CHAPTER - 4
MATERIALS AND METHODS

4. 1 Sample survey

The studies were started with the survey of selected less utilised 6 numbers of bamboo species of Kokrajhar district. Field observation and herbarium collection was done for those bamboo species. The preparation of herbarium was done by following the method described by Jain and Rao (1977). Matching of the specimen was done with the help of available literature, mainly with the descriptions given by Barooah and Borthakur in the book named Diversity and distribution of Bamboos in Assam; published by Bishen Singh Mahendra Pal Singh in 2003 at 23-A, New Connaught Place, Dehra Dun-248001, India.

For confirmation of the specimen, the plantation site of Assam Bio-resource Centre at Madan Kamdev archaeological site near Baihata Chariali in Kamrup district and Bambusetum of Indian Botanical Garden, Kolkata were visited. The final identification and authentication were done at Botanical Survey of India, Eastern Regional Centre, 123, Laitumkhrah, Shillong-793003, Meghalaya. Herbarium specimen has been deposited at the Department of Botany NEHU, Shillong.

Table 4.1: List of selected bamboo species

Sl. no.	Name of the Bamboo species	Local name
1.	*Bambusa garuchokua* Barooah *et* Borthakur.	Owa goraiating/Nagal banh
2.	*Bambusa assamica* Barooah *et* Borthakur.	Owa tere/Saru bijuli
3.	*Bambusa pallida* Munro.	Owa hatai/Bijuli banh
4.	*Melocanna baccifera* (Roxb.) Kurz.	Owa tharai/Muli banh
5.	*Bambusa polymorpha* Munro.	*Owa jaoti/Betwa banh*
6.	*Bambusa bambos* (L.) Voss.	*Owa sugwnang/Kotoha banh*

4.2 Collection of samples

The sample was collected from all four directions of Kokrajhar district. Since all the 6 numbers of studied bamboo species are home grown village bamboo, several villages of Kokrajhar, Gossaigaon and Parbatjhora sub-division, under Kokrajhar district were visited for the collection of bamboo sample. The number of villages visited was 30 under 3 sub-divisions. The selection of village for collection of bamboo sample was based on availability of bamboo clump and presence of selected species. The bamboo culms were randomly collected and care was taken to collect the culms almost of same age of 3 years. The age of the culms were ascertained by direct observation as well as method proposed by Waheed (1962) and Banik (1988). The choosing of 3 years old bamboo is based on the knowledge that the industrial applicability of bamboo culm of this age was found more (Razak *et al*., 2013).

The culms were cut at about 30 cm above the ground level, as cuttings of the culms below that level will affect the growing rate of the other culms in the bamboo clump (Maya *et al.*, 2013). Harvesting of bamboo for sample collection was done during the month of January 2014. The maintaining of month and season for collection of sample was done as described by Banik (2010). Five numbers of individual culm from each species were harvested. Altogether 30 numbers of individual culm from 6 numbers of bamboo species were collected. Out of 30 individual bamboo culms, 15 individuals from Kokrajhar sub-division, 10 from Gossaigaon and 5 from Parbatjhora sub-division were collected as shown in **Table 4.2**. After harvesting, the selected bamboos were assigned sample numbers for the convenience of study.

Table 4.2: Bamboo species and assigned sample number with name of villages from which bamboo samples were collected

District sub-division	Name of village	Collected Bamboo species.	Accession No.	Coll.no.
Kokrajhar	Debargaon	*Bambusa garuchokua* Barooah *et* Borthakur.	NEHU-12160	1

Kokrajhar	Ranchaidham	*B. garuchokua* Barooah *et* Borthakur.	NEHU-12160	2
Gossaigaon	Raimana	*B. garuchokua* Barooah *et* Borthakur.	NEHU-12160	3
Gossaigaon	Guabari	*B. garuchokua* Barooah *et* Borthakur.	NEHU-12160	4
Parbatjhora	Devitola	*B. garuchokua* Barooah *et* Borthakur.	NEHU-12160	5
Kokrajhar	Karigaon	*B. assamica* Barooah *et* Borthakur.	NEHU-12161	6
Kokrajhar	Dhaoliguri	*B. assamica* Barooah *et* Borthakur.	NEHU-12161	7
Gossaigaon	Raikumbari	*B. assamica* Barooah *et* Borthakur.	NEHU-12161	8
Parbatjhora	Bashbari	*B. assamica* Barooah *et* Borthakur.	NEHU-12161	9
Parbatjhora	Bhumka	*B. assamica* Barooah *et* Borthakur.	NEHU-12161	10
Kokrajhar	Aflagon	*B. pallida* Munro.	NEHU-12162	11
Kokrajhar	Bhutiapara	*B. pallida* Munro.	NEHU-12162	12
Kokrajhar	Daolabari	*B. pallida* Munro.	NEHU-12162	13

Gossaigaon	Hatidhura	*B. pallida* Munro.	NEHU-12162	14
Gossaigaon	Simultapu	*B. pallida* Munro.	NEHU-12162	15
Kokrajhar	Nayekgaon	*Melocanna baccifera* (Roxb.) Kurz.	NEHU-12165	16
Kokrajhar	Silgara	*M. baccifera* (Roxb.) Kurz.	NEHU-12165	17
Kokrajhar	Bhutiapara	*M. baccifera* (Roxb.) Kurz.	NEHU-12165	18
Gossaigaon	Sapkata	*M. baccifera* (Roxb.) Kurz.	NEHU-12165	19
Gossaigaon	Jaraguri	*M. baccifera* (Roxb.) Kurz.	NEHU-12165	20
Kokrajhar	Bongshidharma	*B. polymorpha* Munro.	NEHU-12163	21
Kokrajhar	Bhalukjhora	*B. polymorpha* Munro.	NEHU-12163	22
Kokrajhar	Gambaribil	*B. polymorpha* Munro.	NEHU-12163	23
Gossaigaon	Bamankura	*B. polymorpha* Munro.	NEHU-12163	24
Parbatjhora	Malatijhora	*B. polymorpha* Munro.	NEHU-12163	25
Kokrajhar	Adabari	*B. bambos* (L.) Voss.	NEHU-12164	26
Kokrajhar	Gossainessina	*B. bambos* (L.) Voss.	NEHU-12164	27

Gossaigaon	Soraibil	*B. bambos* (L.) Voss.	NEHU-12164	28
Gossaigaon	Tulsibil	*B. bambos* (L.) Voss.	NEHU-12164	29
Parbatjhora	Tipkai	*B. bambos* (L.) Voss.	NEHU-12164	30

MAP 4.1

Map of Kokrajhar district showing locations of bamboo collection with assigned sample number

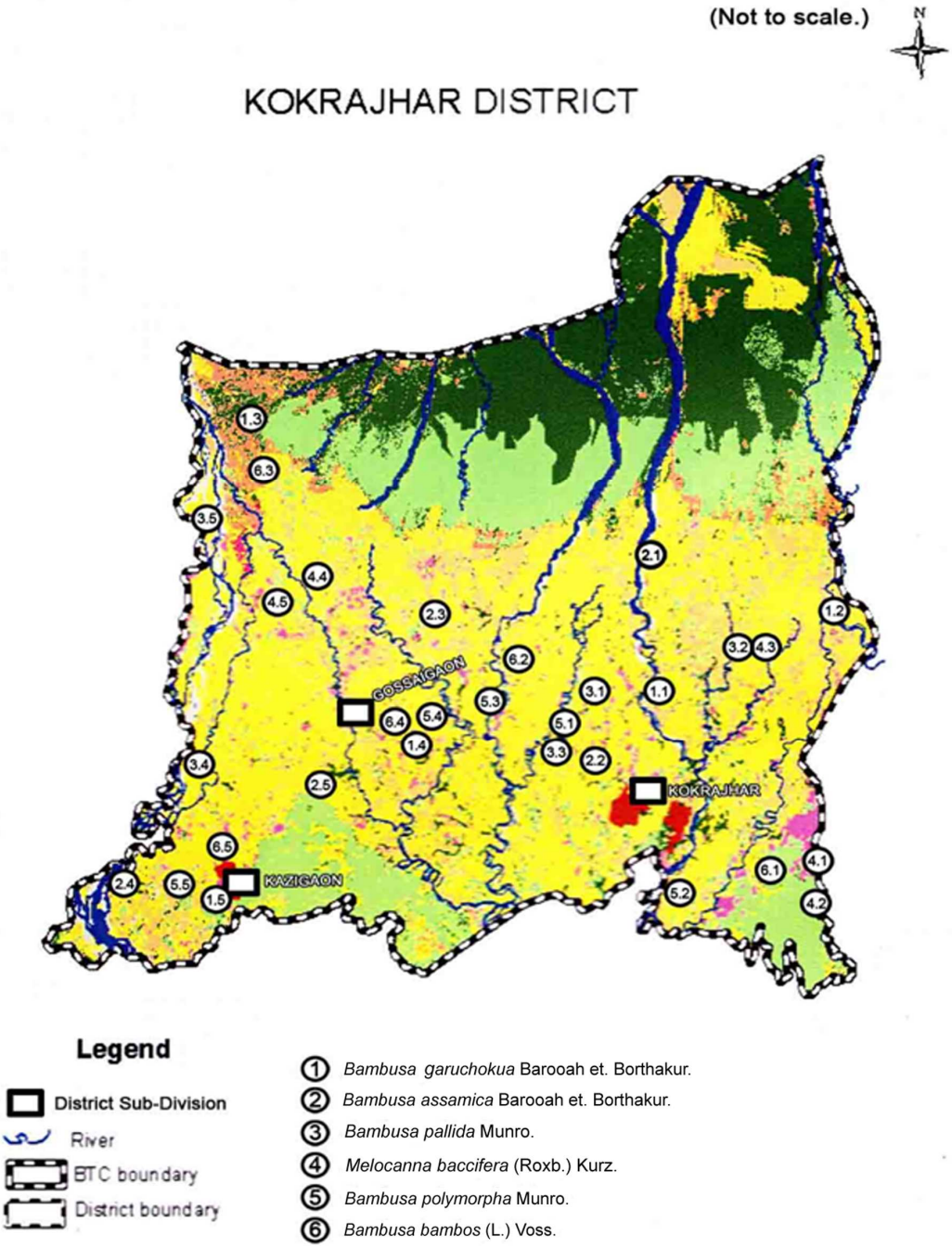
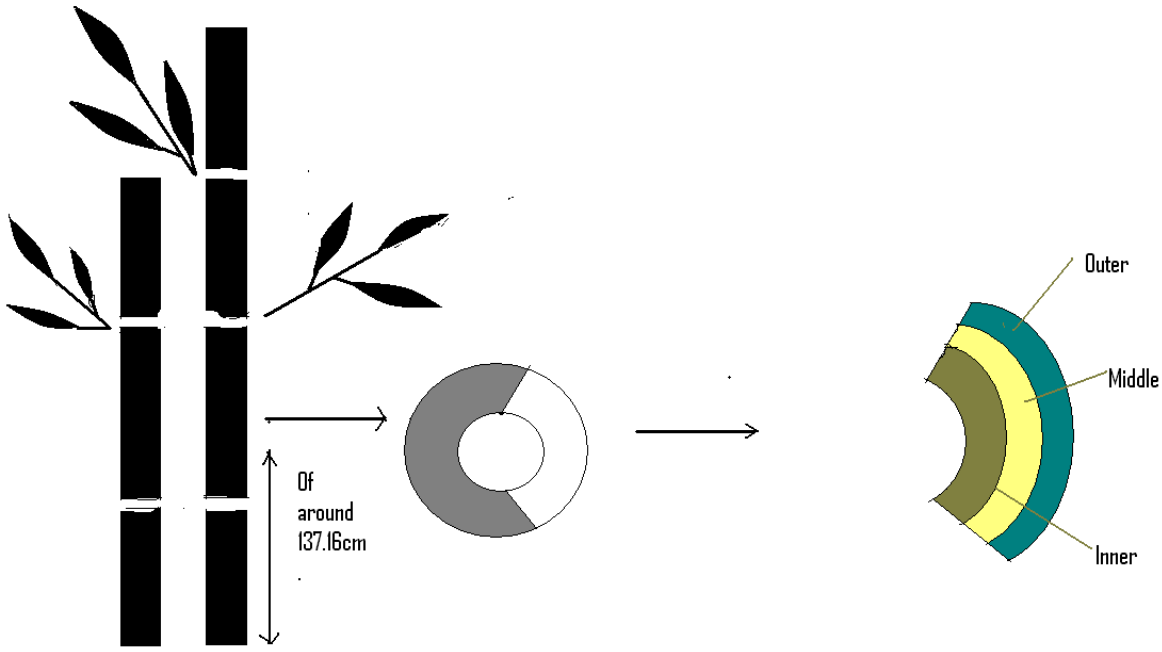


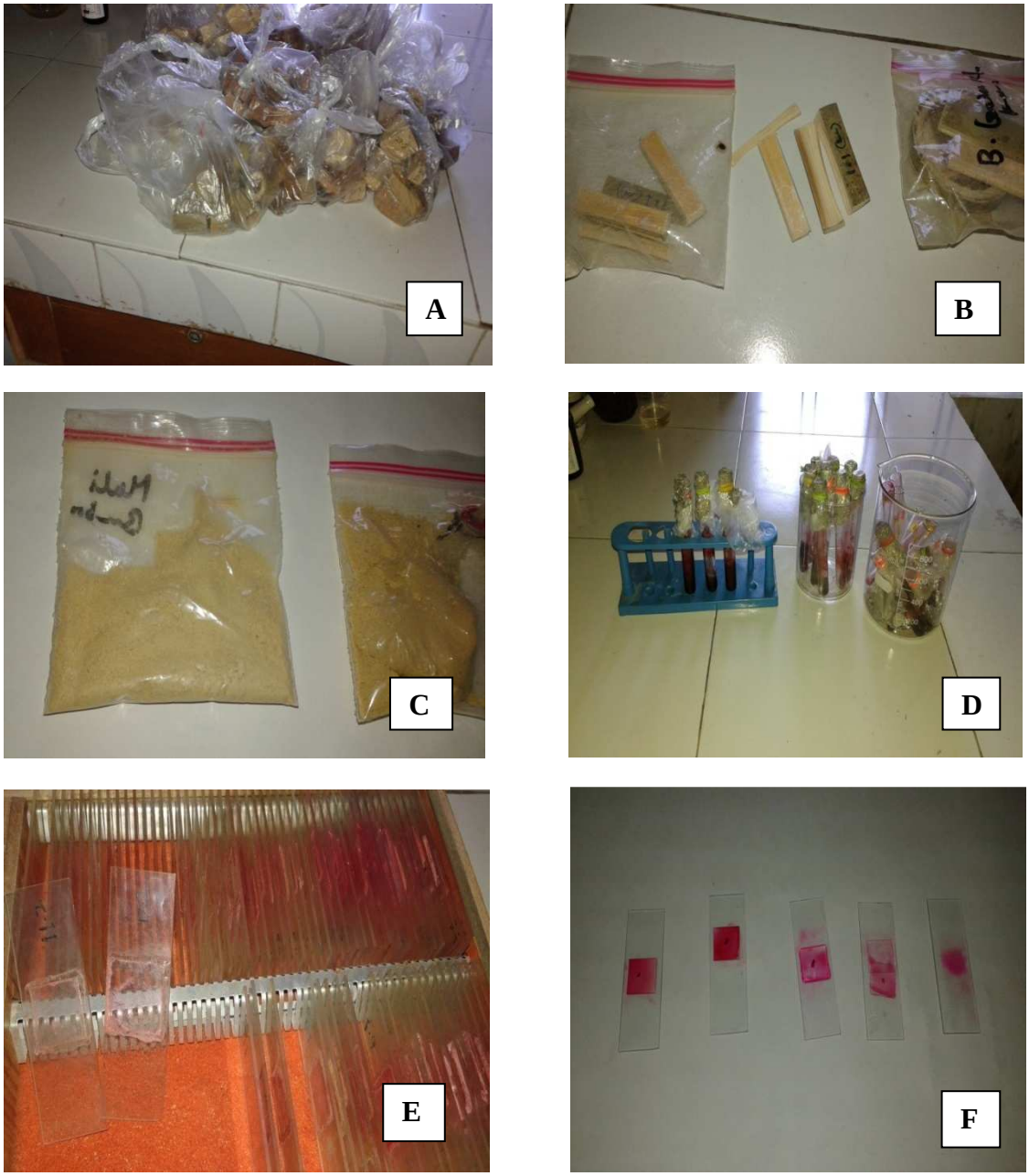
PLATE 4.1 Plan for sample preparation



Sketch for plane of sample preparation

Plan of sample preparation for chemical, physical and anatomical experiment, showing sample preparation from Breast height diameter (BHD), which comes around 137.16 cm height portion of bamboo culm and culm wall cross sectional position outer, middle and inner.

PLATE 4.2: Sample preparations for chemical, physical and anatomical experiment



[**A] - Oven dried sample; [B] - Sample block and strips; [C] - Grinded sample powder; [D] - Macerated sample; [E] & [F] - Temporary preparation of micro-sections and macerated fibers .**

4.3 Study of culm characters

Soon after the felling of bamboo, they are cut into suitable pieces. Before cutting it into pieces, the following basic parameters were studied. The important morphological structures mentioned below were studied and measured with a standard scale in centimetre (cm).

4.3.1 Culm characters

4.3.1.1. Total culm height

4.3.1.2. Total culm diameter

4.3.1.3. Culm wall thickness

4.3.1.4. Numbers of internodes

4.3.1.5. Length of internodes

4.3.2 Leave characters

4.3.2.1. Length of leaves

4.3.2.2. Width of leaves

4.3.3 Culm sheaths characters

4.3.3.1 Length of culm sheaths

4.3.3.2 Width of culm sheaths

4.4 Study of chemical properties

For study of chemical properties, the collected bamboo samples were made small blocks and also grinded into powder. To perform the other experiments the pieces of bamboo were kept in sealed plastic bag. All the samples were collected from the BHD (Breast Height Diameter) which comes around 137.16 cm portion of the bamboo culm.

With this sample, the following tests were performed.

4.4.1 Determination of alcohol-toluene solubility content

4.4.2 Determination of water solubility content

4.4.3 Determination of ash content

4.4.4 Determination of Klason lignin content

4.4.5 Determination of holocellulose content

4.4.6 Determination of α-cellulose content

4.4.7 Determination of element content

4.4.1 Determination of alcohol-toluene solubility content

To determine the alcohol-toluene solubility of the studied bamboo, soxhlet extraction was done by using a 2 g of oven dried bamboo sample. The solution used was 2:1 solution of ethyl alcohol (92%) and toluene respectively. To perform this experiment, minor modification of ASTM D 1107-56 by Li (2004) was done by replacing the benzene with toluene solution. The soxhlet extraction tube is fitted with boiling flask. A condenser was fitted to the set up. The whole set up was boiled in a heating mantle regulating the temperature. The temperature was so maintained that it gives approximately 6 siphoning per hours. Accordingly, the siphoning was done for 8 hours. After the completion of the extraction, the solution containing the extraction was heated in the heating mantle so that all the solution gets evaporated. Content was oven dried at 103±2^{o}C and weighed (ASTM D 1107-56; Reapproved 1972).

The alcohol-toluene solubility percentage of the bamboo sample was calculated by using the formula given below:

$$\text{Alcohol-toluene soluble percentage (\%)} = \frac{W_2}{W_1} \times 100$$

Where, W_1 = Weight of oven dry test sample (g).

W_2 = Weight of oven dry extraction residue (g).

4.4.2 Determination of hot-water solubility content

To determine hot-water solubility content a 2 g of oven dried bamboo sample was mixed with 100 mL of distilled water into a 250 mL Erlenmeyer flask. The flask was put into a water bath for 3 hours for gently boiling by attaching condenser to the flask. After three hours of boiling, the sample was removed from water bath and filtered with vacuum suction filter. The filtrate was poured to a glass crucible of known weight and oven dried at 103±2^{o}C. After drying the residue containing crucible was cooled under a desiccators and process was repeated untill a constant weight was obtained (ASTM 1110-56; Reapproved 1977).

The hot-water solubility percentage of the bamboo sample was calculated by using the formula given below:

Hot water solubility content percentage (%) = $\frac{W_1 - W_2}{W_1} \times 100$

Where, W_1 = Weight of oven dried bamboo sample (g).

W_2 = Weight of hot water extraction of bamboo (g).

4.4.3 Study of ash content

To determine the ash content, the dry bamboo sample was obtained by air drying followed by oven drying at 103±2^{o}C. After oven drying the sample was cooled under desiccators and weighed. The weighing of the sample was repeated till a constant weight is obtained. A 2 g of oven dried bamboo sample was taken in crucible and ignitied in the muffle furnace until all the carbon was removed. The heating was slow at the beginning to check the flaming and then increases up to the final heating of 580 to 600^{o}C. The crucible with content was removed from the muffle furnace and allowed to cool under desiccator and weighed (ASTM D1102-84; Reapproved 1990).

The ash content percentage of the bamboo sample was calculated by using the formula given below:

Ash content percentage (%) = $\frac{W_2}{W_1} \times 100$

Where, W_1 = Weight of oven dried bamboo sample (g).

W_2 = Weight of ash content of bamboo (g).

4.4.4 Determination of Klason lignin

To determine K-lignin, 1 g of oven dried extractive free bamboo sample was taken in to a beaker of 150 mL. The sample was mixed with 15 mL of H_2SO_4 (72%) slowly from the side to avoid fume. To initiate the reaction stirring was done. The reaction was allowed to continue for 2 hours by placing the whole set up into water bath maintained at 20^{o}C. After 2 hours of reaction 560 mL of distilled water was added to dilute the H_2SO_4.

The whole set up was placed in a boiling water bath. During the process, a glass condenser was added to the flask. After 4 hours of boiling the flask was removed

from the water bath and is kept for a rest to settle down the insoluble materials. The content of the flask was collected and oven dried at 103±2^{o}C. After drying the content was weighed accurately (ASTM D 1106-56; Reapproved 1977).

The Klason lignin content percentage of the bamboo sample was calculated by using the formula given below:

$$\text{Klason lignin content percentage (\%)} = \frac{W_4 - W_3}{100 \times W_2} \times (100 - W_1)$$

Where, W_1 = Alcohol-toluene solubility content (%).
W_2 = Weight of extractive free sample (g).
W_3 = Weight of crucible (g).
W_4 = Weight of oven dried residue with crucible (g).

4.4.5 Determination of holocellulose content

A 2 g of oven dried bamboo sample was taken into a 250 mL Erlenmeyer flask. The sample was added with 150 mL of distilled water 0.2 mL of glacial acetic acid and 1 g of $NaClO_2$ (Sodium chlorite). After mixing well the whole set up was placed into a water bath maintaining at 70^{o}C. The reaction was allowed to continue for about 5 hours. After the reaction, the flask was cooled at ice water to lower the temperature to 10^{o}C. The content of the flask was filtered and washed with cold water (500 mL) to make the content free of ClO_2 (Chlorine dioxide) and then oven dried at 103±2^{o}C (ASTM D 1104-56; Reapproved 1978).

The holocellulose content percentage of the bamboo sample was calculated by using the formula given below:

$$\text{Holocellulose content percentage (\%)} = \frac{W_4 - W_3}{100 \times W_2} \times (100 - W_1)$$

Where, W_1 = Alcohol-toluene solubility content (%).
W_2 = Weight of extractive free sample (g).
W_3 = Weight of crucible (g).
W_4 = Weight of oven dried residue with crucible (g).

4.4.6 Determination of α-cellulose content

To determine α-cellulose content a 3 g of oven dried holocellulose sample was taken into a 250 mL Erlenmeyer flask and added and mixed with 50 mL of NaOH (17.5%) and allowed to react. After few minutes of reaction, a 50 mL of distilled water was added and mixed well. The whole setup was carried out into a water bath maintained at 20^{o}C. The filtrate was washed with 50 mL NaOH (8.3%) then, 40 mL acetic acid (10%) and finally washed with hot water to make the content acid free. The content was oven dried at 103±2^{o}C and weighed accurately (ASTM D 1103-60; Reapproved 1978).

The α-cellulose content percentage of the bamboo sample was calculated by using the formula given below:

$$\alpha\text{-cellulose content percentage (\%)} = \frac{W_4 - W_3}{100 \times W_2} \times W_1$$

Where, W_1 = Holocellulose content (%).

W_2 = Weight of oven dried holocellulose sample (g).

W_3 = Weight of crucible (g).

W_4 = Weight of oven dried residue with crucible (g).

4.4.7 Determination of elements

To obtain the ash for analysis, the sample was made clean by removing all the adhering foreign matters including sand, soil and then air dried. The air dry sample was made into ash by ignition in muffle furnace for 2 hours at 500^{o}C. After ashing, the porcelain crucible was taken out from the muffle furnace and allowed to cool. As the ash becomes cool, it was wet by required drops of H_2O_2 and slowly addition of HNO_3. The excess HNO_3 was evaporated by placing it on hot plate at 100^{o}C. After additional ashing for 1 hour, at 500^{o}C the crucible is allowed to cool and the ash was dissolved in 10 mL, HCl. (AOAC 58, 436, action in the year 1975).

The analysis was done by Graphite Furnace-Atomic Absorption Spectrometer (GF-AAS, Analytik Jena Vario-6) under concentration in ppm (parts per million).

The element content percentage of the bamboo sample was calculated by using the formula given below:

Element (ppm) = (µg/mL) × F/g. sample

Element percentage (%) = ppm×10^{-4}

Where, F = (mL original dilution × Final dilution)/mL.

4.5 Physical properties

4.5.1 Determination moisture content

To determine the moisture content, the bamboo sample was prepared by cutting sections from internodes of Breast height diameter (BHD) which comes around 137.16 cm portion of the culm. The sample blocks were cut into 3 cm length ×1 cm width × culm wall thickness, which comes around 2 g in weight. Fresh weight was taken immediately after the collection of sample and dry weight was taken after the sample block was oven dried at 105±2^{o}C for 48 hours. Desiccator was used to obtain constant weight (ASTM D 4442).

The moisture content percentage was calculated by using the following formula:

$$\text{Moisture content percentage (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

Where, W_1 = Initial weight of the sample (g).

W_2 = Final weight of the sample (g).

4.5.2 Determination of specific gravity

The specific gravity (SG) of a substance can be defined as comparison of density of the substance with that of water. To determine the SG, the same sample blocks that used for bending test were used (3 cm length × 1 cm width × 0.40 cm thickness). Weight displacement method was followed to find the volume of the sample. Weight displacement was done by soaking the sample into distilled water without touching the wall of the container. The soaking of the sample was continued until all the air bubbles that exist in the glass setup comes out and final reading was recorded. The

weight of water displacement = weight of substance + weight of bottleful water – weight of water with sample. The oven dried weight of the sample was obtained by drying the sample block at 105±2^{o}C for 48 hours. (ASTM D 143-94; Reapproved 2000).

The specific gravity was calculated by using the following formula:

$$\text{Specific gravity (SG)} = \frac{W_2}{Vg} \times 0.99917$$

Where, W_2 = Weight of oven dried substance.

Vg = Weight of equal volume of water displacement.

0.99917 = Specific gravity of water.

4.5.3 Determination of shrinkage

To determine the shrinkage percentage, the round internode from BHD was cut into suitable size about 20 mm × 20 mm × culm wall thickness and initial diameter was recorded. After the initial reading the sample was oven dried at temperature 103±2^{o}C. The drying was continued until it gives a constant weight. After oven dry, the final dimension was recorded. The longitudinal (length) and tangential (circumference) shrinkage percentage were calculated by using the following equitation (ASTM D - 143; Reapproved 2000).

The shrinkage percentage was calculated by using the following formula:

$$\text{Shrinkage \%} = \frac{D_1 - D_2}{D_1} \times 100$$

Where, D_1 = Initial dimensions (before oven dried).

D_2 = Final dimensions (after oven dried).

4.5.4 Determination of bending and compression properties

The bending properties of bamboo species was studied by using the sample obtained from the internodes of a bamboo culm portion of BHD (Breast height diameter). The sample was cut into suitable strip size of 16 cm long and 1cm wide thickness was made similar size of 0.40 cm for each species by removing from inner side. Prior to testing, the sample was air dried for about four weeks to obtain moisture

equilibrium of 10%. The test was done on an Instrone machine maintaining span of 12.7 cm and speed of 0.25 cm per minute. The value was recorded and calculated. The test was carried out by following the method mention by Li (2004) with modification of ASTM, D1037 - 94.

The MOR and MOE were calculated based on load applied and maximum deflection of splint as follows:

$$\text{Modulus of rupture (MOR)} = \frac{1.5P_1I}{bd^2}$$

$$\text{Modulus of elasticity (MOE)} = \frac{P_2l^3}{4bh^3d}$$

Where, P_1 = Failure load in N.

P_2 = Load at proportional limit in N.

I = Span in cm.

l = Length of the specimen in cm.

b =Width of specimen in cm.

d = Depth of specimen in cm

h = Deflection in cm

Compression test was done by using the same Instron machine by following ASTM, D1037-94 with slide modification by Li (2004). The sample size for compression test was trimmed to 1.2 cm × 1.2 cm and the speed of the test machine was maintained at 0.12 cm per minute.

$$\text{Compression strength} = \frac{\text{Ultimate failure load in N}}{\text{Area of application of load in cm}^2}$$

4.6 Study of anatomical properties

4.6.1 Study of vascular bundle

A 45 micron thickness transverse section of bamboo sample was cut with a sliding microtome machine and a temporary preparation of slide was done by adding aqueous safranin stain. Prepared section was oven dried at 40^{o}C for 8 hours. By using

the same preparation, both vascular bundle concentration and measurement of vascular bundle were done.

To determine the vascular bundle concentration by using the prepared slide, a microscopic observation of the slide was done under a light compound microscope at 10x magnification. The focused area was divided into three outer, middle and inner horizontal layers. Vascular bundle was counted separately from each horizontal layer and the area was measured and converted to centimeter. To calculate the result of vascular bundle concentration, 5 numbers of slides from each individual sample, altogether 150 numbers of slides were studied. Same numbers of slides were used to determine the vascular bundle dimension in length and wide. Five (5) numbers of vascular bundle were measured from randomly selected vascular bundle from each cross section (outer, middle, inner) with the help of a camera fitted microscope and ScopeImage 9.0 (HCL) software. Size of the vascular bundle was measured by using same slide and was focused under light microscope at 10x magnification. Care was taken not to measure the shorter and longer vascular bundle and also taken care to discard the repeated value of measurement. (The experiment was performed by method explained by Li, 2004).

4.6.2 Study of fiber characters

To study the fiber length and fiber diameter, the bamboo sample is macerated. The sample is first cut into small pieces of 0.25 × 0.25 × 5 cm. The pieces of samples were macerated with the maceration solution of Glacial acetic acid, 30% hydrogen peroxide and distilled water in ratio 5: 1: 4 respectively. The time for maceration was 48 hours at 60^{o}C. The randomly selected fiber from the focused area was measured by using camera fitted microscope and ScopeImage 9.0 (HCL) software at 10x magnification. Number of fiber measured was 75 numbers for each species, altogether 450 numbers of fiber were measured for 6 numbers of bamboo species. Care was taken not to measure the shorter and longer fiber and also taken care to discard the repeated value of measurement. (The experiment was performed by method explained by Li, 2004).