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Evaluation of holocellulose and lignin content of wood of banoak provenances from Himachal Pradesh

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Abstract
Wood is composed of three primary cell wall constituents namely cellulose, hemicellulose and lignin, which interacts in such a way so as to form a natural composite. The present study aimed at evaluating the content of holocellulose and lignin in the wood of banoak (*Quercus leucotrichophora*) among twenty two provenances from Himachal Pradesh. The average holocellulose content ranged from 65.31 % to 71.30 %. The maximum and minimum holocellulose content was found in Barog (71.30 %) and Banikhet (65.31 %), respectively. The average lignin content was ranged from 20.48 % to 28.46 %. The maximum and minimum lignin content was observed in Dhami (28.46 %) and Jibhi (20.48 %), respectively.

Keywords: cellulose, hemicelluloses, lignin, banoak, provenance

Introduction
Cellulose, a long chain polymer of β-D-glucose, formed by the process of photosynthesis, is regarded as the fibre within the composite (Dinwoodie, 2000) [7] and constitutes 40 to 50 per cent of the dry weight of wood. Cellulose chains have been estimated to be up to 5000 nm in length (Barnett and Jeronimidis, 2003) [5] with adjacent chains bonded to one another through a combination of hydrogen bonds and Van der Waals' forces.

The smallest building blocks in the cell wall are elementary fibrils, made up of approximately 40 cellulose chains and assembled into highly crystalline cellulose microfibrils and are 10–30 nm in diameter (Tsoumis, 1991) [13]. They can be randomly organized, as in the primary wall, or aligned to a particular angle, as in the S_1, S_2, and S_3 layers of the secondary wall. The primary cell wall is especially rich in pectin and lignin (Bowyer *et al.*, 2003) [6], middle lamellae in lignin, while the secondary cell wall layers have a higher concentration of cellulose. Within the cell wall, cellulose fibers are bonded together by a lignin matrix by the compatibilizing action of hemicelluloses.

Hemicellulose is a carbohydrate polymer, which differs from cellulose in many aspects, notably that it is non-crystalline and its degree of polymerization is much lower. The combination of cellulose (40-50%) and hemicellulose (15-25%) is called holocellulose and usually account for 25-35 per cent of dry weight of wood (Rowell, 2013). These polysaccharides in wood are synthesized entirely from glucose, mannose, galactose, xylose, arabinose, 4-o- methylglucuronic acid and galacturonic acid residues.

Lignin is an aromatic polymer and along with hemicelluloses can form the matrix of the composite, creating a sheath surrounding the cellulose core. It constitutes 18 to 35 per cent of dry weight of wood and consists of an irregular array of variously bonded hydroxyl- and methoxy- substituted phenylpropane units (Perez *et al.*, 2002) [11].

Ban oak is the most common broadleaf tree of family Fagaceae in the mid-elevations of the Central Himalayas in India. It is also found in Myanmar, Pakistan, Nepal, Thailand and Vietman. It is best adapted to the regions with a mild and moist climate. Oaks (*Quercus* spp.) are the dominant, climax tree species of the moist temperate forests of the Indian Himalayan region where about 35 species of *Quercus* are extensively distributed between 1000-3500 m elevations (Troup, 1921) [12]. Five species of evergreen oak namely; *Quercus glauca, Q. leucotrichophora, Q. dilatata, Q. semecarpifolia* and *Q. baloot* grow naturally in the Western Himalaya which comprises of the eastern part of Himachal Pradesh and state of Uttarakhand. Among these oak species, ban oak forms most extensive forests between 1000 - 2500 m elevations.

Methodology

Procurement of wood samples from field: Wood samples were collected with increment borer from live standing trees of *Quercus leucotrichophora* from different provenances of Himachal Pradesh, elevation ranged from 1000-2500 m above sea level. Small cylindrical piece of wood of known diameter and length at DBH was taken by using increment borer, from trees having diameter ranging from 25-30 cm. The length of samples ranged from 10-12 cm and it contained the portion of bark, sapwood and heartwood. Three replicates from three random selected oak trees with distance of 100 m were taken within a provenance. Small samples were grinded to powder form and oven dried for 2-3 days at 105 ± 2˚C.

Procedure for extraction of Holocellulose (T9m-59-Anonymous, 1959)

Five grams oven dried wood dust pre-extracted with alcohol-benzene (1:2 v/v) was taken in a conical flask and 160 ml of distilled water was added to it. The contents were treated with 1.5 gram of sodium chlorite and 10 drops of acetic acid at 70-80˚C on a water bath for one hour. The process was repeated four times till the meal became white. The contents were then filtered through IG-2 crucible, washed with water and finally with acetone. The sample was dried in an oven at 105 ± 2˚C to a constant weight. The extracted holocellulose content was calculated on the basis of the oven dry weight.

Procedure for extraction of Klason-lignin content (T12m-59-Anonymous, 1959c)

Two grams oven dried wood dust pre-extracted with alcohol-benzene (1:2 v/v) was treated with 15 ml of 72 per cent sulphuric acid for 2 hours at 18-20˚C with constant stirring. The material concentration was brought down to 3 per cent by adding 545 ml of double distilled water. The solution was refluxed for 4 hours and then allowed to settle. The contents were filtered, washed with hot distilled water and dried in an oven at 105 ± 2˚C till constant weight and expressed in percentage on oven dry weight basis.

Results and Discussions

Analysis of variance (Table- 1) reflected significant difference in holocellulose content in the wood of oak from different provenances at 5 per cent level of significance. Among all provenances holocellulose per cent was found to be maximum in Barog (71.30 %) which was statistically at par with Jibhi (71.17 %), Bechad bag (71.11 %), Nandli (70.78 %), Bahadurpur (70.60 %), Seog (70.60 %), Padhar (70.57 %), Jarri (70.47 %), Panarasa (70.45 %), Sarahan (70.36 %), Ghanati (70.31 %), Kuhasari (70.29 %), Nohradhar (70.29 %), Churwadhar (70.08 %), Chail (70.06 %), Andhreta (69.70 %), Naao (69.54 %), Dhami (69.52 %), Badidhar (69.27 %) and Rahala (69.11 %), whereas, minimum was observed in Banikhet (65.31 %) which was statistically at par with Kalapul (67.18 %).

Lignin percent was found to be maximum in Dhami (28.46 %) which was at par with Kalapul (28.39 %), Chail (28.36 %), Bechad bag (28.27 %) and Panarasa (27.16 %), whereas minimum was recorded in Jibhi (20.48 %) and it was statistically at par with Jarri (20.93 %), Ghanati (21.35 %), Churwadhar (22.20 %), Naao (22.23 %), Badidhar (22.42 %) and nohradhar (23.08 %).

Cellulose and hemicellulose, together described as holocellulose, is a term used to denote the polysaccharides in wood. The holocellulose content is a quantitative indication of fibrous raw material influencing consideration of its suitability for pulp. Lignin content in wood is considered to be undesirable and represents the carbohydrate fractions and is highly amorphous phenolic polymer of indeterminate molecular weight and it is responsible for providing stiffness to the cell wall. It also serves to bond individual cells together in the middle lamella region (Alen, 2000) [3].

Table 1: Variation in Holocellulose (%) and Lignin (%) of banoak wood samples

Sr. No.	Provenances	Holocellulose (%)	Lignin (%)
1	Andreta (Kangra)	69.70	23.43
2	Badidhar (Solan)	69.27	22.42
3	Bahadurpur (Bilaspur)	70.60	24.94
4	Baniket (Chamba)	65.31	25.60
5	Barog (Solan)	71.30	24.47
6	Bechad Bag (Sirmaur)	71.11	28.27
7	Chail (Solan)	70.06	28.36
8	Churwadhar (Sirmaur)	70.08	22.20
9	Dhami (Sirmaur)	69.52	28.46
10	Ghanati (Shimla)	70.31	21.35
11	Jarri (Kullu)	70.47	20.93
12	Jibhi (Kullu)	71.17	20.48
13	Kalapull (Kangra)	67.18	28.39
14	Kuhasari (Mandi)	70.29	23.53
15	Naao (kullu)	69.54	22.23
16	Nandli (Mandi)	70.78	23.29
17	Nohradhar (Sirmaur)	70.29	23.08
18	Padhar (Mandi)	70.57	25.11
19	Panarsa (Mandi)	70.45	27.16
20	Rahala (Kullu)	69.11	24.70
21	Sarahan (Sirmaur)	70.36	25.54
22	Seog (Shimla)	70.60	24.05
	Mean	69.91	24.45
	SE (d)	1.19	1.28
	$CD_{0.05}$	2.41	2.60

Alton *et al*. (1990) [4] reported that heartwood contained significantly more extractives than sapwood whereas, sapwood contained more holocellulose than heartwood. Among provenances significant variations was found and per cent holocellulose content ranged in live ban oak trees was from 65.31 % - 71.30 % and lignin content ranged from 20.48 % - 28.46 %. The results are in conformity with study by Pari *et al.* (1997) [10] in nine Indonesian species and Fakhrian *et al.* (2005) in *Alnus glutinous.* Kumar *et al.* (2005) [9] also estimated lignin and holocellulose content of *Dalbergia sissoo* ranged from 23.90 % - 30.50 % and 66.00 % - 76.55 %, respectively.

Conclusion

Holocellulose and lignin content in ban oak wood samples showed a significant variation among different provenances. It has also been found that the site having lower holocellulose content have higher lignin and vice- versa. Lower lignin content from Jibhi also indicates that banoak is recommended for pulp making.

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