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# Modification in protocol for estimation of Klason-lignin content by gravimetric method

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**Abstract**
Plant biomass is mainly comprises of cellulose, hemicelluloses and lignin. Estimation of Klason-lignin content is important for delignification during pulping process in pulp and paper industries. To estimate Klason-lignin, four different available volumetric methods were compared using wood samples of *M. dubia* along with different concentration of $H_2SO_4$. Result revealed that the optimal concentration of 72% $H_2SO_4$ solution on weight by weight (w/w) basis is suitable for acceptable estimation of Klason-lignin content from wood samples. Further, it requires 600.80 ml of concentrated $H_2SO_4$ (in the case of 98% purity, sp. gr. 1.84, 18.4 mol/l) and 399.20 ml of distilled water for preparation of 1 litre of 72% (w/w) $H_2SO_4$, for estimation of Klason-lignin content in wood samples of *M. dubia* and other hardwood species.

**Keywords:** *Melia dubia*, Klason-lignin, 72% $H_2SO_4$, volumetric method

## Introduction
Lignin is a complex polyphenolic compound with three dimensional networks of phenyl propane units present in the middle lamella and cell wall of wood (Alder, 1977) [1]. The three common monolignol units such as paracoumaryl alcohol, coniferyl alcohol and sinapyl alcohol are precursors and incorporated into lignin in the form of *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S), respectively. Softwoods lignin consists almost entirely G with small quantities of H. Dicotyledonous hardwoods lignin consists more often S than G with very little H; while, monocotyledonous lignin is a mixture of S, G and H units cross-linked by a series of ether and carbon-carbon bonds (Weng and Chapple, 2010) [12]. Holocellulose, which is directly related to pulp yield generally cross-linked in the matrix of lignin. The complexity of lignin degradation depends upon the S/G ratio and its deposition in different types of tissues and cell wall (Fagerstedt *et al.*, 2015) [2]. However, various non-lignin components such as tannins, non-extracted polysaccharides and proteins also present in the lignin residue, which limit the accuracy of the Klason-lignin estimation (Hatfield, 2005) [3].
Generally, 72% $H_2SO_4$ solution is used to digest the holocellulosic content of wood in many research laboratories. The well accepted methods for estimation of lignin content of wood in research laboratory are TAPPI method (T-222), followed by near-infrared NMR spectroscopic and acid detergent fiber (ADF) methods (Kirk and Obst, 1988) [5]. TAPPI method is recommended for determination of Klason-lignin in wood due to its reliability (Klason, 1893) [6]. For estimation of Klason-lignin, 72% $H_2SO_4$ solution is used to digest the holocellulosic content of wood in many research studies. However, the preparation of 72% $H_2SO_4$ solution creates confusion, where it has not been clearly mentioned in many research methodologies, even in standard TAAPI method also, that whether this solution is prepared on volume by volume (v/v), weight by weight (w/w) or weight by volume (w/v) basis. Hence, the present study is carried out to know the accurate volumetric method for preparation of 72% $H_2SO_4$ solution for estimation of lignin content from wood samples of *Melia dubia* Cav. (Malabar Neem), which is mainly used in pulp and paper industries. Further, the study also implies to understand the concentration of $H_2SO_4$ using five different concentrations in order to check the validity of 72% $H_2SO_4$ solution after standardizing the volumetric method for estimation of lignin content, which was compared with TAAPI method.

## Material and Methods
### Study site and methodology
The present study was carried out in the laboratory of Forest Products, which is well equipped with instrumentation and facilities for analysis of chemical contents of wood. In this study,

wood samples of *Melia dubia* were used in order to estimate the Klason-lignin content.

*Melia dubia* Cav. (Family: Meliaceae), an emerging fast growing tree species used by pulp and paper industries in India, which has high quality of fibre than traditional raw materials (Partibhan *et al.*, 2009; Kumar *et al.*, 2013) [9, 7], hence, its demand for wooden raw materials has been increased (Saravanan *et al.*, 2013) [10]. Therefore, *Melia dubia* progeny trial was established at Forestry Farm, College of Forestry, Navsari Agricultural University, Navsari, Gujarat during January 2013 to understand the growth, establishment, genetic diversity and wood quality parameters.

For this study, wood core samples were collected from three different standing trees of *M. dubia* at the age of five years using Pressler's increment borer at the breast height (1.37 m) to estimate chemical proximate composition of wood. Klason-lignin content was estimated from extractive free wood powder samples as per improved DE (Designer Energy Ltd.) method (Ioelovich, 2015) [4] using following formula.

$$\text{Lignin content (\%)} = \frac{\text{ODW of lignin with polypropylene tube (g)} - \text{ODW of polypropylene tube (g)}}{\text{ODW of test specimen of wooden powder (g)}} \text{ x } 100$$

Where, ODW is Oven Dry Weight.

**Preparation of 72% $H_2SO_4$ solution by different volumetric methods**

For preparation of one litre of 72% $H_2SO_4$ solution, four volumetric methods were used to estimate Klason-lignin content in *M. dubia* wood samples. One litre of 72% $H_2SO_4$ solution was prepared on the basis of volume by volume (v/v), weight by weight (w/w), weight by volume (w/v) along with TAPPI method.

**(A) On the basis of volume/volume (v/v):**

One litre solution of 72% $H_2SO_4$ was prepared on volume/volume (v/v) basis from concentrated $H_2SO_4$ (98% purity, sp. gr. 1.84, 18.4 mol/l) by the following formula.

$$N_1 x V_1 = N_2 x V_2,$$

Where, $N_1$= Concentration of chemical, $V_1$= Volume of chemical, $N_2$= Required concentration of solution, $V_2$= Required volume of solution.

Therefore, preparation of 1 litre of 72% (v/v) $H_2SO_4$, it requires 734.69 ml of concentrated $H_2SO_4$ and 265.31 ml of distilled water.

**(B) On the basis of weight/weight (w/w):**

One litre solution of 72% $H_2SO_4$ was prepared on weight/weight (w/w) basis from concentrated $H_2SO_4$ (98% purity, sp. gr. 1.84, 18.4 mol/l) by the following formula.

$$C_1 x D x V_1 = C_2 x [(V_2 - V_1) + (D x V_1)],$$

Where, $C_1$= Concentration of chemical, D= Density of chemical, $V_1$= Volume of chemical, $C_2$= Required concentration of solution, $V_2$= Required volume of solution.

Therefore, preparation of 1 litre of 72% (w/w) $H_2SO_4$, it requires 600.80 ml of concentrated $H_2SO_4$ and 399.20 ml of distilled water.

**(C) On the basis of weight/volume (w/v)**

One litre solution of 72% $H_2SO_4$ was prepared on weight/volume (w/v) basis from concentrated $H_2SO_4$ (98% purity, sp. gr. 1.84, 18.4 mol/l) by the following way.

Based on the weight by volume basis, one litre of 72% $H_2SO_4$ means 720 g of $H_2SO_4$ is dissolved in 280 ml of distilled water. By considering specific gravity (1.84) of $H_2SO_4$, the volume of 720 g of $H_2SO_4$ is 391.30 ml. Hence, the total amount of solution required is 671.30 ml. This amount of solution is multiplied by a fraction of 1.48964 to convert into one litre solution.

Therefore, preparation of 1 litre of 72% (w/v) $H_2SO_4$, it requires 582.90 ml of concentrated $H_2SO_4$ and 417.10 ml of distilled water.

**(D) On the basis of TAPPI method (T 222)**

According to TAPPI method, preparation of 1 litre of 72% $H_2SO_4$, it requires 665 ml of concentrated $H_2SO_4$ (95.5 to 96.5% purity, sp. gr. 1.84) and 335 ml of distilled water (TAPPI, 2011) [11].

After standardizing the preparation of 72% $H_2SO_4$ solution on weight by weight (w/w) basis, the volume of 100 ml solution with five different concentrations of $H_2SO_4$ (18%, 36%, 54%, 72% and 90%) were prepared to check the validity of 72% (w/w) $H_2SO_4$ solution (Table 1).

**Table 1:** Preparation of five different concentrations (w/w) of $H_2SO_4$ solution

| $H_2SO_4$ Concentration (%) | Quantity of concentrated $H_2SO_4$ (ml) | Quantity of distilled water (ml) | Final Solution (ml) |
|---|---|---|---|
| 18 | 10.90 | 89.10 | 100 |
| 36 | 23.99 | 76.01 | 100 |
| 54 | 40.01 | 59.99 | 100 |
| 72 | 60.08 | 39.92 | 100 |
| 90 | 85.94 | 14.06 | 100 |

**Results and Discussion**

The estimation of Klason-lignin content in wood samples of *Melia dubia* by three different volumetric methods along with TAPPI method using 72% $H_2SO_4$ solution are presented in Table 2. The lowest (27.07%) Klason-lignin content was reported in weight/weight volumetric method of 72% $H_2SO_4$ solution; while, the highest lignin content (46.21%) was reported in weight/volume method, followed by volume/volume method. The Klason-lignin content (30.72%) estimated by TAPPI method was slightly higher than our volumetric estimation by weight/weight method which may be due to low purity of concentrated $H_2SO_4$ (95.5 to 96.5%) as used by TAAPI (Table 2). However, in our volumetric estimation, the purity of concentrated $H_2SO_4$ was 98%.

**Table 2:** Klason-lignin content estimation in *M. dubia* wood using four different volumetric methods of 72% $H_2SO_4$ solution

| Samples | Volume/volume | Weight/weight | Weight/volume | TAPPI |
|---|---|---|---|---|
| 1 | 41.32 | 26.18 | 44.24 | 30.06 |
| 2 | 43.59 | 28.53 | 47.59 | 31.23 |
| 3 | 42.27 | 26.50 | 46.79 | 30.87 |
| Mean | 42.40 | 27.07 | 46.21 | 30.72 |
| SD | 1.14 | 1.27 | 1.75 | 0.60 |

The estimation of higher lignin content by 72% $H_2SO_4$ in volume by volume (v/v) method may be due to coagulation of the lignin with structural polysaccharides present in the cell wall of wood during fast hydrolytic reaction with high volume of acid. However, higher lignin content in weight by volume method may be due to more dilution of acid. Hence, the preparation of 72% $H_2SO_4$ solution in weight/weight (w/w) volumetric method provided the optimal condition to solubilize the polysaccharides *i.e.,* cellulose and hemicellulose to estimate lignin content in *M. dubia* that matches with the range of hardwood lignin (Lu *et al.*, 2017) [8].

After standardizing the volumetric method on weight by weight (w/w) basis for estimation of lignin content in *M. dubia*, five different concentrations of $H_2SO_4$ solution were used to check the validity of 72% (w/w) $H_2SO_4$ solution and result is presented in Table 3. The mean Klason-lignin content was recorded lowest (27.07%) in *M. dubia* when wood samples were digested in 72% (w/w) $H_2SO_4$ solution. The estimated lignin content in wood samples of *M. dubia* using 18%, 36%, 54% and 90% concentration of $H_2SO_4$ solution were higher than 72% (w/w) $H_2SO_4$ concentration (Table 3). Figure 1 showed that wood samples of *M. dubia* was not completely digested at 18%, 36% and 54% concentration of $H_2SO_4$ solution and the colour of samples did not turned black as compared to 72% (w/w) $H_2SO_4$ concentration. The reason for higher lignin content at lower concentrations of $H_2SO_4$ solution may be due to incomplete degradation of polysaccharides present in wood samples. Whereas, wood samples treated in 90% (w/w) $H_2SO_4$ solution digested polysaccharides with black colour and coagulated. This could be due to fast carbonization of carbohydrates and it coagulated the oxidative degradation of structural polysaccharides. Thus, both lower and higher concentrations of $H_2SO_4$ solution led to overestimation of Klason-lignin. Therefore, wood samples of *M. dubia* should be properly digested in 72% (w/w) $H_2SO_4$ solution for acceptable estimation of lignin content.

**Table 3:** Klason-lignin content estimation in *M. dubia* wood using five different concentrations of $H_2SO_4$ solution

| Samples | $H_2SO_4$ concentration (%) | | | | |
|---|---|---|---|---|---|
| | 18 | 36 | 54 | 72 | 90 |
| 1 | 72.12 | 63.86 | 58.73 | 26.27 | 41.01 |
| 2 | 74.13 | 64.31 | 62.31 | 28.08 | 43.91 |
| 3 | 73.89 | 62.50 | 60.00 | 27.01 | 41.64 |
| Overall | 73.38 | 63.56 | 60.35 | 27.12 | 42.19 |
| SD | 1.10 | 0.94 | 1.81 | 0.91 | 1.53 |

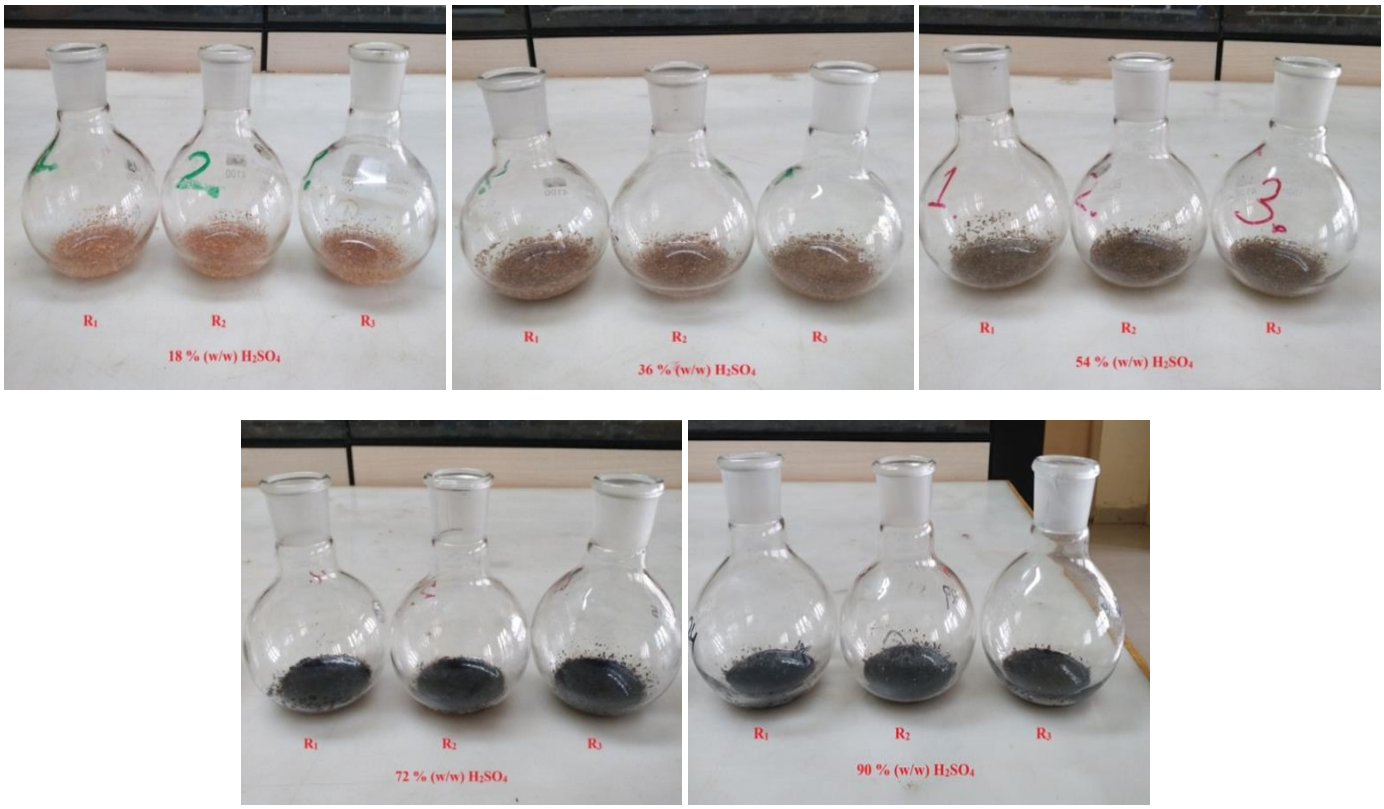


**Fig 1:** Digestion of *M. dubia* wood samples at five different $H_2SO_4$ concentrations (18, 36, 54, 72 and 90 %) for estimation of Klason-lignin content in *M. dubia*

## Conclusion

Study concludes that the optimal concentration of 72% $H_2SO_4$ solution on weight by weight (w/w) basis is suitable for acceptable estimation of Klason-lignin content from wood samples. Further, it requires 600.80 ml of concentrated $H_2SO_4$ (in the case of 98% purity, sp. gr. 1.84, 18.4 mol/l) and 399.20 ml of distilled water for preparation of 1 litre of 72% (w/w) $H_2SO_4$. While estimation of Klason-lignin, preparation of stock solution of 72% (w/w) $H_2SO_4$, initial concentration (purity) of the acid should be taken care; similarly, correct volumetric method *viz.*, v/v, w/w or w/v, needs to be mentioned.

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