Physico-Chemical and Thermochemical Profiling of Mulberry (*Morus* spp.) Clones for Sustainable Bioenergy Application: A PCA-Based Selection Approach for Sustainable Bioenergy Application

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ABSTRACT

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| **Background:** Energy demand in recent decades has surged due to rapid industrialization, population growth, and lifestyle changes. In India, most people live in rural areas and depend on biomass fuel wood for energy, leading to a significant increase in the country's energy requirements. In order to fulfill the energy, need the people mostly focuses on using fuel wood as their source of energy because they are easily available and cheaper than fossil fuel.  **Aims:** The study aimed to evaluate the physico-chemical and thermochemical characteristics of mulberry (*Morus* spp.) clonal genetic resources to assess their suitability as alternative renewable feedstock for bioenergy and fuelwood applications.  **Study design:** A Completely Randomized Design (CRD) was adopted for the experimental layout, and statistical analysis was performed using SPSS (version 23) and PCA was conducted using XLSTAT 24.  **Place and Duration of Study:** The research was conducted at the Department of Sericulture, Forest College and Research Institute, Mettupalayam, Tamil Nadu, during 2023–2024.  **Methodology:** The wood of seventeen mulberry clones *viz.,* *Morus alba* (MI-0674, ME-0174, MI-0145, MI-0211, MI-0828, MI-0300, ME-0169, MI0034), *Morus latifolia* (MI-0665, ME-0006, MI-0783, ME-0168, MI-0549, MI-0632, MI-0818, MI-0845), and *Morus laevigata* (MI-0532), were assessed for their bioenergy potential based on parameters such as moisture content, bulk and basic density, specific gravity, alcohol-benzene extractives, acid-insoluble lignin, holocellulose, ash content, volatile matter, fixed carbon, calorific value, higher heating value (HHV), and fuel value index (FVI). Standard protocols were followed for all analyses.  **Results:** Significant variation (*P* < 0.05) was observed among clones for all assessed traits. Moisture ranged from 29.97% to 41.10%, and basic density from 619.09 to 701.80 kg m⁻³. Calorific value and HHV ranged from 16.76 to 19.34 MJ kg⁻¹ and 18.73 to 20.64 MJ kg⁻¹, respectively. PCA identified five principal components explaining 85.092% of total variance, with MI-0845, ME-0168, and MI-0674 showing superior bioenergy profiles. Moisture content is a critical determinant of the combustion characteristics and fuel efficiency of biomass. High moisture levels are generally associated with reduced calorific value, diminished combustion efficiency, and increased operational costs due to higher energy consumption for drying and increased transportation weight. PCA results indicated that clones MI- 0845, MI- 0674, ME- 0168 ranked highly in PC1. While, clones like MI- 0845, MI- 0674, MI- 0034, and MI- 0532 appeared across multiple components, demonstrating their versatility and overall strong performance in contributing to the variability observed in the dataset. This ranking provides valuable insights into the mulberry clones that are most effective or influential for fuelwood and bioenergy production, guiding future selection and breeding strategies to establish mulberry as a sustainable alternative for bioenergy production  **Conclusion:** The study highlights the potential of select mulberry clones as sustainable bioenergy sources, supporting their strategic utilization in renewable energy and agroforestry systems. |

*Keywords: Mulberry clones; bioenergy; physico-chemical characterization; thermochemical characterization; principal component analysis*

1. INTRODUCTION

Bioenergy plays a key role in decarbonization in modelled future pathways, supporting energy system transformation, especially in hard-to-abate applications such as industry, aviation and shipping, and, when linked with carbon capture and storage (BECCS), can remove CO2 from the atmosphere, next to providing renewable energy. This article reviews the literature on bioenergy use for climate change mitigation, including studies that use top-down integrated assessment models or bottom-up modelling, and studies that do not rely on modelling. The article summarizes the state of knowledge concerning potential co-benefits and adverse side effects of bioenergy systems and discusses limitations of modelling studies used to analyse consequences of bioenergy expansion (Calvin et al., 2021; Xu et al., 2024). In the present global scenario, biomass is gaining attention on a global scale as a sustainable feedstock to produce energy and reduce the reliance on fossil fuels (Baqir *et al.,* 2019). A diverse range of biomass is utilized as an energy source, such as woody and herbaceous materials, dung cakes, agricultural wastes, and forest residues (Vamvuka and Sfakiotakis, 2011). Biomass, a renewable energy carrier, stands out as a particularly promising alternative due to its wide availability and potential to replace fossil fuels directly. Biomass not only ensures consistent power generation but also supports the production of various chemicals and transportation fuels, making it a versatile energy source (Manickavasagam et al., 2024). Approximately 90% of the world's energy demands are currently met by fossil fuels, but by 2040, 50% of energy is expected to come from renewable sources (Hussein, 2015; Palaniappan, 2017). The World Energy Forum forecasts that within the next ten years, the world’s reserves of fossil fuels, including oil, coal, and natural gas, will be depleted (Kumar *et al.,* 2010 a). With such swift depletion of our available resources and the increasing environmental hazards, it is essential to prioritize the exploration and utilization of more affordable and environmentally friendly energy sources. The increasing global population and growing human demands are contributing to the rapid depletion of fossil fuel resources, resulting in environmental challenges like the emission of greenhouse gases and water contamination. The World Energy Forum has made a prediction that the world's fossil-based oil, coal, and gas reserves will be completely depleted within the next 10 decades (Bora et al., 2023).

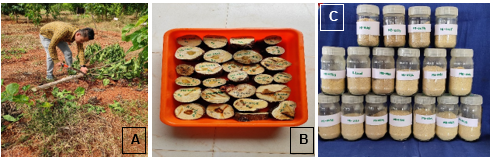
Energy demand in recent decades has surged due to rapid industrialization, population growth, and lifestyle changes. In India, most people live in rural areas and depend on biomass fuel wood for energy, leading to a significant increase in the country's energy requirements. In order to fulfill the energy, people mostly focus on using firewood as their source of energy because they are easily available and cheaper than fossil fuel. The increased consumption of fuel wood in rural areas of India leads to deforestation and rapid environmental degradation, as the extensive cutting of trees without proper knowledge can result in ecological imbalance in specific areas (Sedai *et al.,* 2016). Thus, it is crucial to separate or screen different tree species according to people's preferences. There is a growing emphasis on identifying and promoting fast-growing woody trees that are easy to propagate, adaptable to various agroclimatic conditions, suitable for intensive coppicing, and capable of producing high-quality fuel wood and biomass, while also providing significant economic and social benefits (Christersson, 2010). Mulberry is an exemplary, rapidly growing woody tree that meets these criteria. Compared to the annual biomass growth of many fast-growing trees and perennial energy crops, pruned mulberry branches yield an average of 1.7 kg per plant, or about 17.0–22.5 tonnes ha-1 (Lu *et al.,* 2009). Its impressive growth rate and high biomass yield make mulberry one of the top candidates among promising energy crops.

This study aims to evaluate the physical, chemical, and thermochemical properties of wood from various mulberry clones to determine their potential for bioenergy generation and as promising fuel wood. Currently, much of the mulberry biomass remains either unused or underused. The research findings will be valuable for both rural and urban communities that depend on fuelwood for energy. The results will help these communities to choose the most suitable clones having higher bioenergy generation and firewood potential by considering physicochemical and thermochemical properties, including low moisture content, high density, low ash content, high lignin content, high calorific value, and high fuel value index, among other factors.

2. material and methods

2.1 Sample preparation

Seventeen lignocellulosic woody materials from mulberry clonal genetic resources (eight clones each of *Morus alba* and *Morus latifolia*, and one clone of *Morus laevigata*) were sourced from the Central Sericultural Germplasm Resource Centre in Hosur. Since 2016, these materials have been cultivated in the germplasm garden at the Department of Sericulture, Forest College and Research Institute, Mettupalayam (11°19'37''N to 11°19'39''N latitude and 76°56'09''E longitude, elevation 338 metre with an average annual rainfall ranging from 700-800 millimetre). The selected trees represented a diverse mix of indigenous species and those from exotic origins. For each species, a randomly selected tree with a clean, undamaged bole was felled. Three 5 cm thick discs were cut from each tree, air-dried, ground into powder, and used for various analyses as shown in Fig. 1A, 1B and 1C.

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**Fig. 1A-C. Collection and preparation of samples from germplasm garden maintained at department of sericulture, Forest College and Research Institute, Mettupalayam**

**2.2** **Physical properties**

**2.2.1 Moisture content (%)**

The moisture content of wood was calculated using ASTM D4442-92 (ASTM International, 2003). Representative samples were collected in triplicate and weighed immediately to prevent moisture changes. If immediate weighing is not possible, samples should be stored in plastic bags or wrapped in metal foil. Samples were then dried in an oven at 103 ± 2 °C until stable weight was achieved over 3-hour intervals. The moisture content percentage was determined using the constant weight after drying and the initial weight of the specimen by a specific formula (Bergman, 2021).

**2.2.2 Bulk density**

To determine the bulk density, the weight and dimensions of each wood sample were measured and calculated using a specific formula (Kongprasert *et al.,* 2019).

|  |  |
| --- | --- |
| ρ = | m |
| v |

Where,

ρ = bulk density (g cm-3)

m = mass of wood sample (gram)

v = volume of sample (cm3)

**2.2.3 Basic density**

The basic density of each wood sample were measured by dividing the dry weight of the wood sample in grams by the green volume of the wood sample in cubic centimetres (Zobel & Van Buijtenen, 2012).

|  |  |
| --- | --- |
| Basic density = | Dry weight in grams |
| Green volume in cm3 |

**2.2.4 Specific gravity**

Three 2.5 cm thick discs were cut from each sample and oven-dried at 103 ± 2°C for six hours. After drying, the discs were coated with paraffin for waterproofing and weighed again. Using the water displacement method, ASTM D2395-17(ASTM International 2022), the weight of an equivalent volume of water was measured. By dividing the oven-dry weight of the disc by the weight of an equivalent volume of water, the specific gravity was determined (Mainoo and Ulzen- Appiah, 1996).

**2.3 Chemical properties**

**2.3.1 A-B (Alcohol- benzene) extractive (%)**

The alcohol-benzene extractive content was determined following the TAPPI T204 protocol, as outlined by Buchanan (2007), Malakani *et al.,* (2014), and Dutta *et al.,* (2025). Approximately 7 grams of oven-dried wood powder were placed in a thimble wrapped with cotton cloth and inserted into a Soxhlet extraction apparatus. The extraction was conducted using 300 ml of a 1:2 ethanol-to-benzene mixture, maintained at a temperature between 70°C and 85°C for a duration of six hours. After completion, the extract was transferred to a pre-weighed petri dish (W1), dried at 100°C, and then reweighed (W2). The alcohol-benzene extractive percentage was calculated using the standard formula.

|  |  |  |
| --- | --- | --- |
| AB Extractive (%) = | W2- W1 | × 100 |
| Oven dry weight of the sample |

**2.3.2 Acid insoluble lignin (%)**

The acid-insoluble lignin content was determined using the TAPPI, (2006) method (Anonymous, 2006). Each alcohol-benzene extracted sample (1.00 ± 0.01g) was placed in a 100 ml beaker and wetted with 2 ml of 72% H2SO4 (Sulfuric acid). Then, 130 milliliter of 72% H2SO4 was added. The beaker was placed in a water bath at 20°C for two hours, with occasional stirring. Afterward, the mixture was filtered through a pre-weighed G2 crucible (W1), washed with hot water, and the residues were weighed (W2). The lignin content percentage was then calculated by a specific formula (Malakani *et al.,* 2014, Dutta *et al.,* 2025).

|  |  |  |
| --- | --- | --- |
| Acid insoluble lignin (%) = | W2- W1 | × 100 |
| Oven dry weight of the sample |

**2.3.3 Holocellulose (%)**

To determine the holocellulose content, 5.00 ± 0.01g of the wood sample was placed in a 250 millilitre conical flask. The sample was moistened with 10 millilitre of distilled water, followed by the addition of 150 millilitre of distilled water, 1.5 gram of sodium chloride, and 0.5 millilitre of acetic acid. The flask was sealed with an inverted small flask and heated in a water bath at 70°C for one hour. After heating, the supernatant was poured into a pre-weighed crucible (W1). This process was repeated with the same reagents. The crucible and its contents were dried overnight at 105°C, then cooled and weighed again (W2). The holocellulose percentage was calculated by a specific formula (Ona *et al.* 1995).

|  |  |  |
| --- | --- | --- |
| Holocellulose (%) = | W2- W1 | × 100 |
| Oven dry weight of the sample |

**2.4 Thermochemical properties**

**2.4.1 Ash content (%)**

The ash content percentage of the samples was determined according to the ASTM D3174-12 standard (ASTM International, 2012; Kongprasert *et al.,* 2019). Each sample was put into crucibles weighing one gram, and the crucibles were heated for an hour in a muffle furnace. The temperature was raised from 450 to 600°C progressively during this procedure. After that, the temperature was raised to 750°C and kept there for two hours. The crucibles remained inside the furnace for an additional hour. After that the percentage of the ash content was determined**.**

|  |  |  |
| --- | --- | --- |
| Ash content (%) = | W3 - W1 | × 100 |
| W2 - W1 |

Where,

W1= weight of empty crucible, (g)

W2= weight of empty crucible + original sample, (g) and

W3= weight of empty crucible + ash, (g).

**2.4.2 Volatile matter (%)**

The volatile matter content of the biomass samples was determined using 2 grams of sun-dried material with a particle size of 425 μm, placed in a porcelain crucible. Each sample was initially sun-dried, then subjected to heating in a furnace at 500°C for 10 minutes. After cooling in a desiccator, the samples were weighed. The percentage of volatile matter (VM) was subsequently calculated using a standard formula (Ogunsola *et al.,* 2018).

|  |  |  |
| --- | --- | --- |
| Volatile matter (%) = | X- Y | × 100 |
| X |

Where,

X= weight of the sun-dried sample

Y= weight of the sample after 10 min in the furnace at 500˚C.

**2.4.3 Fixed carbon (%)**

Fixed carbon is the solid residue that is left over after volatile components burn. As per ASTM D3172-13 (ASTM International, 2021), the fixed carbon content was computed by deducting the initial mass of the sample from the percentages of moisture, volatile matter, and ash content (Ogunsola *et al.,* 2018).

Fixed carbon (%) = 100 – % MC – % AC – % VM

Where,

MC % = Moisture content %

AC % = Ash content %

VM % = Volatile matter %

**2.4.4 Calorific value**

A bomb calorimeter was used to calculate the calorific value of the sample in accordance with ASTM D5865-19 (ASTM International, 2019) standard protocol. Each sample weighted between 0.5 and 1 gram was put in a crucible, submerged in distilled water within the bomb calorimeter. The calorific value was recorded in megajoules per kilogram (MJ kg-1) of the biomass sample.

**2.4.5 Higher heating value**

An empirical correlation based on proximate analysis was used to determine the higher heating value of biomass (Yin, 2011). The linear regression method was utilised to establish this correlation.

HHV = 0.1905VM + 0.2521FC

Where,

VM = Volatile matter

FC = Fixed carbon

**2.4.6 Fuel value index (FVI)**

The methodology used by Deka *et al.,* (2007) was used to calculate the fuelwood value index. The moisture content is viewed as a negative element in this strategy, while the calorific value and density are considered favourable considerations. The calculation was performed using a standard formula.

|  |  |
| --- | --- |
| FVI = | Calorific value (KJ g-1) × Density (g cc-1) |
| Ash content (g g-1) |

**2.5 Principal Component Analysis (PCA)**

Principal Components Analysis (PCA) was conducted using xlstat version 2024 software to determine the number of clusters and identify variation patterns. The PCA calculation was performed using the given equation.

PCA =

**2.6 Experimental design and Statistical Analysis**

The experimental data were analyzed using a Completely Randomized Design (CRD). Statistical analysis was performed with SPSS version 23. To determine the significance of differences among treatment means, analysis of variance (ANOVA) was carried out at a 5% probability level (*P* < 0.05), followed by Duncan’s Multiple Range Test (DMRT) to categorize and compare the means.

3. results and discussion

**3.1 Physical properties**

**3.1.1 Moisture content (%)**

The moisture content of the investigated mulberry clones exhibited considerable variation, ranging from 29.97% to 41.10% (Table 1.). Clone ME-0174 recorded thehighest moisture content (41.10 ± 0.68%), whereasMI-0845showed thelowest(29.97 ± 2.55%). Themean moisture contentacross all clones was calculated to be36.52%**.** Analysis of variance (ANOVA) revealed statistically significant differences among the clones (*P* < 0.05), suggesting inherent genetic variability in moisture retention capacity among the mulberry genetic resources.

Moisture content is a critical determinant of the combustion characteristics and fuel efficiency of biomass. High moisture levels are generally associated with reduced calorific value, diminished combustion efficiency, and increased operational costs due to higher energy consumption for drying and increased transportation weight (Askowuah *et al.,* 2012). The acceptable moisture content range for woody biomass fuels, as suggested by Dai *et al.,* (2015), lies between 10% and 60%**,** within which the mulberry clones in this study fall comfortably. Typically, freshly felled trees exhibit moisture content ranging from 40% to 60%, influenced by species type and environmental conditions (Mitchual *et al.,* 2014). Tropical and subtropical species often retain more moisture than temperate ones (Bhatt *et al.,* 2010), which aligns with the values observed in this study.

The relatively moderate moisture content (29.97- 41.10%) observed in the mulberry clones indicates their suitability as potential fuelwood sources. These findings are in line with the report of Baqir *et al.,* (2019), who recorded moisture contents ranging from 38.70% to 58.67% in various subtropical tree species. Lower moisture levels, such as those recorded in clone MI-0845, are advantageous for fuel applications, as they enhance energy density, improve combustion efficiency, and reduce the generation of exhaust gases (Kataki & Konwer, 2002; Tsai *et al.,* 2018). Moreover, the comparatively lower moisture content in mulberry clones compared to species like *Ficus natalensis* (69.41%) or *A. grandibracteata* (36.18%) evaluated by Ojelel *et al.,* (2015) in Uganda, reinforces the potential of mulberry as a viable and efficientfuelwood species.

**3.1.2 Bulk and Basic density**

The bulk density of the evaluated mulberry clones exhibited considerable variation, ranging from 166.96 ± 0.70 kg m⁻³ in clone MI-0828 to 225.43 ± 6.01 kg m⁻³ in clone ME-0006, as presented in Table 1. The mean bulk density across all clones was 207.31 kg m⁻³. Statistical analysis revealed significant differences (*P* < 0.05) in bulk density among the clones, reflecting variation in their compactness and structural attributes.

Similarly, basic density varied significantly among the clones, with values ranging from 619.09 ± 8.94 kg m⁻³ in ME-0174 to 701.80 ± 40.06 kg m⁻³ in ME-0168, with an average of 654.34 kg m⁻³. The observed clonal variation in basic density was statistically significant (P < 0.05), indicating distinct wood density profiles among the genetic resources.

Wood density is a pivotal parameter that influences the fuel quality and combustion efficiency of biomass materials. Denser wood contains higher energy content per unit volume, thereby contributing to prolonged burning time and enhanced heat yield (Desta and Ambaye, 2020). In this study, the range of bulk density observed (166.96 to 225.43 kg m⁻³) falls within the acceptable limits for woody biomass fuels, which typically vary between 100 and 750 kg m⁻³, as reported by Dai *et al.,* (2015). These results are in agreement with Bora *et al.,* (2023), who documented bulk densities for mulberry wood between 165.69 kg m⁻³ and 210.98 kg m⁻³, further validating the current findings.

The basic density values recorded in the present study (619.09–701.80 kg m⁻³) are also consistent with the findings of Parthiban *et al.,* (2020), who reported similar density values for multiple fuelwood species such as *Eucalyptus* (540 kg m⁻³), *Casuarina* (495 kg m⁻³), *Chukrasia tabularis* (467.11 kg m⁻³), *Dalbergia sissoo* (610 kg m⁻³), *Acacia auriculiformis* (580 kg m⁻³), and *Leucaena leucocephala* (546 kg m⁻³). Furthermore, Bora *et al.,* (2023) also observed comparable basic densities in mulberry clones, supporting the findings of this study.

The significant inter-clonal differences in both bulk and basic densities among the seventeen mulberry genetic resources highlight their diverse structural characteristics and potential utility in energy-related applications.

**3.1.3 Specific gravity**

The specific gravity of the mulberry clones displayed notable variation, ranging from 0.55 ± 0.02 in ME-0174 to 0.74 ± 0.03 in MI-0845, as shown in Table 1. The mean specific gravity across the seventeen clones was recorded as 0.63. Statistical analysis (*P* < 0.05) confirmed significant differences among the clones, indicating substantial variation in their specific gravity values.

Specific gravity serves as an important indicator of carbon distribution and biomass density in woody materials (Woodcock and Shier, 2002). In this study, the observed range (0.55–0.74) aligns well with the optimal range for energy wood species proposed by Chow and Lucas (1998), who recommended a typical range of 0.45 to 0.70. Although the upper limit in clone MI-0845 slightly exceeded this range, it still reflects the high biomass potential of the clone. Furthermore, the current findings are consistent with Se Golpayegani *et al.,* (2012), who reported specific gravity values between 0.45 and 0.61 for *Morus alba*. Similar ranges of specific gravity have been reported in other energy wood species, with *Cassia siamea* exhibiting a value of 0.43, *Leucaena leucocephala* at 0.69, and *Gliricidia sepium* ranging from 0.45 to 0.75, as documented by Mainoo and Ulzen-Appiah (1996). These results reinforce the potential of mulberry clones as viable biomass resources. The considerable clonal variation in specific gravity observed in this study offers valuable insight for selection and breeding programs aimed at enhancing biomass density and energy efficiency.

**Table 1. Physical properties of mulberry genetic resources**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Clones** | **Moisture content (%)** | **Bulk density (Kg m-3)** | **Basic density (Kg m-3)** | **Specific gravity** |
| MI- 0674 (*M. alba*) | 31.41±0.68de | 168.70±0.55h | 683.34±7.44ab | 0.56±0.02de |
| MI- 0665 (*M. latifolia*) | 37.81±1.43abc | 220.67±3.47abc | 647.85±14.27bcd | 0.61±0.05bcde |
| ME- 0174 (*M. alba*) | 41.10±0.68a | 214.90±3.04abcde | 619.09±8.94d | 0.55±0.02e |
| MI- 0145 (*M. alba*) | 39.17±0.91abc | 205.97±2.08defg | 675.41±9.9abc | 0.57±0.04de |
| MI- 0211 (*M. alba*) | 35.67±0.53bcd | 222.69±5.17ab | 647.09±2.77bcd | 0.63±0.01bcde |
| ME- 0006 (*M. latifolia*) | 35.39±0.06bcd | 225.43±6.01a | 669.99±6.23abc | 0.67±0.00abc |
| MI- 0532 (*M. laevigata*) | 35.22±0.70bcd | 208.63±5.44cdefg | 625.12±13.27d | 0.68±0.00abc |
| MI- 0828 (*M. alba*) | 38.31±0.78abc | 166.96±0.70h | 621.95±1.54d | 0.69±0.02ab |
| MI- 0783 (*M. latifolia*) | 37.42±0.35abc | 211.20±2.82bcdefg | 669.32±10abc | 0.61±0.00cde |
| ME- 0168 (*M. latifolia*) | 35.56±1.87bcd | 224.63±0.49ab | 701.80±40.06a | 0.66±0.05abc |
| MI- 0300 (*M. alba*) | 39.67±0.61ab | 205.40±6.12efg | 640.98±9.98cd | 0.57±0.02de |
| ME- 0169 (*M. alba*) | 35.60±0.89bcd | 199.17±6.80fg | 656.56±11.3bcd | 0.64±0.01bcd |
| MI- 0549 (*M. latifolia*) | 37.45±1.23abc | 203.53±7.97efg | 668.17±14.85abc | 0.63±0.01bcde |
| MI- 0632 (*M. latifolia*) | 37.60±5.27abc | 211.83±5.20abcdef | 643.3±14.24cd | 0.62±0.06bcde |
| MI- 0034 (*M. alba*) | 34.63±1.98cde | 197.35±2.86g | 622.48±7.34d | 0.64±0.02bcd |
| MI- 0818 (*M. latifolia*) | 38.92±1.34abc | 217.43±5.04abcde | 657.57±10.96bcd | 0.61±0.03bcde |
| MI- 0845 (*M. latifolia*) | 29.97±2.55e | 219.73±8.36abcd | 673.69±3.05abc | 0.74±0.03a |
| Mean | 36.52 | 207.31 | 654.34 | 0.63 |
| *P* value | *P* < 0.05 | *P* < 0.05 | *P* < 0.05 | *P* < 0.05 |

\*Data expressed as Mean ± S.E. values within the same column with different superscript are significant at *P* < 0.05 levels of probability.

**3.2 Chemical properties**

**3.2.1 A-B (Alcohol- benzene) extractive (%)**

The Alcohol-Benzene (A-B) extractive content exhibited substantial variation among the seventeen mulberry clones evaluated. As presented in Table 2. The A-B extractives ranged from 2.05 ± 0.36% in MI-0300 to 3.79 ± 0.42% in MI-0845, with an average value of 3.06%. Statistical analysis revealed significant differences (*P* < 0.05) among the clones, indicating distinct chemical extractive profiles across the genetic resources studied.

Extractives in woody biomass play essential roles in tree physiology and metabolism, functioning as defence compounds, energy reserves, and contributors to overall biomass quality (Telmo & Lousada, 2011). Importantly, the quantity and composition of these extractives are known to significantly influence the heating value and combustion performance of biomass fuels (Dadile *et al.,* 2020). In the present study, the A-B extractive content ranged from 2.05% to 3.79%, which aligns well with the findings of Bora *et al.,* (2023), who reported A-B extractive values in mulberry species ranging from 2.21% to 3.77%. Similarly, Walia (2013) documented an extractive content of 2.60% in *Morus rubra* (red mulberry), further supporting the validity of the results obtained in this investigation.

The observed variation in extractive content among the mulberry clones highlights their chemical diversity and potential differences in thermal behavior, which can directly impact their suitability as bioenergy feedstocks.

**3.2.2 Acid insoluble lignin (%)**

Substantial variation in acid-insoluble lignin content was observed among the seventeen mulberry clones evaluated, as presented in Table 2. Clone MI-0845 recorded the maximum lignin content at 28.41 ± 0.14%, whereas clones ME-0169 and MI-0034 exhibited the lowest values, with 23.71 ± 0.16% and 23.48 ± 0.19%, respectively. The mean lignin content across all clones was found to be 25.61%. Statistical analysis confirmed that these differences were significant (*P* < 0.05), highlighting the diverse lignin accumulation potential among the mulberry clonal genotypes.

Lignin plays a crucial role in determining the combustion efficiency of woody biomass. Its content is strongly correlated with the heating value, as higher lignin concentrations enhance the thermal stability and energy yield of biofuels (Demirbas, 2007; Dadile *et al.,* 2020). In this study, the acid-insoluble lignin content ranged from 23.48% to 28.41%, consistent with the findings of Rahman and Jahan (2014), who reported 23% lignin content in *Morus* species, and Walia (2013), who found 21.42% lignin in *Morus nigra*. Furthermore, Parthiban *et al.,* (2020) reported lignin values between 22.00% and 28.31% in various energy species, aligning closely with the current findings.

**3.2.3 Holocellulose (%)**

Among the seventeen mulberry clones evaluated, holocellulose content exhibited marked diversity, as detailed in Table 2. Values spanned a range from 68.68% ± 0.25 in MI-0532, the clone with the lowest recorded content, to 73.28% ± 0.19 in MI-0034, which registered the highest. The overall mean for holocellulose across the genotypes was 70.71%. The differences were found to be statistically significant (*P* < 0.05), underscoring the presence of substantial genetic variability in holocellulose deposition among the studied clones.

Holocellulose plays a pivotal role in determining the energy properties of biomass, particularly within the realm of bioenergy production. The higher the holocellulose content in biomass, the greater the potential energy yield during thermochemical processes such as pyrolysis and gasification. These processes convert holocellulose into bio-oil, syngas, and other energy-dense products, largely due to the relatively simpler structure of the polysaccharides in holocellulose, which enhances their susceptibility to thermal degradation (Marques *et al.,* 2020). In the current study, the holocellulose content of the analysed mulberry clones ranged from 68.68% to 73.28%, with clone MI- 0034 exhibiting the highest content and clone MI- 0532 the lowest. These findings are consistent with the work of Parthiban *et al.,* (2020), who reported holocellulose content ranging from 63.50% to 78% across various energy species. Additionally, the results align with those of Bora *et al.,* (2023), who observed a holocellulose content range of 68.59% to 73.28% in different mulberry species. This consistency across studies underscores the reliability of the present data and further supports the suitability of these mulberry clones for bioenergy production, given their high holocellulose content and the associated energy yield potential.

**Table 2. Chemical properties of mulberry genetic resources**

|  |  |  |  |
| --- | --- | --- | --- |
| **Clones** | **AB extractive (%)** | **Acid insoluble lignin (%)** | **Holocellulose (%)** |
| MI- 0674 (*M. alba*) | 3.37±0.06abc | 26.71±0.47c | 70.01±0.46defg |
| MI- 0665 (*M. latifolia*) | 2.80±0.14cdef | 25.23±0.11fgh | 70.40±0.13cde |
| ME- 0174 (*M. alba*) | 2.69±0.15ef | 26.12±0.06d | 72.98±0.30a |
| MI- 0145 (*M. alba*) | 3.36±0.20abc | 27.31±0.13b | 72.17±0.20ab |
| MI- 0211 (*M. alba*) | 3.53±0.16ab | 24.74±0.12h | 68.99±0.20fgh |
| ME- 0006 (*M. latifolia*) | 3.07±0.07bcde | 24.81±0.06gh | 71.39±0.04bc |
| MI- 0532 (*M. laevigata*) | 2.34±0.03fg | 25.49±1.83ef | 68.68±0.25h |
| MI- 0828 (*M. alba*) | 2.71±0.09def | 25.31±0.24fg | 71.49±0.11bc |
| MI- 0783 (*M. latifolia*) | 3.41±0.08ab | 25.14±0.32fgh | 69.61±0.96efgh |
| ME- 0168 (*M. latifolia*) | 3.49±0.05ab | 26.12±0.21d | 68.82±0.31gh |
| MI- 0300 (*M. alba*) | 2.05±0.36g | 25.51±0.11ef | 70.26±0.37cdef |
| ME- 0169 (*M. alba*) | 3.27±0.19abcd | 23.71±0.16i | 71.44±0.23bc |
| MI- 0549 (*M. latifolia*) | 3.45±0.08ab | 25.55±0.19ef | 70.64±0.57cde |
| MI- 0632 (*M. latifolia*) | 2.79±0.39def | 25.84±0.11de | 71.30±0.62bcd |
| MI- 0034 (*M. alba*) | 3.23±0.04abcde | 23.48±0.19i | 73.28±0.19a |
| MI- 0818 (*M. latifolia*) | 2.67±0.18ef | 25.91±0.09de | 69.84±0.96efgh |
| MI- 0845 (*M. latifolia*) | 3.79±0.42a | 28.41±0.14a | 70.84±0.32h |
| Mean | 3.06 | 25.61 | 70.71 |
| P value | *P* < 0.05 | *P* < 0.05 | *P* < 0.05 |

\*Data expressed as Mean ± S.E. values within the same column with different superscript are significant at *P* < 0.05 levels of probability.

**3.3 Thermochemical properties**

**3.3.1 Ash content (%)**

Considerable variation in ash content was observed among the mulberry clones, indicating differences in the inorganic matter remaining post-combustion. The highest ash content was recorded in clone MI-0632 (2.46% ± 0.02%) (Fig.2), reflecting a greater accumulation of non-combustible mineral residues. In contrast, clone MI-0674 exhibited the lowest ash content (0.69% ± 0.08%) (Fig.2), suggesting superior combustion characteristics with minimal residual ash. The average ash content across all clones was 1.25%, and the differences were found to be statistically significant (*P* < 0.05), confirming substantial inter-clonal variability in mineral composition.

**Fig. 2. Ash content of the wood of mulberry clonal genetic resources**

Ash content is a critical factor in determining the suitability of fuelwood for combustion, as higher ash content can negatively impact the combustion process by leaving behind non-combustible residues, thereby reducing the overall energy efficiency of the fuel (Kumar *et al.,* 2011). In the present study, the ash content varied among the mulberry clones, with clone MI- 0632 exhibiting the highest ash content at 2.46%, while clone MI- 0674 had the lowest at 0.69%. These findings are consistent with the recommendations of Dai *et al.* (2015), who suggested that the ash content of woody biomass fuels should ideally be below 2.5% to ensure optimal combustion performance. The results also align with previous studies by Goswami and Das (2020), who reported an ash content of 2.21% in mulberry, and Baqir *et al.,* (2019), who observed ash content ranging from 0.82% to 2.81% across 12 wood species. Similar observations were made by Nabi *et al.,* (2017) in key tree species of the Kashmir Valley. The consistency of these findings with the literature further validates the current results, emphasizing the importance of selecting clones with lower ash content for improved fuelwood quality and combustion efficiency.

**3.3.2 Volatile matter (%)**

The percentage of volatile matter, representing the fraction of biomass that vaporizes upon thermal exposure, exhibited noticeable diversity among the studied clones, as illustrated in Fig. 3. The highest level was identified in clone MI-0828, which released 87.48% ± 0.08% (Fig.3) of its mass as volatile compounds, highlighting its elevated proportion of thermally responsive constituents. In contrast, clone MI-0632 retained more of its mass under heat, with a comparatively lower volatile matter value of 81.48% ± 0.18% (Fig.3), suggesting enhanced thermal resistance. The overall mean volatile content was 82.99%, and the observed differences were statistically significant (*P* < 0.05), reinforcing the presence of substantial compositional variation across genotypes.

**Fig. 3. Volatile matter composition of the wood of mulberry clonal genetic resources**

The volatile matter content in biomass is a crucial determinant of its combustion characteristics, as higher volatile content tends to lower the ignition temperature and enhance combustion reactivity, thereby improving the efficiency of the fuel (Marques *et al.,* 2020). In this study, the volatile matter content among the mulberry clones ranged from 81.48% in MI-0632 to 87.48% in MI-0828, which falls within the optimal range for woody biomass fuel, which Dai *et al.,* (2015) identified as between 70% and 90%. These findings are consistent with the volatile matter content reported by Goswami and Das (2020) in red mulberry (*Morus rubra*), which was 85.13%. Additionally, Baqir *et al.,* (2019) observed volatile matter content ranging from 76.89% in *Eucalyptus spp*. to 85.64% in *Pithecellobium dulce* across 12 wood species, further corroborating the results of the current study. Desta and Ambaye (2020) also reported a volatile content range of 70.71% to 79.67% in various fuelwood species, supporting the general trend observed here. These consistent findings across different studies emphasize the high combustion efficiency potential of the mulberry clones examined, making them suitable candidates for bioenergy production.

**3.3.3 Fixed carbon (%)**

Fixed carbon represents the solid carbonaceous residue remaining after volatile matter is released and is crucial for sustained combustion. The amount of fixed carbon varied significantly among the clones, ranging from a low value of 11.35% in clone MI- 0034 to a high value of 17.33% in clone MI- 0845 as shown in Fig. 4. The average fixed carbon content was 15.75%, with significant P value (*P* < 0.05) indicating substantial differences among the clones.

**Fig. 4. Fixed carbon content of the wood of mulberry clonal genetic resources**

Fixed carbon in fuel represents the proportion of carbon available for combustion into char, essential for generating carbon-intensive gases like carbon monoxide (Assima *et al.,* 2018). Higher fixed carbon content is directly associated with increased energy values of biomass (Kumar *et al.,* 1992; Kumar *et al.,* 2010 b). In this study, fixed carbon content ranged from 11.35% (MI- 0034) to 17.33% (MI- 0845) among the examined samples. Baqir *et al.,* (2019) reported that *P. Juliflora* had the highest fixed carbon content at 22.04%, while *P. dulce* had the lowest at 12.19%. Similarly, Marques *et al.* (2020) found a high fixed carbon content of 16.21% in their assessment of five different fuelwood species. Goswami and Das, (2020) reported a fixed carbon content of 12.65% in red mulberry (*Morus rubra*), which falls within the range observed in the current study. These results suggest that the mulberry clones under investigation possess a fixed carbon content conducive to efficient energy production, further supporting their potential as a viable fuelwood source.

**3.3.4 Calorific value and Higher heating value**

Substantial inter-clonal differences were observed in both calorific valueandhigher heating value(HHV)**,** reflecting variation in the energy potential of the mulberry biomass, as illustrated in Fig. 5. The calorific value, which quantifies the energy yield from complete combustion of the biomass, ranged from 16.76 MJ kg⁻¹±0.30 in clone MI-0145 to 19.34 MJ kg⁻¹ ± 0.08 in clone MI-0845, with an average of 18.10 MJ kg⁻¹. These values indicate significant differences in energy content among the clones (*P* < 0.05).

Similarly, the Higher heating value (HHV), which includes the latent heat of vaporization of water and thus represents the total recoverable energy during combustion, also varied significantly (*P* < 0.05). The highest HHV was recorded in clone MI-0828 **(**20.64 MJ kg⁻¹ ± 0.02) (Fig. 5.), while clone MI-0034 exhibited the lowest (18.73 MJ kg⁻¹ ± 0.02) (Fig. 6.), with a mean HHV of 19.78 MJ kg⁻¹ across all clones.

**Fig. 5. Calorific value and higher heating value of the wood of mulberry clonal genetic resources**

The calorific value of wood is a critical factor in determining its energy content. In this study, the highest calorific value was recorded in clone MI- 0845 (19.34 MJ kg⁻¹), while the lowest was in clone MI- 0145 (16.76 MJ kg⁻¹). These findings are consistent with Lu *et al.,* (2009), who reported a calorific value of 17.053 MJ kg⁻¹ for mulberry branches. Additionally, Adeleke *et al.,* (2021) found a calorific value of 18.72 MJ kg⁻¹ in *melina* wood, further supporting the results of the present study.

The higher heating value (HHV) measures the total heat produced when a fuel burns, including the water already present in the fuel and the water vapor generated during combustion. In the present study, the HHV ranged from 18.73 MJ kg-1 (MI- 0034) to 20.64 MJ kg-1 (MI- 0828). Comparable results were reported by Goswami and Das, (2020) for *Morus rubra*, with an HHV of 18.36 MJ kg-1. Additionally, Selenwa and Sims, (1999) investigated the fuel characteristics of biomass from 12 tree species grown under short rotation forestry and found HHV values ranging from 19.6 to 20.5 MJ kg-1, aligning with the trends observed in the current study. These findings underscore the considerable genetic variability in the thermal energy characteristics of mulberry wood, supporting the selection of superior clones for bioenergy applications.

**3.3.5 Fuel value index**

Fuel value index (FVI), a combined measure of various fuel characteristics, was used to assess overall fuel quality. Clone MI- 0674 exhibited the best fuel properties with the highest FVI value (1933.65 ± 246.67), while MI- 0632 had the lowest FVI (452.88 ± 13.46), indicating inferior fuel quality as shown in Fig. 6. The mean FVI was 1062.19, with significant differences among the clones (*P* < 0.05).

**Fig. 6. Fuel value index (FVI) of the wood of mulberry clonal genetic resources**

The fuel value index (FVI) serves as a crucial parameter for assessing species combustibility and their ability to produce intense heat during combustion, commonly used to rank preferred fuel wood species (Deka *et al.,* 2007; Cardoso *et al.,* 2015). In the current study, the FVI varied from 452.88 (MI- 0632) to 1933.65 (MI- 0674). Bhatt *et al.,* (2010) observed a similar trend in firewood trees, reporting an FVI range of 306.9 to 1178.6, which falls within the range of this study. Results of the current study also align with the results obtained by Puri *et al.* (1994), where it has been recorded a fuel value index of 533.4 to 2815 among ten fuelwood species. Similarly, Kataki and Konwer, (2002) investigated indigenous tree species in northeast India and reported FVI values ranging from 369 to 2089, which strongly supports the current findings. These results underscore the suitability of the studied species for efficient combustion and highlight their potential as reliable sources of energy.

**3.4 Principal Component Analysis (PCA)**

Principal Component Analysis (PCA) is a technique for data reduction that simplifies a large set of unstructured variables into a smaller subset that captures the majority of the variance present in the original correlation matrix. In the present study, PCA was employed to assess mulberry clonal genetic resources based on their physical, chemical, and thermochemical characteristics. Among the thirteen principal components (PCs) analyzed, only five had eigenvalues greater than 1.0, collectively explaining about 85.092% of the total variability in the traits studied, which is illustrated in Table 3.

**Table 3. Eigen values, % variance and cumulative variability of mulberry clones**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Traits** | **Principal components** | **Eigen values** | **% of variance** | **Cumulative %** |
| Moisture content | PC1 | 4.109 | 31.606 | 31.606 |
| Bulk density | PC2 | 2.286 | 17.582 | 49.188 |
| Basic density | PC3 | 1.961 | 15.083 | 64.271 |
| Specific gravity | PC4 | 1.533 | 11.789 | 76.060 |
| AB extractive | PC5 | 1.174 | 9.032 | 85.092 |
| Acid insoluble lignin | PC6 | 0.720 | 5.538 | 90.631 |
| Holocellulose | PC7 | 0.619 | 4.761 | 95.391 |
| Ash content | PC8 | 0.301 | 2.313 | 97.704 |
| Volatile matter | PC9 | 0.169 | 1.298 | 99.002 |
| Fixed carbon | PC10 | 0.085 | 0.653 | 99.655 |
| Calorific value | PC11 | 0.035 | 0.266 | 99.921 |
| Higher heating value | PC12 | 0.010 | 0.078 | 99.999 |
| Fuel value index | PC13 | 0.000 | 0.001 | 100.000 |

PC1 accounted for 31.606% of the variance and was highly positively correlated with the fuel value index (0.861), calorific value (0.760), basic density (0.582), higher heating value (0.565), and acid-insoluble lignin (0.514). It also displayed strong negative correlations with ash content (-0.746), moisture content (-0.634), and holocellulose (-0.613). PC2, which explained 17.582% of the variation, was more positively associated with volatile matter (0.914) and showed a strong negative correlation with bulk density (-0.857). Similarly, PC3, PC4 and PC5 recorded a variation of 15.083%, 11.789% and 9.082% respectively and showed strong positive correlation with AB extractive (0.742), calorific value (0.494) and specific gravity (0.787), respectively. While PC3 showed a strong negative association with moisture content (-0.650) and fixed carbon (-0.639). Similarly, PC4 showed strong negative correlation with AB extractive (-0.469), higher heating value (-0.460), fixed carbon (-0.457) and acid insoluble lignin (-0.418), while PC5 showed strong negative correlation with acid insoluble lignin (-0.321) and fuel value index (-0.319) as shown in Fig. 7.

**Fig. 7. Eigenvectors of mulberry wood physical, chemical and thermochemical characters**

Positive values greater than 1.0 were considered for the five principal components (PC1, PC2, PC3, PC4, and PC5). The positive values for PC1 ranged from 4.210 (MI- 0845) to 1.105 (MI- 0532). For PC2, the positive values ranged from 4.633 (MI- 0828) to 1.635 (MI- 0034). For PC3, the positive values spanned from 3.344 (MI- 0034) to 1.048 (MI- 0674). For PC4, the positive values were between 2.246 (MI- 0532) and 1.082 (MI- 0211). Lastly, for PC5, the positive values ranged from 1.366 (MI- 0532) to 1.022 (MI- 0828) (Table 4). The clones with the highest scores under PC1 were MI- 0845 (4.210), MI- 0674 (3.084), and ME- 0168 (2.600), indicating that these clones contribute significantly to the primary variance component. MI- 0828 (4.633) had the highest score in PC2, suggesting its substantial influence on this component, followed by MI- 0674 (1.831) and MI- 0034 (1.635). For PC3, MI- 0034 (3.344) and MI- 0845 (1.199) were the most dominant, emphasizing their relevance to this component. In PC4, MI- 0532 (2.246) and MI- 0034 (1.570) were the most influential clones. Lastly, for PC5, MI-0532 (1.366) and MI- 0828 (1.022) made significant contributions.

**Table 4. Mulberry clones selected on the basis of principal components score with positive value more than ˃ 1.0 value**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **PC1** | **PC2** | **PC3** | **PC4** | **PC5** |
| **Mulberry**  **Clones** | MI- 0845 (4.210)  MI- 0674 (3.084)  ME- 0168 (2.600)  MI- 0532 (1.105) | MI- 0828 (4.633)  MI- 0674 (1.831)  MI- 0034 (1.635) | MI- 0034 (3.344)  MI- 0845 (1.199)  MI- 0783 (1.135)  MI- 0674 (1.048) | MI- 0532 (2.246)  MI- 0034 (1.570)  MI- 0300 (1.325)  MI-0211 (1.082) | MI- 0532 (1.366)  MI- 0828 (1.022) |

PCA has been widely applied in studies related to the physical, chemical, and thermochemical properties of various wood species; for instance, Barta- Rajani *et al.* (2016) explored the relationship between torrefaction temperature, chemical composition, and thermal parameters in black locust wood and herbaceous biomass materials; Fodil Cherif *et al.* (2020) compared the physicochemical properties and thermal stability of hardwood and softwood biomass; Mendoza Martinez *et al.* (2021) examined the thermal properties and combustibility of coffee- pine wood briquettes; and Batista and Gomes (2021) analyzed the effect of chemical composition and the pyrolysis process on biochar yields using PCA. In the present study, PCA was used to assess mulberry clonal genetic resources based on their physical, chemical, and thermochemical characteristics. Among the thirteen PCs analyzed, only five were selected with eigenvalues greater than 1.0, as the general guideline for deciding which principal components (PCs) hold practical significance and should be retained is that their eigenvalues should be greater than 1.0 (Lezonni and Pritts, 1991). The PCA results indicated that clones MI- 0845, MI- 0674, ME- 0168 ranked highly in PC1. While, clones like MI- 0845, MI- 0674, MI- 0034, and MI- 0532 appeared across multiple components, demonstrating their versatility and overall strong performance in contributing to the variability observed in the dataset. This ranking provides valuable insights into the mulberry clones that are most effective or influential for fuelwood and bioenergy production, guiding future selection and breeding strategies to establish mulberry as a sustainable alternative for bioenergy production.

4. Conclusion

The comprehensive physical, chemical and thermochemical profiling of seventeen mulberry clonal genetic resources revealed substantial inter-clonal variation in properties critical to fuelwood and bioenergy applications. Parameters such as moisture content, density, specific gravity, extractives, lignin, holocellulose, ash content, volatile matter, fixed carbon, calorific value, and fuel value index varied significantly, underscoring the genetic diversity among the studied clones. Notably, clones MI-0845, ME-0168, and MI-0674 consistently demonstrated superior traits, including high basic density, low moisture and ash content, elevated calorific values, and favorable FVI scores, making them ideal candidates for bioenergy utilization. Principal Component Analysis further corroborated these findings, with the top-performing clones contributing significantly to the total variance across key energy-related traits.

These findings position mulberry not only as a traditional sericultural resource but also as a promising renewable energy source capable of addressing rural energy demands and mitigating environmental degradation from unsustainable fuelwood extraction. The identified superior clones offer strong potential for large-scale propagation and integration into agroforestry systems, thereby supporting national goals for sustainable energy and climate resilience.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

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