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Chemical constituents of the stem in *Dalbergiasissoo*

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Abstract

Dalbergiasissoo is one of the most important precious woods in agro forestry production, especially heartwood, excellent in durability and processing properties, and resistant to insects. The chemical constituents of ethyl acetate extracts from heartwood and sapwood of different ages of *Dalbergiasissoo* 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 *Dalbergiasissoo* 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 the formation of the heartwood is closely related to its chemical composition.

Keywords: Chemical compound; *Dalbergiasissoo*; Sapwood; Heartwood.

1. Introduction

*Dalbergiasissoo*Roxb. Commonly known as Sissoo or shisham, is an evergreen or deciduous medium tree with small canopy, widely distributed throughout the Indian subcontinent(Tewari 1994), as well as Nepal, Pakistan, Bangladesh. Countries, Brazil, Madagascar and other countries, and it has been introduced to Yunnan, China since 1999(Pande 2006, Singh et al. 2006,Shi et al.2008). Due to its interlaced texture, fine and beautiful structure, anti-termite, outstanding abrasion resistance, hard and not easy to crack, the heartwood of the *Dalbergiasissoo* is suitable for engraving, finishing, decorating and furniture and so on (KhanI&Faruque2010, Ali 2017). In terms of various aspects of use and processing performance, the heartwood of *Dalbergiasissoo* 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. Therefore, the quality and output of the heartwood of *Dalbergiasissoo* 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 (Rajneeshet al. 2005, Sorz&Hietz2008). 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 *Dalbergiasissoo* cultivated systematically, in order to provide certain basic data for the development and utilization of *Dalbergiasissoo*. 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 thefull 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 *Dalbergiasissoo* heartwood and sapwood. On the other hand, it helps to understand the formation process of the heartwood in the stem of *Dalbergiasissoo*, which provides a scientific basis for the excellent breeding of *Dalbergiasissoo*.

2. Material & Method

2.1 Materials

The test material is collected from the Yuanjiang Test Base of the Resource Insect Institute of the Chinese Academy of Forestry. The cutting time is June 2018. Four tree strains of different ages and the same growth state were selected in the experimental plots. After the sample wood is selected, it is numbered Ds.1, Ds.2, Ds.3, Ds.4 (3 years, 7 years, 12 years, 18 years) according to the age of the trees. According to the height of each tree, about 10 cm from the ground is regarded as the base of the trunk, the middle of the trunk is the middle, and the upper 10 cm below the crown is regarded as the upper part of the trunk. The base is marked on the trunk for felling. After the felling, a disc of about 5 cm thick is 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 are marked.

2.2 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：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 are divided into two parts(heartwood and sapwood), and each part takes 2 samples of similar size(Fig.1).And take 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 is 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 um micro porous membrane were used in the injection bottle. The supernatant in the sample bottle was taken for GC-MS analysis.

2.3 Gas chromatography–Mass spectrometry (GC–MS) analysis

GC–MS analyses were performed using a Shimadzu Gas Chromatograph QP2010 Ultra equipped with AutosamplerAOC-20i, Ion source: electronic impact High-performance Quadrupole Mass Filter. Separation of compounds was carried out in a DB-5J&W capillary column(30 mm×0.25 mm inner diameter, 0.25 μm film thickness)using helium as the carrier gas (35 $cm \cdot s^{-1}$). The chromatographic conditions were as follows: start time at6.5 min; initial temperature, 90 °C for 4 min; temperature rate, 16 °C min^{-1}up to 180 °C, followed by temperature rate, 6 °C min^{-1}up to 250 °C; followed by temperature rate, 3 °C min^{-1}up to 300 °C which was maintained for5 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 $scan \cdot s^{-1}$over a range of 33–750 m/z. The ion source was kept at250 °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 one percent 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 *Dalbergiasissoo* were obtained ultimately.

3. Results and analyze

3.1. Ethyl acetate extract of *Dalbergiasissoo* 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 *Dalbergiasissoo* 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.

3.2 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. As can be seen from the Tab.3, from the ethyl acetate extracted material of the sample from the Ds.4, we can see 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.

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 there 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 compounds in the sapwood.

Obviously, the chemical constituents of ethyl acetate extracted from *Dalbergiasissoo* 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 get 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.

4. Discussion

4.1 Analysis of heartwood formation

Combined with the tree growth status and the above results, it can be inferred that the heartwood of *Dalbergiasissoo* is formed from the base. In connection with the growth status of the trees and the table of extracts from various parts, 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 (IAWA Committee 1964, Tayloret al. 2002).The formation mechanism of wood is very complicated, and many changes are closely combined and occur instantaneously (Gowariker et al. 2009), 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 (Chattaway 1952, Hugentobler 1966, Stewart 1966, Hillis 1971,Bamber 1976, Cui et al. 2016).

When a normal growing tree reaches a certain age, it will form 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& Humphreys 1965, Spicer 2016). *Catalpa bignoniodes* and *Crytomeriajaponica* form heartwood at very young ages, while *Pinus ponderosa* and *Nyssa sylvastica* begin to grow in centuries (Yang &Hazenberg1991a, 1991b, Yang et al.1994, Guo et al. 2010). According to the investigation, it was found that the heartwood of *Dalbergiafragrans* 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 *Dalbergiasissoo*was 3-7years.

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 (Morling&Valinger 1999, Kuroda et al. 2009, Luo et al. 2010, Nagai&Utsumi 2012, Peng 2014).

4.2 Analysis of phenylalanine metabolic pathway

In this experiment, the iconic extract detected in the heartwood of *Dalbergiasissoo*, resveratrol trimethyl ether, is a terpenoid compound with stilbene as the structural core. The most representative substance of terpenoids is resveratrol. The resveratrol trimethyl ether extracted in our experiments should be based on resveratrol and a compound formed by the substitution of hydrogen on three hydroxyl groups by a methyl group. Resveratrol is a plant secondary metabolite that is synthesized from phenolic compounds such as flavonoids and furocoumarins via a phenylpropanoid metabolic pathway (Ososki& Kennelly 2003, Ma et al. 2018). The phenylalanine metabolic pathway, starting molecule is phenylalanine, which produces a substance containing a phenylpropane skeleton and is one of the most important secondary metabolic pathways in plants. Phenylalanine is deaminated to cinnamic acid by

phenylalanine ammonia lyase (PAL), and cinnamic acid produces p-coumaric acid under the action of cinnamic acid 4-hydroxylase (C4H). It is catalyzed by p-coumyl Co A-ligase (4CL) by co-cocoyl Co A, a pathway known as the phenylpropanoid core metabolic pathway (Weisshaar& Jenkins 1998). Resveratrol synthase (RS) and Chalcone synthase (CHS) catalyze the same substrate (3 molecules of malonyl-CoA and 1 molecule of coumaroyl-CoA) Res and naringenin (Rolfs &Kindl 1984, Isabel et al. 2004).In addition, the Xyloltenin measured in this test are structurally obtained through the phenylalanine metabolic pathway. And the Xyloltenin is structurally similar to coumaric acid.

5. Conclusion

The number of ethyl acetate extract from the stem of the *Dalbergiasissoo* 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 *Dalbergiasissoo* is formed for about 3-7 years. In addition, we found that the main component of the heartwood extract is Trismethoxyresveratrol, which mingt 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 the further interpretation of the phenylalanine metabolic pathway. This study would guide the regulation, material metabolism, sampling and research methods in the process of heartwood formation.

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Tab.1 -The presence or absence of heartwood of four sample trees. The heartwood of *Dalbergiasissoo* is brown, and the sapwood is light in color, straw-colored, which is significantly different from the heartwood.

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

Position	Serial number	Molecular formula	Identified compounds
Heartwood	1	$C_{16}H_{14}O_3$	Xyloltenin;
	2	$C_{17}H_{18}O_3$	Trismethoxyresveratrol;
	3	$C_{14}H_{12}N_2O_3$	1,9-Dimethoxyphenazine 5-oxide;
	4	$C_{12}H_{16}N_6O_6$	N-2,4-Dnp-L-arginine;
	5	$C_{18}H_{20}O_3$	Allogibberic acid;
	6	$C_{14}H_{12}N_2OS$	Phenol, 4-methyl-2-[5-(2-thienyl)pyrazol-3-yl]-;
	7	$C_{18}H_{20}O3$	Dibenz[a,c]cyclohexane, 2,4,7-trimethoxy- ;
	8	$C_{16}H_{16}O2$	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-ethyenyl];
	11	$C_{23}H_{22}N_2O_3$	3,4-Dimethoxy-benzoic acid (1-biphenyl-4-yl-ethylidene)-hydrazide;
Sapwood	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	$C_{20}H_{25}NO_3S$	Androst-4-en-9-methylthio-11-ol-3,17-dione;
	15	$C_{15}H_{12}N_2O_3$	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;

Tab.3 -The position of the extract in the sample tree and its relative percentage. 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. The figures representing compounds in the table below are from the serial numbers in Tab.2. 100% indicates that only one compound of the samples in this position has a peak area of more than 1%. Sl.No: serial number of the sample tree; VP: vertical position; HP: horizontal position.

Sl.	Ds.1			Ds.2					Ds.3								Ds.4					
VP	Middle		Top	Mid	Base		Top		Middle		Base		Top			Middle			Base			
HP	Ins	Out	Out	Out	Insi	Out	Ins	Out	Ins	Out	Insi	Out	Ins	Trans	Out	Ins	Trans	Out	Insi	Trans	Out	
1					82.6														81.5			
2					7.83		10		10		90.1		10	100%		10	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	10											
16		47.2										
17		43.6										

Fig.1-The picture shows a cross section of the base of a 15-year-old *Dalbergiasissoo*stem, indicating the difference in color between the heartwood and the sapwood of the wood (The heartwood is the dark area of the center).

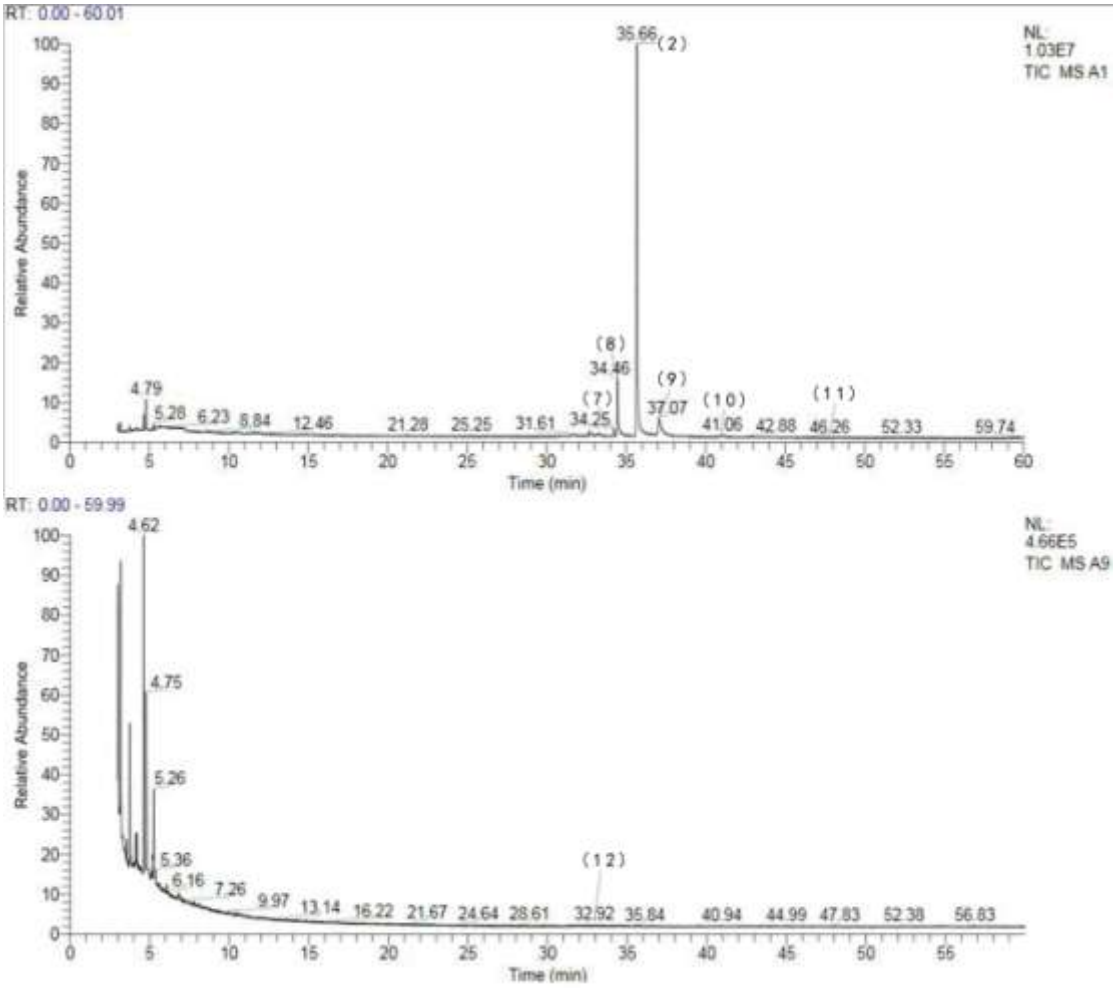

Fig.2- Gas chromatographic mass spectrum of ethyl acetate extract of heartwood and sapwood of Ds.4. In the figure, the upper mass spectrum of the number A1 indicates the ethyl acetate extract of the heartwood, and the lower mass spectrum of the number A9 indicates the ethyl acetate extract of the sapwood.