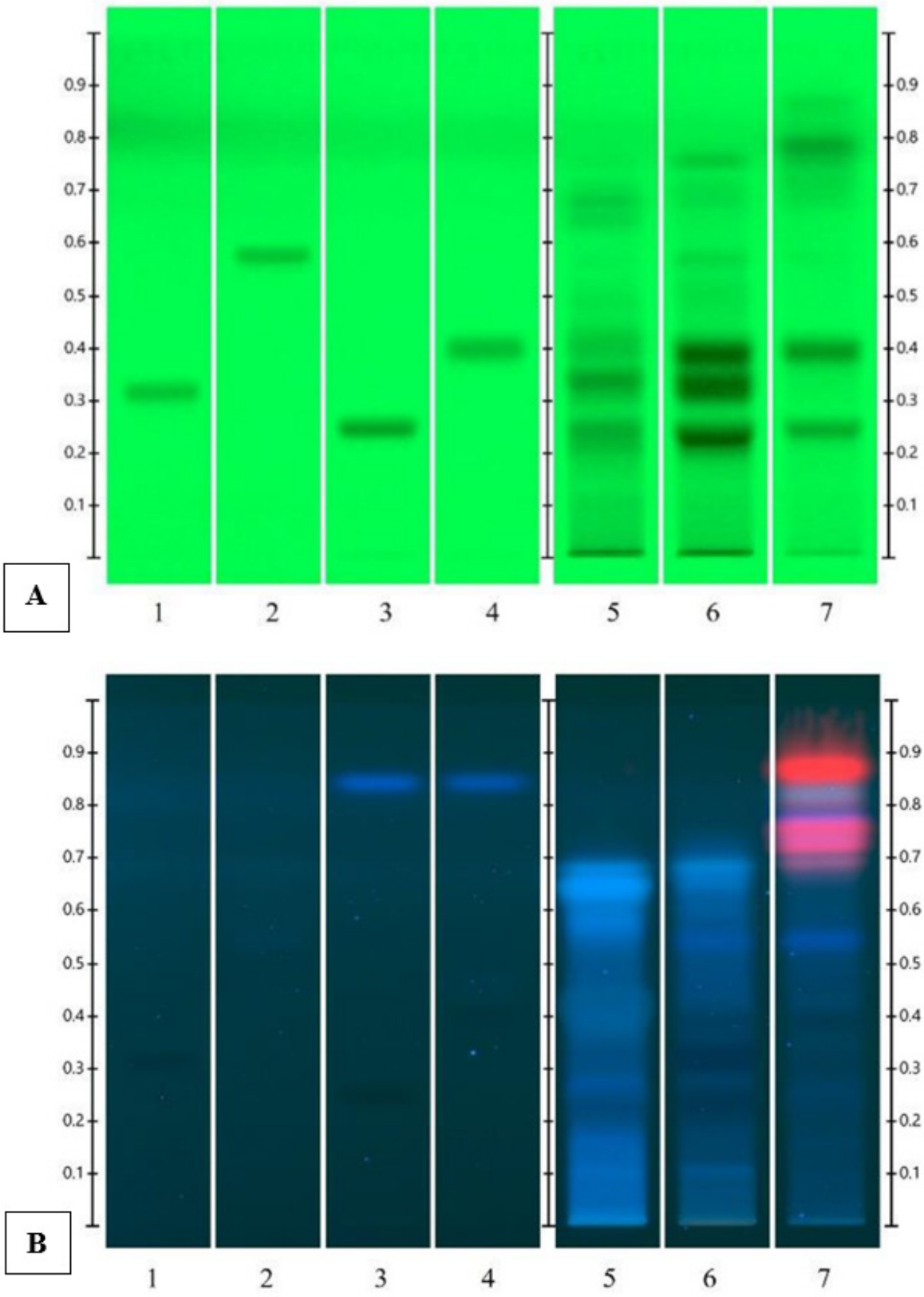

Figure 7A and B: HPTLC profiles of *D. sissoo* standard compounds, leaf raw material and leaf extract under UV 254 nm (A) under UV 366 nm (B), prior to derivatization. Lane 1: CAFG std; R_f 0.31; Lane 2: Biochanin-7-o-glucoside std; R_f: 0.57; Lane 3: Biochanin-A-7-O-[β-Dapiofuranosyl-(1-5)-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside std; R_f: 0.24; Lane 4: Biochanin-A-7-O [β-D-apiofuranosyl-(1-6)- β-D-glycopyranosid std; R_f: 0.39; Lane 5: *D. sissoo* leaf extract 01; Lane 6: *D. sissoo* leaf extract 02; Lane 7: *D. sissoo* leaf raw material (source: DS-HPTLC-Id testing report-authors' pers. data).

Microscopy: A transverse section of the leaf passing through the midrib reveals a rounded abaxial surface and a flat adaxial surface. The leaf is dorsiventral in structure, with a single layer of transversely elongated, rectangular cells making up the epidermis. Compared to abaxial epidermal cells, adaxial epidermal cells are bigger. There are two or four lines of collenchyma cells in the hypodermal area of the adaxial and abaxial epidermis. The lamina's hypodermis is composed of big, rectangular parenchyma cells. In the center is a large arc shaped collateral vascular bundle. Both the adaxial and abaxial sides of the vascular bundles contain sclerenchyma fibers. There are two rows of closely packed columnar cells that make up the palisade tissue. Five to seven rows of loosely distributed spherical parenchyma cells make up the spongy tissue. The mesophyll tissue contains small crystalline grains, prisms, or rod-shaped crystals. The leaf's smaller veins are vertically transcurrent. The adaxial foliar epidermis consists of polygonal parenchyma cells with straight walls and lacks stomata. In contrast, the abaxial epidermis is made up of smaller polygonal cells with straight walls and is perforated by paracytic stomata (Figure 5).

Chemical constituents

Analytical test methods (both HPTLC and UHPLC) for quantification of active constituents (Figure 6) in raw material (leaf) that contain CAFG, Genistein, Biochanin-7-O-Glucoside, Pratensein and Biochanin A given (Figure 6).

HPTLC fingerprinting

HPTLC separation of *D. sissoo* standards, leaf raw material and leaf extract were demonstrated. HPTLC chromatogram of major chemical components CAFG and *D. sissoo* flavonoids is presented in Figure 7 A and B. R_f values of standards of CAFG observed at 0.31, Biochanin-7-o-glucoside at 0.57, Biochanin-A-7-O-[β-Dapiofuranosyl-(1-5)-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside at 0.24 and Biochanin-A-7-O [β-D-apiofuranosyl-(1-6)- β-D-glycopyranoside at 0.39.

UHPLC Assay

Separation and simultaneous quantitative determination of CAFG, Genistein, Pratensein, Biochanin-A-7-O-[β-Dapiofuranosyl-(1-5)-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside, Biochanin-A-7-O [β-D-apiofuranosyl-(1-6)- β-D-glycopyranoside, Biochanin-7-O-glucoside, Biochanin-A by UHPLC in leaf extract has been performed (Figures 8A and B). The mobile phase with gradient flow of water-acetonitrile yielded best separation. The quantification of compounds was carried out using PDA detector at 259 nm in 14 min.[6] One could achieve separation of seven bioanalytical markers from leaf extract.

History of safe use

D. sissoo has been used in traditional medicine and in Ayurveda since old times and has long history of safe use. Use of *D. sissoo* as folk medicine dates back to 1939. There may be reports available prior to 1939, but we report here the available report. In Ayurveda, use of *D. sissoo* stem bark and heart wood has been reported (API, Part-I and Vol-III). *D. sissoo* was introduced to Egypt by Ibrahim Pasha as an ornamental and timber, in the age of Mohamed Ali (1805-1848).[32] In addition to being used as an antihelminthic and antipyretic and to treat a variety of skin and digestive conditions, it has also been used in folk medicine as an aphrodisiac, abortifacient, and expectorant.[15] Ayurvedic medicine uses wood and bark to treat a variety of conditions, including dyspepsia, leukoderma, anal problems, blood disorders, burning sensations, and skin-related issues.[19] The wood and bark of *D. sissoo* are used in Ayurveda medicine to treat a number of ailments, such as blood disorders, leukoderma, burning sensations, diarrhea, and dyspepsia. An infusion of the bark is used to clean wounds. The roots are used to treat abdominal pain, hernias, and induce labor. The heartwood is applied topically to treat vitiligo, herpes and fever. Additionally, oil obtained from the seeds is used to treat various skin conditions.[33] Considering its safe use since 1939 and its use in Ayurveda, *D. sissoo* is considered to be safe.

Safety studies

Several researchers studied safety and toxicity of butanolic, methanol and ethanol extracts of leaf of *D. sissoo*; We present here a detailed account of studies conducted using Methanolic Extract of *D. sissoo* leaves (MEDS), *D. sissoo* Ethanolic Leaf Extract (DSELE), *D. sissoo* Ethanolic Extract (DSEE), standardised *D. sissoo* Leaf Extract (DSLE), Standardised Extract of *D. sissoo* Leaf Extract (SEL-Ds), *D. sissoo* Extract (DSE) among the others.

Subacute Oral Toxicity Study

Using an ethanolic extract of *D. sissoo* leaves at 200 mg/kg, 500 mg/kg, and 1000 mg/kg body weight from day 1 to day 28, a 28-day subacute oral toxicity study was carried out on Wistar rats. It was found that all the animals survived the treatment after 28 days and no signs of toxicity were observed compared to control group.[34] Results of haematological, serum biochemistry, kidney tissues showed no significant lesions or changes.

Acute toxicity studies

Alcoholic bark extracts were used in acute toxicity tests on Wistar rats at doses of 50, 100, 300, 1000, and 3000 mg/kg body weight. According to Asif and Kumar,[16] these studies did not find any toxic symptoms at doses higher than 3000 mg/kg body weight. The same group[15] used a standard of 10 mg/kg Indomethacin to evaluate bark extract. Both toxic symptoms and death were absent from the acute toxicity studies up to the maximum dose of 3000 mg/kg body weight. Additionally, it was reported by Ul-Islam and Elhddad[35] as well as Asif and Kumar[15] that a dose level of 3000 mg/kg body weight was safe. *D. sissoo* at 5000 mg/kg was given orally once without causing any toxicity or or mortality within

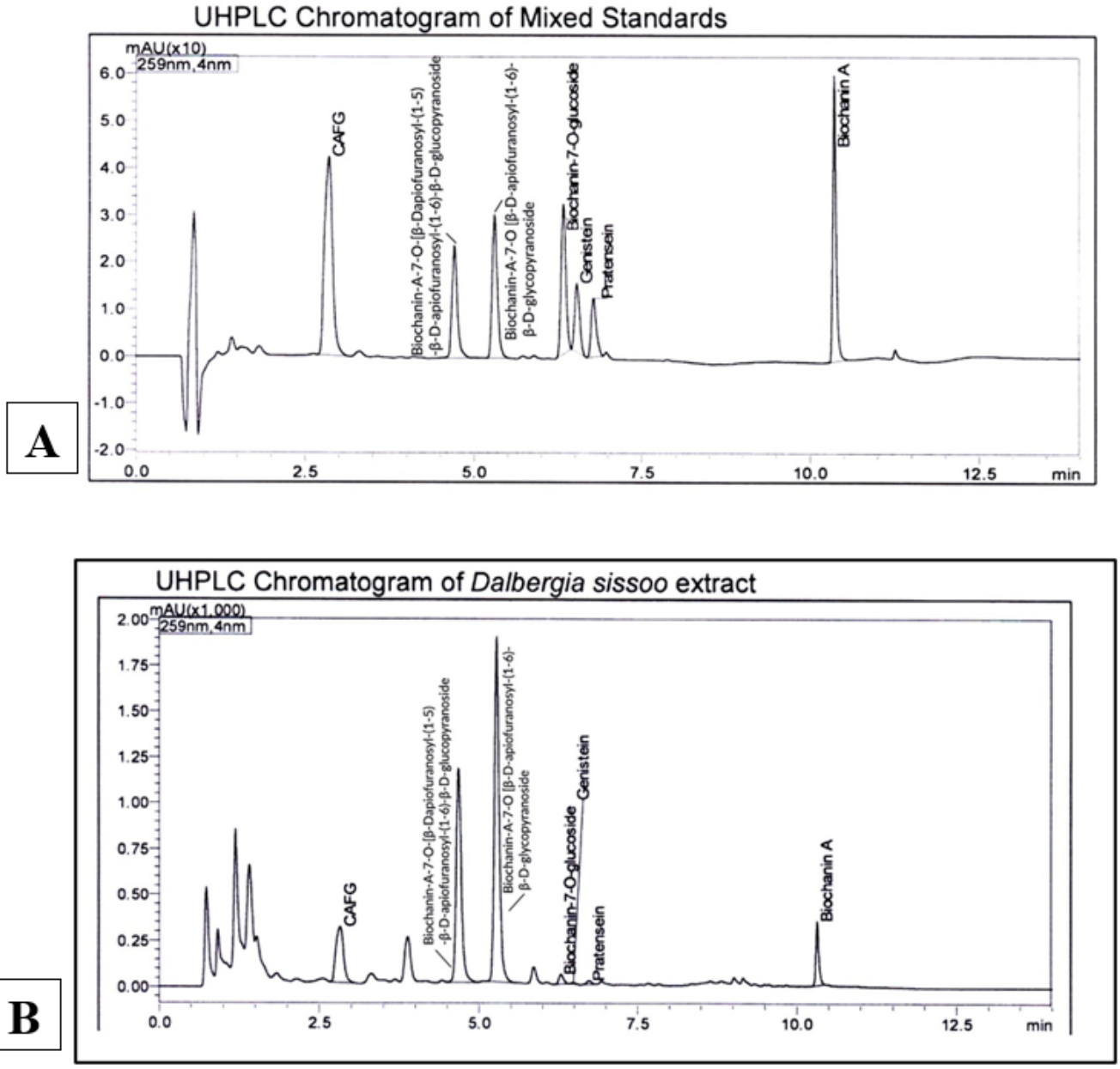

Figure 8 A and B: UHPLC chromatogram of mixed standards (A) and D. sissoo leaf extract (B); Rt= CAFG: 2.98, Biochanin-A-7-O-[β-Dapiofuranosyl-(1-5)-β-D-apiofuranosyl-(1-6)-β-D-glucopyranoside: 4.83, Biochanin-A-7-O [β-D-apiofuranosyl-(1-6)- β-D-glycopyranoside: 5.4, Biochanin-7-O-glucoside: 6.44, Genistein: 6.64, Pratensein: 6.90, Biochanin-A: 10.42 (source: MoA-DS-7 Compound- authors' pers. data).

4 hr when continuously observed in all animals. This dosage was found to be safe for mice.[22] In mice, Sehra and Sharma[36] conducted experiments at doses ranging from 50 to 3000 mg/kg body weight did not show any signs of toxicity or abnormality. There were no changes in body weight or behavior after seven days of oral administration. However, given that the LD_{50} was discovered to be greater than 3000 mg/kg, it can be assumed that the Methanol Extract of *D. sissoo* leaves (MEDS) has low toxicity.[37] Following an overnight fast, alcoholic bark extracts at doses of 300, 1000, and 3000 mg/kg body weight did not result in any toxic effects or animal deaths.[35] Furthermore, rats given an oral 90% ethanolic extract maintained stability up to a dose of 3000 mg/kg.[15] In summary, studies on the extract's acute toxicity in rats did not show any toxic effects or fatalities. The liver and kidney only experienced slight histological alterations as a result of the extracts' administration. The extract had no cumulative effects when taken over an extended period of time.[38]

Subsequently, mice were used in acute toxicity tests with doses of 50, 100, 300, 1000, and 3000 mg/kg body weight of *D. sissoo* leaves (MEDS). All mice survived and showed no signs of toxicity or abnormality in the above dose limits, up to 3000 mg/kg b.w., after the 24-hr administration. No abnormal signs or changes in body weight or behavior were noted over the course of seven

days. With an LD_{50} greater than 3000 mg/kg, it was determined that MEDS has a low toxicity profile based on these data.[37] In a different study, *D. sissoo* Ethanolic Leaf Extract (DSELE) was given to mice of both sexes at doses of 3.00, 4.50, 6.75, and 10.125 g/kg, with a control group.[18] For up to 24 hr, observations were made regarding toxic symptoms and mortality. However, no acute toxic effects or fatalities were noted, even at the maximum dose of 10.125 g/kg, making it difficult to calculate the extract's LD_{50}. The conclusion that DSELE is safe, even at high doses, was reached in mice due to their high LD_{50}, lack of mortality, and lack of toxic effects.

Cytotoxicity studies using *in vitro* models

An alternate model for evaluating the cytotoxic effects of pre-clinical drugs on developing embryos is zebrafish. Over the course of 72 hr, the acute toxicity of dalpatein and *D. sissoo* Ethanolic Extract (DSEE) was evaluated in freshly fertilized zebrafish eggs. The coagulation of eggs, lack of somite formation, inability of the tail-bud to separate from the yolk sac, absence of heartbeat, and mortality were the parameters used to evaluate acute lethality in zebrafish. In 24-well plates, dalapatein and DSEE (10 embryos/well) were used at concentrations of 1, 10, 100 µg/L, and 1, 10 mg/L. The control group was DMSO. In the DMSO and water mediums at 1 µL/L and 10 µL/L concentrations, no mortality was seen. On the other hand, at 100 µL/L, 1 mg/L, and 10 mg/L of dalpatein, mortality rates of 10%, 30%, and 40% were noted, respectively. 20% at 100 µL/L, 40% at 1 mg/L, and 50% at 10 mg/L were the mortality rates for DSEE. Higher doses were associated with a noticeably lower survival rate.[39] Overall, zebrafish embryos showed very little toxicity to DSEE extract and dalpatein, with LC_{50} values of 10 mg/L and 100 mg/L, respectively, suggesting their biosafety.

Sub-chronic study

Wistar rats were employed in a repeat-dose sub-chronic toxicity (for 90 days) study to evaluate the safety of a standardized *D. sissoo* Leaf Extract (DSLE). Four groups participated in the experiments. Group I–III: control, 0; low dose, 310; mid dose, 620 mg/kg body weight/day; Group IV: high dose, 1240 mg/kg body weight/day; were applied, respectively. For the duration of the recovery trial, two more animal groups received 0 (Group V) and 1240 (Group VI) mg/kg/day of DSLE, respectively, and were then left without treatment for 28 days. The use levels of DSLE in the 90-day and recovery study were established using a human equivalent dose of 5 mg/kg/day of CAFG and a study report in ovariectomized mice that showed improved bone formation.[28] A number of parameters were tracked, including PCV, Mean Corpuscular Hemoglobin (MCH), eosinophils, lymphocytes, monocytes, Activated Partial Prothrombin Time (APTT), Prothrombin Time (PT), and platelets. At dose levels up to 1240 mg/kg/day, it was found that there were no treatment-related significant effects of DSLE on body weight or body weight gain when compared to the control group. The recovery group's body weight gain did not change significantly over the course of the trial. The results suggest that rats given DSLE at doses up to 1240 mg/kg/day for 90 days did not experience any negative effects on body weight or clinical observations. In the recovery group, there were also no negative effects.[5] The DSLE's NOAEL was 1240 mg/kg body weight/day.

Mutagenicity/Genotoxicity

In genotoxicity studies, the adverse effects of a standardized *D. sissoo* Leaf Extract (DSLE) were assessed. *Salmonella typhimurium* TA98, TA100, TA102, TA1535, and TA1537 were used to conduct the test. The DSLE was standardized to include a minimum of 2.0% CAFG and a minimum of 6.0% total flavonoids, which contained Genistein, Biochanin-7-O-Glucoside, Pratensein and Biochanin A. Test substance was used at 0.002, 0.002, 1.582, and 5 mg/plate in the presence of metabolic activation (+S9) or in the absence of metabolic activation (-). The results demonstrate that following DSLE treatment, there was no appreciable increase in the number of revertant colonies of any testing strains, regardless of dosage. The results indicate that there was no appreciable increase in the number of revertant cases, irrespective of dosage.

No evidence of high mutation rates with rising concentrations was noticed. In all tester strains, mutagens demonstrated a noticeable rise in induced revertant colonies in both, when metabolic activity is present and absent, without demonstrating cytotoxicity. Consequently, it was determined that, within the experimental conditions, the DSLE tested was not mutagenic.[5]

Additionally, an investigation was carried out on albino Wistar rats to assess the antibacterial, cytotoxic, mutagenic, and antiepileptic properties of ethanol extracts derived from *D. sissoo*'s bark and leaves. The antibacterial activity and mutagenicity of three bacterial and three fungus strains were assessed. *D. sissoo* bark, test sample 1 revealed a slight mutagenic activity, with 9 out of 96 wells against *S. typhimurium* strain TA98 and 5 out of 96 wells against TA100 displaying a yellowish colour. Similarly, *D. sissoo* leaf, test sample 2 demonstrated its non-mutagenicity by displaying a yellowish coloration in 7 out of 96 wells against TA98 and 3 out of 96 wells against TA100. The findings infer that there is no mutagenic activity present in any of the samples. The ethanolic extracts showed noticeable protective activities against Pilocarpine induced seizures in rat models where no cytotoxicity and mutagenicity was noticed.[40]

Chromosomal aberration test

In human lymphocytes, the ability of standardized *D. sissoo* Leaf Extract (DSLE) to cause structural and numerical chromosomal abnormalities was evaluated. Chromosome and chromatid breaks, acentric fragments, deletions, exchanges, polyploidy (including end reduplication), and disintegrations were examples of structural chromosomal abnormalities. The induction of

cytogenetic damage in human lymphocytes was assessed both with and without metabolic activation. For varying DSLE concentrations, the mitotic index was calculated at 4, 2, 1, 0.5, and 0.25 mg/mL. The averages that emerged were 39.44%, 52.23, 50.86, 50.71, 53.82, and 63.75%, in that order. The percentage of chromosomal aberration in the negative control was 27% when metabolic activation was absent and 36% when it was present. The chromosomal aberration was 60% in the metabolically activated positive control group and 62% in the non-activated group. With and without metabolic activation, the corresponding percentages of chromosomal aberrations were 25 and 21%, 24% and 31%, and 28 and 26% at concentrations of 1, 0.5, and 0.25 mg/mL DSLE, respectively. The ratio of percent chromosomal aberration between the positive and negative controls was more than 2, while the sample treated with DSLE and the negative control was less than 2, leading to the conclusion that DSLE cannot cause structural or numerical chromosomal aberrations in either phase under the experimental conditions.[5]

Reprotoxicity

No reprotoxicity studies were reported.

General conclusion on toxicity

The safety and effectiveness of various *D. sissoo* plant parts that may have therapeutic applications have been the subject of numerous experimental studies and human clinical trials.[13,3] Additional research has shown that there are no new safety concerns.[15,16,41] Gandhi *et al.*[5] found that the *D. sissoo* leaf extract's NOAEL was 1240 mg/kg bw/d during a 90-day sub-chronic study. *D. sissoo* leaf Methanolic Extract (MEDS) has a low toxicity profile, with an LD_{50} of more than 3000 mg/kg bw, according to Mannan *et al.*,[37] whereas *D. sissoo* Ethanolic Leaf Extract (DSELE) was safe in rats up to 10.125 g/kg, p.o.[18]

Human studies

Several clinical studies were conducted by Meeta *et al.*,[13] Dixit *et al.*,[3] and PG G *et al.*[42] to confirm the efficacy and safety of standardized extracts of *D. sissoo* leaf. The impact of ethanolic *D. sissoo* leaf extracts on bone health was investigated. According to recently published preclinical studies, CAFG, a novel molecule, exhibits strong osteogenic activity.[7,28] By encouraging regeneration at the fracture site, CAFG administered orally at doses of 1 and 5 mg/kg improved bone fracture healing in rodents.[28] The activity of bone regeneration was ascribed to CAFG. A clinical evaluation of standardized *D. sissoo* Leaf Extract (SEL-Ds) for osteoporosis was carried out.[13] The above group reported the anti-osteoporotic activity of *D. sissoo* and safety studies in Post Menopausal Osteoporosis. Thirty women between 45-69 years age were recruited for an open-labeled, prospective clinical study for 1-year. Clinical investigation was carried out at a women's hospital's menopausal health care center. The study was evaluated once a week, twice a fortnight, and four times every three months. Tests on organ function and adverse events were monitored to evaluate the test substance's tolerance. Oral SELDs (300 mg) were administered twice a day. Twice day, 250 mg of calcium and 200 IU of vitamin D were administered. The parameters such as DXA-scan (spine, femur), biochemical markers, ALP, TNF-α, and anti-inflammatory marker hs-CRP were assessed. For a year, SELDs was well tolerated at the prescribed dose. Reductions in TNF-α (12.04 –2.35 pg/mL), ALP (208.75-154.52 IU/L), and hs-CRP (6.1 –3.9 mg/L) showed the anti-osteoporotic and anti-inflammatory properties of SEL-Ds. The bulk of the bone sites showed no change in the DXA-scan BMD-score. The anti-osteoporotic and anti-inflammatory properties of SEL-Ds have been demonstrated by a decrease in circulating TNF-α and a corresponding drop in ALP. The anti-osteoporotic activity was confirmed by the majority's BMD index not declining. Dixit *et al.*[3] assessed a standardized extract of *D. sissoo* leaves in a second clinical investigation. Long-term bone fracture healing is a complicated phenomena that requires several months to repair. The efficacy and safety of DSE in treating patients with long bone fractures were assessed in the clinical trial. In a single arm, pilot clinical study carried out on participants (n=16) with lengthy bone fractures in their upper or lower limbs. A 300 mg dose of DSE twice a day for two months was given. Radiological assessments were made of the parameters, including fracture healing, at weeks 2, 4, 6, and 8. Adverse events were monitored in order to assess safety. The DSE treatment, according to the results, resulted in fracture line to fade and the subjects' functional mobility to return at the end of the eight-week trial period. No clinically significant adverse events were reported. The urea, SGOT and SGPT levels were much lower than baseline. The above study demonstrated safety of DSE with a potential of fracture healing. DSE was well tolerated during the treatment period. In a third study by PG *et al.*,[42] a combination product was employed in a randomized, control trial to see whether subcutaneous injection of tablet Reunion and teriparatide may enhance maxillofacial fracture healing. Tablet Reunion is a combination drug of *D. sissoo* and *Cissus quadrangularis*. Three equal groups were randomly selected from a total of twenty-four patients who had mandibular fractures, either with or without concurrent maxillofacial fractures. Group 1 is the control group; Groups 2 and 3 were given injections of teriparatide and tablets, respectively. Four weeks of treatment was given. Up until 12 weeks after surgery, parameters such as bite force, discomfort, serum markers, radiographic healing and fracture site mobility were measured both before and after surgery. Though it was not statistically significant, it was noted that individuals in Group 2 exhibited early pain relief. Throughout all time points, Group 3 displayed the highest anterior bite force. When comparing groups, the mean posterior bite force changed and increased statistically at the eight and twelfth-week marks. By the twelve-week, Group 3 exceptionally performed better than Group 1 and recorded the highest posterior bite force. Serum ALP levels in Group 3

revealed a significant increase, while serum calcium and PTH levels did not change significantly. The radiographic evaluation revealed no substantial variance between the three groups. With the osteoanabolic action, both intervention groups demonstrated a promising effect on enhancing biting force restoration and speeding up the healing of fractures; however, Group 3 showed early radiographic healing and elevated serum osteogenic markers, which indicates its potential role in maxillofacial fracture healing.[42]

Adverse events

No serious adverse events reported.

Contraindications

Not known.

Estimated daily intake

Kushwaha *et al.*[28] estimated the human equivalent dose of *D. sissoo* leaf extract, an ingredient in CAFG. It was estimated to be around 0.081 mg/kg of body weight per day. Therefore, the daily requirement for CAFG was calculated to be about 4.8 mg for a person weighing 60 kg, which is easily achievable. Through a series of tests, it was found that 3000 mg/kg bw in animals was well tolerated, safe, and non-toxic. *D. sissoo* leaf extract (300 mg) (b.i.d) was used for two months on subjects, thus the exposure is 600 mg per day.[3] In another study,[42] mixture of extracts of *Cissus quandrangularis* (600 mg) and *D. sissoo* (400 mg) (b.i.d) for 30 day found to be safe and efficacious. Here, in this study, the exposure per day is 800 mg. The above dietary exposure is safe and well tolerated and efficacious.

In a one-year open-label prospective clinical study, thirty postmenopausal women between the ages of 45 and 69 received 300 mg of *D. sissoo* leaf extract orally twice a day in addition to 250 mg of calcium and 200 IU of vitamin D. Leaf extract was well tolerated without any adverse events.[13]

Vulnerable groups

Pregnant women, children and lactating mothers; the above extract has not been tested on these groups.

Regulatory status

D. sissoo has been listed in following Pharmacopoeia, Monographs and Regulatory bodies.

D. sissoo is listed in Old Dietary Ingredient List compiled by American Herbal Products Association (AHPA), Council for Responsible Nutrition (CRN), Natural Products Association (NPA) and the United Natural Products Alliance (UNPA).

Listed in Ayurvedic Pharmacopoeia of India (API), Part-I, Vol. III, pp: 197.

Listed in Ayurvedic Pharmacopoeial Drugs- Expanded Therapeutics, Ed. C. P. Khare, CRC Press, p. 219.

Listed in Quality standards of Indian Medicinal Plants, Vol. 9, ICMR, pp. 113-121.

Listed in Glossary of Indian Medicinal Plants, Ed. C. P. Khare, p. 200.

Listed in Important classical herbs, extracts, p. 200.

Listed in Natural Health Products Ingredients Database, Canada under Schedule 1 and item 1.

ACKNOWLEDGEMENT

LH acknowledges the analytical help received by erstwhile colleagues.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

TSJ drafted the manuscript, SN and AD reviewed the manuscript, LH contributed to data access, review and approval. All authors approved the manuscript.

ABBREVIATIONS

CAFG: Caviunin 7-O-β-D-apiofuranosyl-(1→6)-β-D-glucopyranoside; **ALP:** Alkaline phosphatase; **CITES COP:** Convention on International Trade in Endangered Species Conference of the Parties; **IUCN:** International Union for Conservation of Nature. **UE:** Upper epidermis; **LE:** Lower epidermis, **PE:** Pericycle; **VB:** Vascular bundle; **CH:** Chlorenchyma; **CX:** Cortex; **XV:** Xylem vessel; **PH:** Phloem; **SC:** Sclerenchyma; **SM:** Spongy mesophyll; **PM:** Palisade mesophyll; **CT:** Conducting tissue; **ST:** Stomata.

REFERENCES

1. Al-Quran S. Taxonomical and pharmacological survey of therapeutic plants in Jordan. J Nat Prod. 2008;1:10-26.
2. Bakewell-Stone P. *Dalbergia sissoo* (shisham); 2023 [cited Jun 14 2024]. Available from: https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.17808.
3. Dixit P, Pande S, Deshmukh A. Safety, and efficacy of a standardized extract from leaves of *Dalbergia sissoo* in healing of long bone fracture: A pilot clinical study. J Complement Med Alt Healthc. 2018;5(1):555651.
4. Kothari P, Tripathi AK, Girme A, Rai D, Singh R, Sinha S, *et al.* Caviunin glycoside (CAFG) from *Dalbergia sissoo* attenuates osteoarthritis by modulating chondrogenic and matrix regulating proteins. J Ethnopharmacol. 2022;282:114315. doi: 10.1016/j.jep.2021.114315, PMID 34116187.
5. Gandhi T, Shah H, Trivedi R, Deshmukh A, Hingorani L. Safety evaluation of a standardized *Dalbergia sissoo* leaf extract (DSLE). Pharmacogn Res. 2022;14(2):172-80. doi: 10.5530/pres.14.2.25.
6. Saste G, Balasubryamanym AK, Singh R, *et al.* Comprehensive standardization and rapid quality evaluation of isoflavones and isoflavone glycosides from*Dalbergia sissoo* by ultra-high-performance liquid and thin layer chromatography analysis with tandem mass spectrometry. Sep Sci Plus. 2023;6:2200079.
7. Khedgikar V, Gautam J, Kushwaha P, Kumar A, Nagar GK, Dixit P, *et al.* A standardized phytopreparation from an Indian medicinal plant (*Dalbergia sissoo*) has antiresorptive and bone-forming effects on a postmenopausal osteoporosis model of rat. Menopause. 2012;19(12):1336-46. doi: 10.1097/gme.0b013e318256b6ae, PMID 22850441.

8. Khedgikar V, Kushwaha P, Ahmad N, Gautam J, Kumar P, Maurya R, *et al.* Ethanolic extract of *Dalbergia sissoo* promotes rapid regeneration of cortical bone in drill-hole defect model of rat. Biomed Pharmacother. 2017;86:16-22. doi: 10.1016/j.biopha.2016.11.140, PMID 27936389.
9. Lakhey P, Pathak J, Adhikari B. *Dalbergia sissoo*. IUCN Red List Threat Species. 2020. doi: 10.2305/IUCN.UK.2020-3.RLTS.T62022617A62022619.en.
10. Khare CP, editor. Ayurvedic pharmacopoeial plant drugs – expanded therapeutics. NY: CRC Press; 2016.
11. Khare CP, editor. Indian medicinal plants- an illustrated dictionary. NY: Springer; 2007.
12. Dixit P, Chillara R, Khedgikar V, Gautam J, Kushwaha P, Kumar A, *et al.* Constituents of *Dalbergia sissoo* (Roxb.) leaves with osteogenic activity. Bioorg Med Chem Lett. 2012;22(2):890-7. doi: 10.1016/j.bmcl.2011.12.036, PMID 22212722.
13. Meeta RA, Raut AA, Agashe SV, Wajahat A, Sarada CV, Vaidya AD, *et al.* A clinical study of a standardized extract of leaves of *Dalbergia sissoo* (Roxb ex DC) in postmenopausal osteoporosis. J Mid Life Health. 2019;10(1):37-42. doi: 10.4103/jmh.JMH_22_19, PMID 31001055.
14. Vanshita BM. Phytochemistry and pharmacology of *Dalbergia sissoo* Roxb. 2023;75:482-501 ex DC: a review J Pharmacy and Pharmacol.
15. Asif M, Kumar A. Anti-inflammatory activity of ethanolic extract of *Dalbergia sissoo* (Roxb.) Bark. Malays J Pharm Sci. 2009;7(10):39-50.
16. Asif M, Kumar A. Phytochemical investigation and evaluation of antinociceptive activity of ethanolic extract of *Dalbergia sissoo* (Roxb.) bark. J Nat Sci Biol Med. 2011;2(1):76-9. doi: 10.4103/0976-9668.82315, PMID 22470239.
17. Kaur A, Singh S, Chandra P, *et al.* Evaluation of antioxidant potential of stem bark extract of *Dalbergia sissoo*. J Pharm Res. 2011;4(10):3439-41.
18. Hajare SW, Chandra S, Sharma J, Tandan SK, Lal J, Telang AG. Anti-inflammatory activity of *Dalbergia sissoo* leaves. Fitoterapia. 2001;72(2):131-9. doi: 10.1016/s0367-326x(00)00272-0, PMID 11223222.
19. Bharath M, Tulasi EL, Kommu S, et al. *Dalbergia sissoo*- An important medicinal plant. Int J Res Pharm Chem. 2013;3:384-8.
20. Panda SK, Padhy RP, Pani S, Bal K. Phytochemical investigation and antidiabetic activity of leaf extracts of *Dalbergia sissoo* (Roxb.) in alloxan induced diabetic rats. Science and medical res. 2016;1(2):186-9.
21. Niranjan SP, Singh D, Prajapati K. Antidiabetic activity of ethanolic extract of *Dalbergia sissoo* L. leaves in Alloxan-Induced diabetic rats. Int J Curr Pharm Res. 2010;2:24-7.
22. Pund KV, Vyawahare NS, Gadakh RT, Murkuteet VK. Antidiabetic evaluation of *Dalbergia sissoo* against alloxan induced diabetes mellitus in Wistar albino rats Nat J, producer Plant Resour. 2012;2(1):81-8.
23. Hugar MH, Hosamani KM, Ahmed L. Phytochemical and pharmacological studies of ethanol extract of *Dalbergia sissoo* seeds. An approach for the *in vivo* analgesic and antipyretic activities. Int J Pharm Biol Sci. 2010;1:272-80.
24. Gautam J, Kumar P, Kushwaha P, Khedgikar V, Choudhary D, Singh D, *et al.* Neoflavonoid dalbergiphenol from heartwood of *Dalbergia sissoo* acts as bone savior in an estrogen withdrawal model for osteoporosis. Menopause. 2015;22(11):1246-55. doi: 10.1097/GME.0000000000000453, PMID 25850356.
25. Thonda VS, Banerjee S, Handral M. Neuroprotective evaluation of leaf extract of *Dalbergia sissoo* in 3-nitropropionic acid induced neurotoxicity in rats. Int J Pharmaceu Sci Drug Res. 2014;6(1):41-7.
26. Saxena R, Misha M, Farah K, Zaineb Q, Manoj R. Effect of *Dalbergia sissoo* Leaves on aminoglycosides induced nephrotoxicity in experimental rats. Int J Pharmacol Pharmaceu Sci. 2016;3(2):5-14.
27. Kumar P, Kushwaha P, Khedgikar V, Gautam J, Choudhary D, Singh D, *et al.* Neoflavonoids as potential osteogenic agents from *Dalbergia sissoo* heartwood. Bioorg Med Chem Lett. 2014;24(12):2664-8. doi: 10.1016/j.bmcl.2014.04.056, PMID 24803361.
28. Kushwaha P, Khedgikar V, Gautam J, Dixit P, Chillara R, Verma A, *et al.* A novel therapeutic approach with Caviunin-based isoflavonoid that en routes bone marrow cells to bone formation via BMP2/Wnt-betacatenin signaling. Cell Death Dis. 2014;5(9):e1422. doi: 10.1038/cddis.2014.350, PMID 25232676.
29. Al-Snafi. Chemical constituents and pharmacological effects. IOSR J Pharm. 2017;7(2):59-71.
30. Mani SS, Vijayalakshmi A. Standardization of shimshapa (*Dalbergia sissoo* roxb.) stem bark w.s.r to physicochemical and phytochemical evaluation, Int Ayurvedic. Med J. 2020;4(2):2107-14.
31. ICMR. New Delhi, Quality standards of Indian medicinal plants. Vol. 2018;IX:113-21.
32. Othman. Woody trees. Al-Etemad publishers. Cairo. 1939:199.
33. Hussani M, Khan SN. Medicinal importance and association of pathological constraints with *Dalbergia sissoo*. J Phytopathol. 2010;22:135-8.
34. Vaidya MM, Kolhe SU, Tembhurne SV. Evaluation of subacute oral toxicity induced by ethanolic extract of *Dalbergia sissoo* leaves in experimental rats. Eur J Biomed Pharmaceut Sci. 2019;6:568-77.
35. Ul-Islam M, Elhddad S. Phytochemical investigation and evaluation of analgesic activity of ethonolic extract of *Dalbergia sissoo* (Roxb.) bark. Plant Resour Nat J, producer. 2012;2(6):701-4.
36. Sehra SY, Sharma J. Pharmacological effects and medicinal importance of *Dalbergia sissoo* –a review. Int J Pharm Biol Sci. 2018;8(2):234-43.
37. Mannan MA, Khatun A, Khan MF. Antinociceptive effect of methanol extract of *Dalbergia sissoo* leaves in mice. BMC Complement Altern Med. 2017;17(1):72. doi: 10.1186/s12906-017-1565-y, PMID 28114964.
38. Sarg T, Ateya AM, Abdel-Ghani AA, Badr W, Shams G. Phytochemical and pharmacological studies of *Dalbergia sissoo* growing in Egypt. Pharm Biol. 1999;37(1):54-62. doi: 10.1076/phbi.37.1.54.6310.
39. Josephin Nerling Rashida G. A potential phytomedicine for obesity from the leaves of *Dalbergia sissoo* Roxb. using zebrafish (*Danio rerio*)-A metabolomic approach for future herbal drug development [masters Phil thesis]. Chennai: Dr MGR Medical University; 2012.
40. Majeed FA, Munir H, Rashid R, Zubair MT. Antimicrobial, cytotoxicity, mutagenicity and anti-epileptic potential of ethanol extracts of a multipurpose medicinal plant *Dalbergia sissoo*. Biocatal Agric Biotechnol. 2019;19:101155. doi: 10.1016/j.bcab.2019.101155.
41. Raheja S, Girdhar A, Kamboj A, Lather V, Pandita D. Protective Effect of *Dalbergia sissoo* Extract against Amyloid-β (1-42)-induced Memory Impairment, Oxidative Stress, and neuroinflammation in Rats. Turk J Pharm Sci. 2021;18(1):104-10. doi: 10.4274/tjps.galenos.2020.04379, PMID 33634685.
42. Pg G, Chugh A, Chaudhry K, Kaur A, Kumar P, Gaur S, *et al.* Comparison of teriparatide and combination of *Cissus quadrangularis* and *Dalbergia sissoo* on bone healing against the control group in maxillofacial fractures: A randomized open-label control trial. Craniomaxillofac Trauma Reconstr. 2023;16(1):23-33. doi: 10.1177/19433875211067007, PMID 36824186.

Cite this article: Johnson TS, Nair S, de'Souza A, Hingorani L. *Dalbergia sissoo* Roxb. ex-DC. - A Monograph. Pharmacog Rev. 2024;18(36):137-50.