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Research Article

CHEMICAL COMPOSITION OF BHIMAL FIBRES

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ABSTRACT

Bhimal is an important agro-forestry tree in the Himalayan regions. It is grown abundantly as it has multiple uses as fuel, fodder, fruit, fibre, etc. The stem or bast of this tree has been traditionally used for extraction of bhimal fibres. Local people extract bhimal fibre by the process of retting in water bodies around them. The extracted bhimal fibres are basically ligno-cellulosic in nature. This paper is an attempt to enumerate, define and quantify various components of bhimal fibres. Understanding of the basic chemical composition of bhimal fibres would aid to ascertain appropriate end uses for future researchers.

INTRODUCTION

Bhimal fibres are bast fibres extracted from the bark of bhimal tree, abundantly found in the Himalayan belt. They have been traditionally extracted by the process of retting. Local inhabitants in Uttarakhand region are involved in harvesting, shade-drying, dipping and beating of bhimal fibres. The fibre extracted is harsh and is used for making ropes and handicraft articles. Properties of bhimal fibres could be improved and new dimensions for application could be explored if the chemical composition could be determined. The chemical composition of bhimal fibres has not been reported so far. There are many papers reporting values of composition of leaves of bhimal trees but not for branches of the tree. This paper is an attempt to report chemical composition of bhimal fibres separated from the branch and analyse it.

Chemical composition vary greatly amongst plants and also within specific fibres depending on genetic characteristics, age of the plant, soil characteristics, the part of the plant used and growth, harvesting, extraction conditions and methods used to determine chemical composition. The main chemical constituents of cell walls of ligno-cellulosic fibres are cellulose, hemicellulose and lignin in a ratio of 4:3:3. This ratio varies slightly in hardwood, softwood and herbs. Cellulose molecules are regularly arranged into bundles and determine the framework of the cell wall. Hemicellulose and lignin form the filling material in fibres (Chen, 2014).

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Bast fibres have bundles of fibres joined together having cells as ultimate units. These cells are further composed of microfibrils which are made up of parallel cellulosic chains (Grayson, 1984 and P. Manimaran, *et al*., 2016). Microfibrils are entwined into a network and form the basic framework of the cell wall. The diameter of microfibril is 10-25nm. A microfiber is formed by elementary fibrils arranged in parallel form. The diameter of an elementary fibril is approximately between 2-4nm the structural unit of which is cellulose molecules linked by β-1, 4-glycosidic bonds. In some parts of the microfibrils, cellulose molecules are arranged in an orderly fashion and therefore, cellulose has crystal properties. This regular arrangement of cellulose molecules in microfibrils is called a micelle. Some non-cellulosic molecules also exist within the network of cellulose, including hemicellulose, pectin and so on (Chen, 2014).

Cellulose

Cellulose is the main component of plant cells. It is a natural high degree polymer molecule composed of glucose residues having cellubiose as the basic coupling unit (Fig. 1). It is insoluble in water, dilute acidic solutions, and dilute alkaline solutions at room temperature. Usually, cellulose content of plant cell wall accounts for 35-50% of the total dry weight. Pure cellulose, almost 100% is found in cotton. Mostly, cellulose is surrounded by hemicellulose and lignin. α-cellulose forms the bulk of the ultimate cell walls, with its molecular chains parallel to the direction of the fibre axis. Cellulose is the main component which provides strength and stability to the plant. The end use of fibre is determined by its cellulose content. For example, fibres with high cellulose content would be preferable for textile, paper and other

fibrous applications. Pineapple and banana fibres have higher cellulose content in their cell walls to support the high weight of the fruit they have.

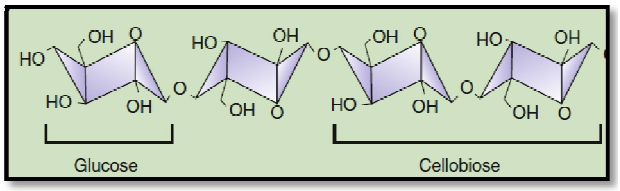

Fig 1 Basic unit of cellulose-cellubiose

It is a linear homopolymer of D-glucopyranose units linked by β-1,4-glycosidic bonds. The chemical formula of cellulose is $(C_6H_{10}O_5)_n$, where, n is the degree of polymerization (DP). DP represents the number of glucose groups present which can range from hundreds to ten thousands in case of cellulose. According to their solubility under specific conditions, cellulose can be divided into three types: α-cellulose which gets dissolved in 16.5% sodium hydroxide at 20ºC, β-cellulose which is deposited material extracted after neutralizing the acid solution and remaining alkaline solution and γ-cellulose which is the remaining soluble portion in the neutralize solution. Generally, α-cellulose is referred to as pure cellulose. β-cellulose and γ-cellulose are together industrially called as hemicellulose. Holocellulose refers to the total carbohydrates in natural cellulosic material, which is equal to the sum of cellulose and hemicellulose. Out of holocellulose, hemicellulose can be extracted by dissolving in dilute alkali. It is easily hydrolysed into sugars and therefore, preferred for producing fuels such as ethanol. Finally, insoluble residue left is α-cellulose (Chen, 2014, Lewin, M. and Pearce, E. M., 1985 & Kozlowski R., *et al*., 2011).

Hemicellulose

They came to be called as hemicellulose as the first researcher who worked on it; Schulz in 1891, thought it was polysaccharides that could easily separate from plant tissues as semi-finished products of cellulose. But this concept was later found to be vague. As per Yang, S.H.'s Plant fibre chemistry (2008) as cited in Chen H., 2014, another researcher, Aspinall in 1962, defined hemicellulose was derived from polysaccharides of plants and included the basic chain containing residues of D-xylose, D-mannose, D-glucose, or D-galactose and other glycosyls as branched chains linked to this basic chain. It can simply be called as alkali soluble polysaccharides. It is a copolymer of different amounts of several saccharide molecules, unlike cellulose. It is 20-35% of the total dry weight plant cell walls. Hemicellulose and lignin are located in between two adjacent cells as a cementing material in the middle lamella, and hence provide lateral adhesion. Hemicellulose forms filler between cellulose and lignin. It has degree of polymerization and orientation less than that of cellulose, and therefore, is easily degraded in acidic medium than cellulose. Similar to cellulose, hemicellulose can slowly degrade under mild alkaline conditions. At high temperature, it would have alkaline hydrolysis. It can dissolve in both alkaline (5% sodium carbonate solution) and acidic conditions (2% hydrochloric acid solution). It has a relative affinity for water, which can make it viscous in water (Chen, 2014, Lewin, M. and Pearce, E. M., 1985 & Kozlowski R., *et al*., 2011).

Lignin

Lignin is the second most abundant large-molecule polymer in the cell wall. It encloses the bundle cells, such as wood fibres and sclerenchyma cells. Chemically, phenylpropanoid derivatives form the basic units of lignin; three main monomers are coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Fig. 2). As per physical characteristics, lignin is hard and hence, increases the hardness of the cell wall. It has high resistance to chemical degradation. It is 5-30% of the total dry weight of plant cell walls. Woody plants have lignin content of about 27-32% whereas herbaceous plants have 14-25% lignin (Chen, 2014). Lignin can be removed by chlorination reaction in which it forms choro-lignin complex and separates from holocellulose. Lignin is a polyphenolic polymer with three-dimensional network consisting of high molecular weight long chain molecules. Lignin has a highly crosslinked molecules with an amorphous structure and occurs in the primary wall and outer part of the secondary wall. It acts as glue between the fibrils forming the cell wall. Divisibility of fibres into individual components is also lowered by presence of lignin and it protects the carbohydrates from chemical and physical damage. Hydroxyls and many other polar groups exist in lignin structure, resulting in strong intramolecular and intermolecular hydrogen bonds, thus making lignin insoluble in any solvent. Because of the presence of phenolic hydroxyl and carboxyl groups, lignin gets dissolved in alkaline solution. Generally, fibres with high cellulose content have low lignin content and vice-versa, as seen in Table 1 below. (Lewin and Pearce, 1985, Reddy & Yang, 2004 and Kozlowski *et al*., 2011).

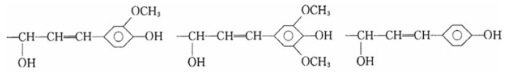

Fig 2 Basic units of lignin

Apart from these three major constituents, bast fibres may also contain minor components such as fats and waxes, inorganic matter, nitrogenous matter and traces of pigments, not exceeding more than 2% of the composition. Pectins are important component of plant fibres as they help bind fibres into bundles and also determine lustre and touch of the fibre. They are basically macromolecular compounds of poly-galacturonic acid. Efficient removal of pectins determines the divisibility of fibres into individual fibres and fineness of fibres and its suitability for spinning (Lewin, M. and Pearce, E. M., 1985 & Kozlowski R., *et al*, 2011).

Neutral Detergent Fibre

Estimation of NDF utilizes detergents which complex with protein to render it soluble and utilizes a chelating agent (EDTA) to remove heavy metal and alkaline earth contamination. This procedure involves the separation of feed dry matter into two fractions-one of high digestibility and the other of low digestibility-by boiling a 0.5-1.0 gram sample of the feed in a neutral detergent solution (3% sodium lauryl sulphate buffered to a pH of 7.0) for one hour and filtering. NDF as determined by Van Soest procedure is considerably higher than the conventional crude fibre values for some feeds since all of the lignin and hemicellulose are included in the NDF fraction. Crude protein content of NDF is neutral detergent insoluble crude protein (NDICP). NDF can be equated with the cell wall content of grasses and cereals. If

preceded by starch extraction, it can be equated with the cell wall constituents of many other feed ingredients. It underestimates the cell wall content of legumes. Legumes and other non-grass specie contain relatively high concentrations of pectic polysaccharides that are extracted by neutral detergent and not included in their NDF fraction. Hence, NDF and cell wall are not synonymuous.

Acid Detergent Fibre

Estimating ADF is used for the purpose of determining lignin in a forage sample. In this method, the ADF procedure is used as a preparatory step. This involves the boiling of a 1.0 gram sample of feed in an acid detergent solution (49.04 grams of sulphuric acid and 20 grams of cetyl trimethyl ammonium bromide per litre) for one hour and filtering. The insoluble residue left is ADF.

NDF-ADF= hemicellulose (+limited amount of protein)

The composition of common natural fibres has been summarized in the table below, Table 1.

Chemical composition testing of bhimal fibres

The composition analysis of bhimal fibres was carried out in order to substantiate major compounds found in natural fibres. The standard tests were performed in order to find out the percentage of cellulose, hemicellulose and lignin which are three major components of natural fibres. The tests were performed at National Dairy Research Institute, Karnal under the guidance of Dr. Chander Datt, Principal Scientist, Animal Nutrition. Association of Official Agricultural Chemists (AOAC), 2005 test methods were followed in the laboratory at NDRI for assessing various components of bhimal fibres. Different stages of extraction of bhimal fibres were taken- sample I was fibrous layer manually pulled and removed, sample II was fibres extracted by water retting by the local people of Dehradun; sourced from AAGAAS, sample III was

Table 1 Chemical Composition of some Natural Fibres

Sample		Cellulose (%)	HC (%)	ADL /Lignin (%)	Pectins (%)	Water soluble (%)	Ash (%)	Fats and waxes (%)
Coir [1]		43.44	0.25	45.84	3.00	5.25	2.22	-
Cotton[2]		91.8	6.4	6.4	6.4	1.1	-	0.7
Flax [2]	Retted	71.2	18.6	2.2	2.0	4.3	-	1.7
	Non-retted	62.8	17.1	2.8	4.2	11.6	-	1.5
Jute[2]		71.5	13.4	13.1	0.2	1.2	-	0.6
Abaca[2]		70.2	21.8	5.7	0.6	1.5	-	0.2
Ramie[2]		76.2	14.6	0.7	2.1	6.1	-	0.3
Pina[3]		70-80	18	5-12	-	-	3.6-7	-
Bagasse[4]		32-48	19-24	23-32	1.5-5	-	-	-
Hemp		74.4[5]	17.9[5]	3.7[5]	2.5[8]	-	0.53[8]	-
Sisal		78[5]	10[5]	8[5]	0.8[8]	1.2[8]	-	0.3[8]
Grewia tilifolia[5]		67.9	17	15	-	-	-	-
Nettle[6]		53-86	4-10	3.5-9.4	-	-	-	3.1-4.2
Kenaf		53.14[6]	14.33[6]	8.18[6]	2[7]	-	2-5[6]	0.8[6]
Banana	Untreated	Ho-67.1, α-62.1	5	17	-	-	-	-
	Treated	Ho-76.9, α-74.8	2.1	10.25	-	-	-	-

Source: 1- Mahapatra (2016), 2- McGovern, J. N. (1984), 3-Doraiswamy, I. & Chellamani, P. (1993) as cited in Reddy N. & Yang Y. (2005), 4- Rowell, R. M. et al. (1997) as cited in Reddy N. & Yang Y. (2005), 5- Jayaramudu, J., Guduri, B. R., & Rajulu, A. V., 2010, 6-- P. Senthamaraikannan, S. S. Saravanakumar, V. P. Arthanarieswaran & P. Sugumaran., 2016, 7- Tahir, P. M., Ahmed, A. B., Siafulazry, O. A., & Ahmed, Z., 2011, 8- Lewin, M. and Pearce, E. M., 1985, 9- Vardhini, K. J. V., Murugan, R., Selvi, C. T. & Surjit, R., 2016

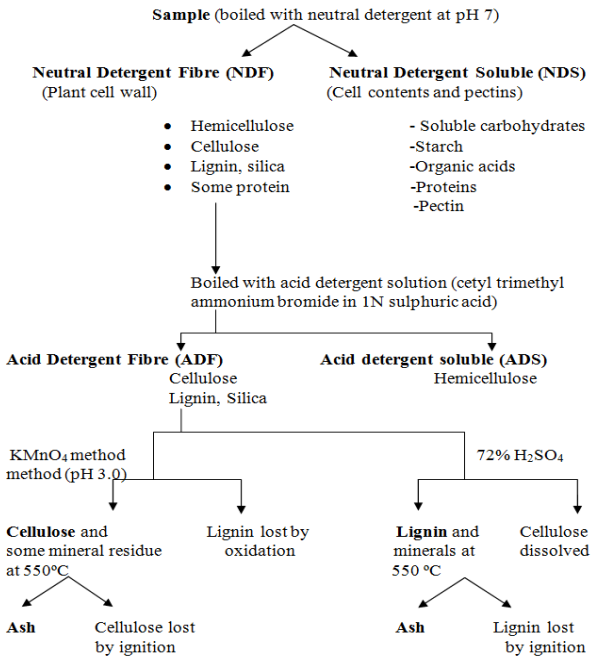

Figure 3 Van Soest method of partitioning fibre in feeds

urea retted @2.5%owm solution, sample IV was urea retted @5%owm solution, sample V was urea retted @2.5%owm and 5 gpl hydrogen peroxide treated and sample VI was urea retted @5%owm and 5 gpl hydrogen peroxide treated. The procedures followed have been given below under separate headings.

Estimation of Cell Wall Constituents

The fraction of cell wall constituents such as NDF, ADF, hemicellulose were estimated using Van Soest *et al.*, 1991 as shown in the flowchart below, Fig 3. NDF was analysed by using heat stable α-amylase (Number A3306, Sigma Chemical Company) enzyme.

Neutral Detergent Fibre (NDF)

Apparatus**:** spout less beakers, sintered glass crucible, vacuum pump, hot air oven, muffle furnace, electronic balance and dessicator.

Reagents**:** Neutral detergent solution (NDS), heat resistant amylase solution, acetone

Procedure for making Neutral detergent solution (NDS)

Ingredients:
Sodium lauryl sulphate-30 grams
Disodium ethylene diamino tetra acetate (EDTA)-18.61 grams
Sodium borate decahydrate-6.81 grams
Disodium hydrogen phosphate (anhydrous)-4.56 grams
Triethylene glycol- 10ml
Distilled water-900 ml

Ethylene diamino tetra acetate (EDTA) and sodium borate were put together in a large beaker with some distilled water and heated on hot plate until dissolved. Similarly, sodium lauryl sulphate was dissolved in distilled water and triethylene glycol was added to it. The solution of sodium lauryl sulphate and triethylene glycol was added to the previous solution. Disodium hydrogen phosphate was taken in another beaker and some amount of distilled water was added and the contents were heated until dissolved. Then, it was added to solution containing other ingredients and volume was made up to one litre with distilled water.

For preparing amylase solution: dissolve 2 grams heat resistant α-amylase enzyme in 90 ml water, filter through Whatmann filter paper no. 54 and stored at 5ºC.

Procedure for estimating NDF

Approximately 1 gram sample was taken in a spout less beaker of 1 litre capacity. To this, 100 ml. NDS and 0.5 grams of sodium sulphite were added. The contents of spout less beaker were refluxed for half an hour. Thirty minutes after onset of boiling, the contents of beaker were filtered through pre-weighed 50 ml sintered glass crucible (G-I) using oil-free vacuum pump. The contents were washed repeatedly with hot boiling water and then acetone to remove all salts. The sintered crucible containing residue was dried in hot air oven (100±5ºC) overnight, cooled and weighed to constant value. Then ashing was done and crucible along with ash was weighed again. The NDF (ash free) was calculated as follows:

$$\mathbf{NDF(\%)}=\frac{(\text{weight of crucible with residue}-\text{weight of crucible with residual ash})}{\text{weight of sample taken}}\ X\ 100$$

Acid Detergent Fibre (ADF)

Apparatus**:** spout less beakers, sintered glass crucible, vacuum pump, hot air oven, muffle furnace, electronic balance and dessicator.

Reagents**:** Acid detergent solution (ADS), acetone, hot boiling water

Procedure for making Acid detergent solution (ADS)

20 grams cetyltrimethyl ammonium bromide (CTAB) was dissolved in one litre of 1 N sulphuric acid.

Procedure for estimating ADF

Approximately 1gram of sample was taken in a spout less beaker of 1 litre capacity. To this, 100 ml. ADS was added and the contents were refluxed for exactly one hour. After refluxing, the residue was filtered through pre-weighed sintered glass crucible G-1 using vacuum pump and washed with hot water 2-3 times followed by acetone to remove all salts. The sintered crucible containing residue was dried in hot air oven (100±5ºC) and weighed again. The ADF was calculated as follows:

$$\text{ADF}(\%)=\frac{(weight\ of\ crucible\ with\ residue-weight\ of\ empty\ crucible)}{weight\ of\ sample\ taken}\ X\ 100$$

Hemicelluloses

Hemicellulose was soluble in ADS and thereby calculated by subtraction of ADF from NDF as follows:

Hemicellulose (%) = NDF (%) – ADF (%)

Acid Detergent Lignin (ADL)/Lignin

Apparatus**:** spout less beakers, sintered glass crucible, vacuum pump, hot air oven, muffle furnace, electronic balance and dessicator.

Reagents**:** Acid detergent solution (ADS), acetone, hot boiling water

Procedure

The procedure for estimation of ADL content was exactly same up to the filtering and drying of ADF contents of sintered crucible after treating with 72% sulphuric acid (w/w) in the cellulose estimation procedure. Then the crucible with dry residue was kept in muffle furnace for ignition at 550-600ºC for 2-2.5 hours, cooled and weighed again. The acid detergent lignin was calculated as follows:

$$\text{ADL}(\%)=\frac{(weight\ of\ crucible\ with\ dry\ residue-weight\ of\ crucible\ with\ ash)}{weight\ of\ sample}\ X\ 100$$

Cellulose

Principle

For estimation of cellulose, the detergent fibre (ADF) procedure is used as a preparatory step. The ADF residue consists of cellulose, lignin, cutin and acid insoluble ash (mainly silica). Cellulose is dissolved in 72% sulphuric acid treatment of ADF.

Procedure

Sintered crucible grade G-1 containing ADF contents was placed in enamel tray in such a manner that one end of the enamel tar jaws at about 2 cm. higher than the other end. So that acid could drain away from the crucible. The crucible was then filled with 72% sulphuric acid (w/w basis) and the contents were stirred with glass rod to break all the lumps. The crucible was refilled with 72% sulphuric acid after one hour interval. After three hours, the crucible was removed from the tray and filtration of acid was done by using vacuum pump. The material was washed with hot water until it becomes free from acid and kept into the oven (100±2ºC) overnight and weighed.

$$\text{Cellulose (\%)} = \frac{W1-W2}{Y}\ X100$$

Where, W_1= weight of crucible + weight of sample (before extraction)

W_2= weight after extraction
Y= weight of initial sample

Chemical composition of bhimal fibres

Analysis of chemical composition of bhimal fibres was carried out to investigate various components of it so that it could be applied in appropriate fields accordingly. The composition of bhimal fibres as provided by NDRI has been tabulated below in Table 2:

As seen in the table above, bhimal fibres have high amount of Acid Detergent Fibre and Neutral Detergent Fibre, which must be the reason why they are used as a shampoo by the local people of Uttarakhand. HESCO, an NGO based in Uttarakhand has developed a commercial shampoo by adding other components to bhimal's extracts.

Table 2 Chemical composition of bhimal fibres

Sample	Description	NDF (%)	ADF (%)	C (%)	HC (%)	L (%)
I	Fibrous layer manually pulled and removed	64.88	58.14	31.36	6.74	23.77
II	Extracted by water retting by the local people of Dehradun	64.33	59.86	35.91	4.47	22.78
III	Urea Retted @2.5% owm solution	70.11	65.70	42.60	4.41	20.90
IV	Urea Retted @5% owm solution	65.56	57.58	41.65	4.98	20.80
V	Urea Retted @2.5% owm and 5 gpl hydrogen peroxide treated	70.50	66.09	43.47	4.41	21.42
VI	Urea Retted @5% owm and 5 gpl hydrogen peroxide treated	70.40	65.07	43.08	4.33	21.04

Key: NDF- Neutral Detergent Fibre, ADF- Acid Detergent Fibre, HC- Hemicellulose, C- Cellulose, L- Lignin

Amount of cellulose and non-cellulosic components in a fibre determine the structure and properties. They influence its crystallinity and moisture regain. Although strength of fibres cannot be exactly correlated to the cellulose content and microfibrillar angle, generally fibres with higher cellulose content, higher degree of polymerization of cellulose and lower microfibrillar angle give better mechanical properties (Reddy, N., & Yang, Y., 2004). As seen in the table, the amount of cellulose in sample I was lowest with higher values of hemicellulose and lignin. This sample was untreated fibres, manually pulled from the branches, so these values indicate the true composition of bhimal fibres. In the case of sample II, the content of cellulose has increased 15% while the content of hemicellulose and lignin has decreased by 51% and 4%, respectively. These changes can be attributed to bacterial degradation caused by retting practiced by the local people. Sample III was chemically retted fibres using urea @ 2.5% owm, which has shown a further increase from water retted sample II in cellulose content by 19% and a decrease in hemicellulose and lignin by 1% and 9% respectively. Sample IV, which was urea retted at a higher percent, showed an increase of 16% in cellulose content and a decrease in hemicellulose and lignin content by 12% and 10%, respectively. There was no significant increase in the composition of fibres with urea retting @ 2.5%owm and 5%owm (sample III and IV). On bleaching @5 gpl to already urea retted fibres @ 2.5%owm in sample V, cellulose increased slightly from 42.60 to 43.47 (2%), while hemicellulose remained constant and lignin came down by 2.5% further, suggesting further improvement in properties. Sample VI which was bleached sample IV, showed an increase in cellulose by 3% and decrease in hemicellulose by 15%, however, lignin remained constant at 20.80. there was no significant difference in composition of sample V and sample VI, suggesting both the combinations of 2.5% owm urea retting and 5 gpl hydrogen peroxide and 5% owm urea retting and 5 gpl hydrogen peroxide, had similar effect on bhimal fibres.

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