

CHEMICAL CONSTITUENTS OF THE STEM IN *DALBERGIA SISSOO*

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ABSTRACT

The chemical constituents of ethyl acetate extracts from heartwood and sapwood of different ages of *Dalbergia sissoo* were studied by gas chromatography-mass spectrometry. The results showed that the chemical composition of wood heartwood and sapwood is significantly different. In the vertical direction, the type of the ethyl acetate extract from *Dalbergia sissoo* tends to decrease from the base to the upper portion; in the horizontal direction, the type of extract gradually decreases from the center to the periphery. And it showed an increasing trend with the age of the trees. The experiment also revealed that there were significant differences in chemical components between heartwood and sapwood. We speculated that the main chemical component trimethoxyresveratrol of heartwood extract may be related to the formation of heartwood, and the specific correlation needs to be further verified.

KEYWORDS: Chemical compound, *Dalbergia sissoo*, sapwood, heartwood, gas chromatography-mass spectrometry, ethyl acetate extract, trimethoxyresveratrol.

INTRODUCTION

Dalbergia sissoo is one of the most important precious woods in agroforestry production, especially heartwood, excellent in durability and processing properties, and resistant to insects. *Dalbergia sissoo* Roxb. commonly known as Sissoo or Shisham, is an evergreen or deciduous medium tree with small canopy, widely distributed throughout the Indian subcontinent (Sultana et al. 2015), as well as Nepal, Pakistan, Bangladesh. Countries, Brazil, Madagascar and other countries, and it has been introduced to Yunnan, China since 1999 (Pande and Singh 2005, Shi et al. 2011). Due to its interlaced texture, fine and beautiful structure, anti-termite, outstanding abrasion resistance, hard and not easy to crack, the heartwood of the *Dalbergia sissoo* is suitable for engraving, finishing, decorating and furniture and so on (Khan and Faruque 2010, Al-Snafi 2017). In terms of various aspects of use and processing performance, the heartwood of

Dalbergia sissoo is obviously superior to sapwood, and sapwood is difficult to put into use in most cases, resulting in greatly reduced wood utilization. In addition, the formation of the heartwood is special slow, it can't be promoted artificially, and the output of the heartwood is not well controlled (Hirano et al. 2001). Therefore, the quality and output of the heartwood of *Dalbergia sissoo* is our ultimate goal, but we have not yet fully understood the process of heartwood formation.

The essential difference between heartwood and sapwood is that they have different composition components, and the difference in structure and composition between heartwood and sapwood makes it very different in terms of application range, economic value and comprehensive benefits (Kumar et al. 2005, Zhang et al. 2020). At present, systematic research on its chemical composition is rare (Inyang et al. 2014, Javaid et al. 2015), so we need to studies the chemical composition in the stem of *Dalbergia sissoo* cultivated systematically, in order to provide certain basic data for the development and utilization of *Dalbergia sissoo*. Therefore, the study of the difference between the composition of heartwood and sapwood, as well as the spatial and temporal distribution of matter in the heartwood and sapwood, will greatly help reveal the cause and mechanism of the formation of the heartwood, and is conducive to artificially promote the formation of heartwood to achieve the full use of wood.

In this paper, the type and content of ethyl acetate extract of sapwood and heartwood were measured by measuring the formation of heartwood, and the content and distribution of the extract were analyzed. On the one hand, it lays a foundation for the research on the quality of *Dalbergia sissoo* heartwood and sapwood. On the other hand, it helps to understand the formation process of the heartwood in the stem of *Dalbergia sissoo*, which provides a scientific basis for the excellent breeding of *Dalbergia sissoo*.

MATERIAL AND METHODS

Materials

The test material was collected from the Yuanjiang Test Base of the Resource Insect Institute of the Chinese Academy of Forestry. The plantation in the test station management level was consistent, the plant growth condition was robust. Four tree strains of different ages and the same growth state were selected in the experimental plots. After the sample wood was selected, it was numbered Ds.1 – Ds.4 (3, 7, 12 and 18 years) according to the age of the trees. According to the height of each tree, about 10 cm from the ground was regarded as the base of the trunk, the middle of the trunk was the middle, and the upper 10 cm below the crown was regarded as the upper part of the trunk. The base was marked on the trunk for felling. After the felling, a disc of about 5 cm thick was cut in the middle and upper part of the base of the sample trunk, and the tree number and the position of the disc relative to the trunk were marked.

Sample preparation

Before the formal sampling, we did a preliminary experiment to determine whether to take multiple directions in the horizontal direction or single-direction sampling. We cut a part of

the tree with the heartwood in the upper and lower sections in advance, sampled in the three directions of the upper section of the sample, and sampled in the corresponding direction in the lower section.

According to the cross-sectional state of the test material, the upper, middle and base portions were divided into two parts (heartwood and sapwood), and each part took 2 samples of similar size (Fig. 1), and took a sample in the transition zone between heartwood and sapwood. The samples were cut into particles as small as possible, and then directly immersed in a glass test tube containing ethyl acetate, which was placed in a fume hood with a test tube rack. There was no significant environmental change and pollution during extraction, and shaken periodically for 5 days. 2 mL supernatant and 0.45 μm microporous membrane were used in the injection bottle. The supernatant in the sample bottle was taken for GC-MS analysis.



Fig. 1: A cross section of the base of a 15-year-old Dalbergia sissoo stem, indicating the difference in color between the heartwood and the sapwood of the wood.

Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analyses were performed using a Shimadzu Gas Chromatograph QP2010 Ultra equipped with Autosampler AOC-20i, Ion source: electronic impact High-performance Quadrupole Mass Filter. Separation of compounds was carried out in a DB-5J&W capillary column (30×0.25 mm inner diameter, $0.25 \mu\text{m}$ film thickness) using helium as the carrier gas ($35 \text{ cm}\cdot\text{s}^{-1}$). The chromatographic conditions were as follows: start time at 6.5 min; initial temperature 90°C for 4 min; temperature rate, $16^\circ\text{C}\cdot\text{min}^{-1}$ up to 180°C , followed by temperature rate $6^\circ\text{C}\cdot\text{min}^{-1}$ up to 250°C ; followed by temperature rate, $3^\circ\text{C}\cdot\text{min}^{-1}$ up to 300°C which was maintained for 5 min; injector temperature 320°C ; transfer-line temperature, 300°C ; split ratio 1:50. The mass spectrometer was operated in the electron impact (EI) mode with energy of 70 eV, and data were collected at a rate of $1 \text{ scan}\cdot\text{s}^{-1}$ over a range of $33\text{--}750 \text{ m}\cdot\text{z}^{-1}$. The ion source was kept at 250°C . The total ion flow chart of ethyl acetate extract was obtained, and the peak of the extract was deleted from the total ion flow chart of ethyl acetate. Then the GC-MS total ion graph was integrated and the peak area (A) of the relative peak area (A) was more than 1% was selected and the similarity ratio of the peak of the satisfied conditions was compared with the system. From total ion chromatogram, the peaks were identified by

comparing their mass spectra with the mass spectral libraries (NIST 14 Mass Spectral and Wiley Registry TM of Mass Spectral Data), with MS spectra and MS fragmentation pattern published in the literature, by comparing the retention times and mass spectra data of the standard compounds injected in the same chromatographic conditions. The chemical constituents of ethyl acetate extract from *Dalbergia sissoo* were obtained ultimately.

RESULTS AND DISCUSSION

Ethyl acetate extract of *Dalbergia sissoo* and its position

The results of preliminary experiments showed that there was no difference in extracts between samples in the same height horizontal direction, so the sampling method in the experiment did not take parallel samples of the samples in the horizontal direction of the same height. It can be seen that the heartwood and sapwood of *Dalbergia sissoo* are distinct in color and can be easily distinguished (Fig. 1). By observing the gas chromatogram of each sample, we found that the mass spectrums of the heartwood sample were approximately the same, and the sapwood was approximately the same (Fig. 2). We list the state of the wood of the cross section of the four sample trees (the presence or absence of the heartwood) as Tab. 1. Totally, there are 11 substances in the extract from the heartwood, and the other 6 from the sapwood. The substances are numbered in Tab. 2.

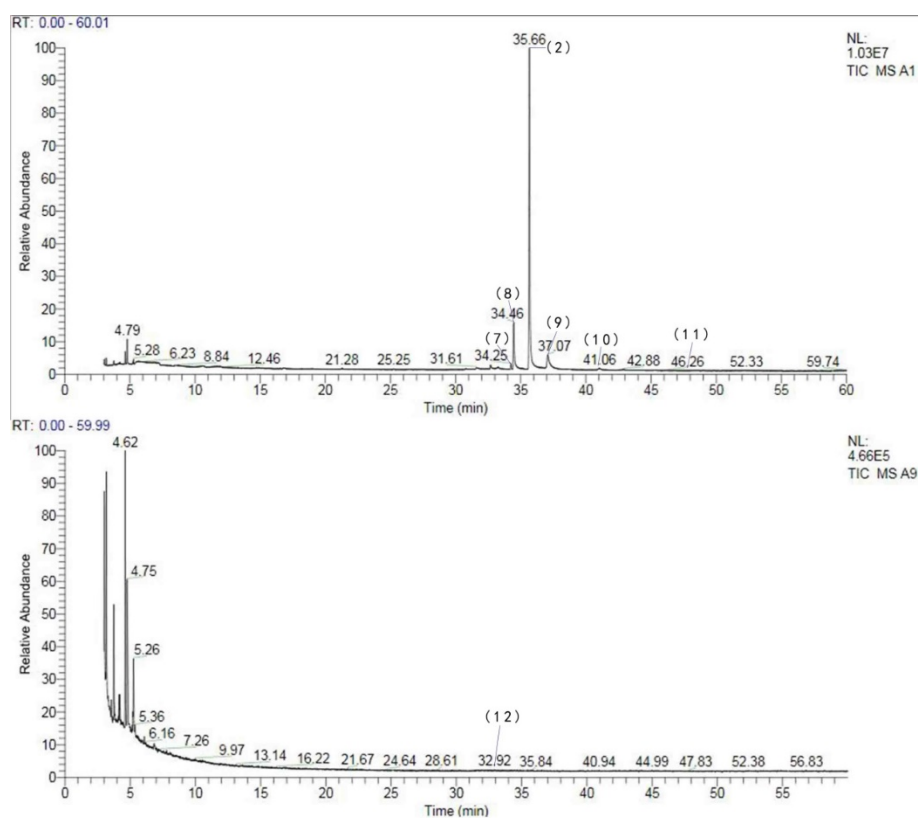


Fig. 2: Gas chromatographic mass spectrum of ethyl acetate extract of heartwood (upper) and sapwood (lower) of *Ds.4*

Tab. 1: The presence or absence of heartwood of four sample trees.

Samples	Top	Middle	Base
Ds.1	no	no	no
Ds.2	no	no	have
Ds.3	have	have	have
Ds.4	have	have	have

Tab. 2: Ethyl acetate extract of *Dalbergia sissoo*.

Position	Serial number (SN)	Molecular formula	Identified compounds
Heartwood	1	C ₁₆ H ₁₄ O ₃	Xyloltenin;
	2	C ₁₇ H ₁₈ O ₃	Trismethoxyresveratrol;
	3	C ₁₄ H ₁₂ N ₂ O ₃	1,9-Dimethoxyphenazine 5-oxide;
	4	C ₁₂ H ₁₆ N ₆ O ₆	N-2,4-Dnp-L-arginine;
	5	C ₁₈ H ₂₀ O ₃	Allogibberic acid;
	6	C ₁₄ H ₁₂ N ₂ OS	Phenol, 4-methyl-2-[5-(2-thienyl)pyrazol-3-yl]-;
	7	C ₁₈ H ₂₀ O ₃	Dibenz[a,c]cyclohexane, 2,4,7-trimethoxy- ;
	8	C ₁₆ H ₁₆ O ₂	Benzene, 1,3-dimethoxy-5-[(1E)-2-phenylethenyl]-;
	9	C ₂₅ H ₂₇ NO ₃	α -Phenyldihydrothebaine;
	10	C ₁₆ H ₁₄ O ₃	Benzoic acid, 4-[2-(3-methoxyphenyl)-1-ethylenyl]; 3,4-Dimethoxy-benzoic acid
Sapwood	11	C ₂₃ H ₂₂ N ₂ O ₃	(1-biphenyl-4-yl-ethylidene)-hydrazide;
	12	C ₁₆ H ₁₂ O ₄	7-hydroxy-3-(4-methoxyphenyl)-2H-chromen-2-one;
	13	C ₁₈ H ₂₂ O ₂	Estra-4,9,11-trien-3-one, 17- β -hydroxy- ;
	14	C ₂₀ H ₂₅ NO ₃ S	Androst-4-en-9-methylthio-11-ol-3,17-dione;
	15	C ₁₅ H ₁₂ N ₂ O ₃	1,4-diamino-2-methoxyanthracene-9,10-dione;
	16	C ₁₆ H ₁₂ O ₄	2,3-dimethoxyanthracene-9,10-dione;
	17	C ₃₀ H ₅₀ O	α -amyrin;

* The substances in the table are all compounds with a content greater than 1% after GC-MS analysis of the ethyl acetate extract from the trunk.

Temporal and spatial distribution of various chemical components in stem

First, for samples with heartwood from the inside to the outside in the cross section of the wood, the type of the extract gradually decreases from the base to the upper part (Tab. 3). The columns in the table represent the compounds extracted from the corresponding position by the trunk, while the rows represent the serial number of the tree and the position of the sample inside and outside the cross section of the vertical position of the trunk. The percentages in the table show the relative amounts of various substances. The component content of a substance is the average taken from multiple samples. 100% indicates that only one compound of the samples in this position has a peak area of more than 1%.

As can be seen from the Tab. 3, from the ethyl acetate extracted material of the sample from the Ds.4, that there are seven kinds of extracts at the base. And two compounds are extracted in the middle. Similarly, only two compounds were detected in the upper part. The GC-MS of ethyl acetate extract of Ds.3 wood has detected seven chemical constituents at the base. Only two substances were detected in the middle. In the upper part, two compounds were detected. In the samples of Ds.2, five extracts were extracted at the base, and one was detected in the middle and upper samples.

Tab. 3: The position of the extract in the sample tree and its relative percentage.

SN	Percentage content (%)																					
	Ds.1		Ds.2				Ds.3				Ds.4											
	Mid		Top	Mid	Base		Top		Mid	Base		Top		Mid		Base						
HP	In	Out	Out	Out	Ins	Out	In	Out	In	Out	In	Out	In	Tra	Out	In	Tra	Out	In	Tran	Out	
1					82.6															81.5		
2					7.83		100		100		90.16		100	100		100	100			9.32	95.84	
3																				4.93		
4																				2.29		
5																					2.22	
6																					1.31	
7											2.08											
8					2.74						1.45											
9					2.26						2.51											
10											1.74											
11											1.86											
12			100			100							100		100		100					100
13				100						100												
14								100														
15	100																					
16		47.2																				
17		43.6																				

*In - inside, Out - outside, Tran - transition, SN - serial number of the sample tree, VP - vertical position, HP - horizontal position.

Secondly, for samples with heartwood from the cross section of the wood, the type of the extract is reduced from the inside to the outside part (Tab. 3). Among the multiple samples of Ds.4 wood, there were four kinds of substances extracted by ethyl acetate in the center part of the heartwood, and there are three compounds detected from the transition zone, only one compound was obtained in the outer part of the sapwood. In the samples taken from Ds.3 wood, six compounds were detected in the ethyl acetate extract at the center of the heartwood, and three substances were get from the outside of the sapwood. The GC-MS of ethyl acetate extract of Ds.2 wood has detected four compounds in the heartwood, and only two compound in the sapwood.

Obviously, the chemical constituents of ethyl acetate extracted from *Dalbergia sissoo* wood are gradually increasing with the increase of tree age. A total of three chemical components were detected in the extract of ethyl acetate from wood of Ds.1, six compounds were got in the Ds.2, nine compounds were detected in the Ds.3, and seven chemical components were detected in the Ds.4.

Referring to the gas chromatogram of the ethyl acetate extract of sapwood and heartwood (Fig. 2), it is clear that the sapwood has a small number of species and relatively lower content of components; while in the heartwood, there are more species and obvious main substance with high content.

Discussion

First of all, in appearance, there is no heartwood in young wood. With the growth of tree age, heartwood begins to form at the base of wood. The color and anatomical structure of heartwood and sapwood are significantly different, and their chemical composition is also greatly different. We speculate that some extracts may have an impact on the color of wood, and the specific substance or substances need to be verified. Then, in connection with the extractives of different ages and parts of trees and their spatial variation, we believe that trismethoxyresveratrol may be related to the formation of heartwood. First, the trismethoxyresveratrol is only present in the ethyl acetate extract of the heartwood sample in the middle position. In addition, compared with the extracts of samples, trismethoxyresveratrol only exists in Ds.2, Ds.3 and Ds.4 (Tab. 3), and they differ from the Ds.1 in: There is a clear color difference between the interior and exterior of the wood cross section, and the interior is darker. Furthermore, in the Ds.2 sample, only the cross section of the base has a color difference, and only the trismethoxyresveratrol is detected in the extract of the base, but not in the middle and the upper parts. Combined with the peak area of the total ion current map, which is its relative percentage in the heartwood, we consider the material to be a characteristic substance of the heartwood.

The heartwood is the central part of the tree that does not contain living cells, and the storage material in the trees has been eliminated or converted into extracts of the heartwood (Anatomists 1964, Taylor et al. 2002). The formation mechanism of wood is very complicated, and many changes are closely combined and occur instantaneously (Jaemo et al. 2004, Gowariker et al. 2008), so it is difficult to conduct in-depth systematic research on its occurrence process. This experiment attempts to link the extracts from sapwood and heartwood through secondary metabolic pathways in plants, which is of great significance for explaining the process and mechanism of heartwood formation or sapwood conversion into heartwood. At present, there are two hypotheses about the formation mechanism of heartwood: The first hypothesis is that heartwood is the site where trees accumulate toxic secondary metabolites. The second hypothesis is that the formation of heartwood is the result of the unique physiological functions of parenchyma cells in the transition zone (Fengel 1970, Hugentobler 1965, Stewart 1966, Hillis 1971, Bamber 1976, Cui et al. 2016).

When a normal growing tree reaches a certain age, it will form a heartwood inside the xylem. The trees continue to grow, except for the addition of new sapwood on the periphery, while the heartwood gradually extends outward (Bamber and Humphreys 1965, Spicer 2016). *Catalpa bignonioides* and *Crytomeria japonica* form heartwood at very young ages, while *Pinus ponderosa* and *Nyssa sylvatica* begin to grow in centuries (Yang and Hazenberg 1991, Yang et al. 1994, Harrington and Warren 2011). According to the investigation, it was found that the heartwood of *Dalbergia fragrans* began to form 6-7 a (Cui et al. 2016). In our study, by observing the formation of heartwood of four trees of different ages, we believe that the time required for the formation of heartwood in *Dalbergia sissoo* was 3-7 years.

After research, growth regulators, fungal infections, exogenous gases and water and fertilizer management, thinning tending, pruning and cutting, cutting and stripping bark and other tending measures can affect the formation of the heartwood (Hillis 1999, Kuroda et al. 2009, Tanabe et al. 2019, Nagai and Utsumi 2012, Basu 2014, Cui et al. 2020).

CONCLUSIONS

The number of ethyl acetate extract from the stem of the *Dalbergia sissoo* showed an increasing trend with the age of the trees; as for space, from the base to the upper part, from the center of the tree to the periphery, the trend was decreasing. The chemical composition of wood heartwood and sapwood is significantly different, therefore, we believe that the formation of heartwood is inseparable from its chemical composition. It is speculated that the heartwood of *Dalbergia sissoo* is formed for about 3-7 years. In addition, we found that the main component of the heartwood extract is trimethoxyresveratrol, which could be a derivative of plant phenylalanine metabolism. And other ingredients such as Xyloltenin may have a potential link to the intermediate coumaric acid. By analyzing the chemical constituents extracted from the wood, it may have a positive effect on guiding the regulation, material metabolism, sampling and research methods in the process of heartwood formation.

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