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Comparative Study on Lignin and Cellulose Distribution in Seven Hevea *Species for Potential Incorporation in Rubber Forest Plantation*

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Lignin and cellulose distribution in plant parts of seven Hevea *species which are* H. brasiliensis *(RRIM 3001),* H. benthamiana, H. guianensis, H. pauciflora, H.spruceana, H. nitida *and* H. camargoana *were studied to ascertain the pattern of distribution among plant parts, to project total lignin and holocellulose that could be extracted from unused plant parts of the rubber tree during rubber replanting programmes as well as to suggest new planting materials in rubber forest plantation for the purpose of wood harvesting. The study has successfully analysed different distribution patterns among plant parts of* Hevea *trees, where lignin was found to be of highest concentration in the leaf while holocellulose concentration was highest in clear bole samples. For a 25,000 ha area in rubber forest plantation replanting programme, the estimated amount of lignin combustion energy and ethanol production are 13.34 TJ and 545 million kg respectively, while for the replanting programme in rubber plantation which involves a 20,000 ha area, the estimated amount of lignin combustion energy and ethanol production are 13.94 TJ and 570 million kg, respectively. Since lignin is a co-product of biofuel processes it can be recovered and used for power generation in biofuel production. The study has also recognised* H. spruceana *and* H. benthamiana *as possible planting materials for rubber forest plantation since both species have a comparable high biomass weight and high amount of lignin as well as holocellulose in unused plant parts. Immediate future work will focus on investigating optimal methods and conditions for biomass pretreatment, cellulosic hydrolysis and sugar fermentation to yield ethanol.*

Keywords: *Hevea* species; rubber forest plantation; lignin; cellulose; biofuel

The rubber forest plantation (RFP) concept has generally been accepted and adopted in many states in Malaysia. The purpose of its establishment divides it into two types. RFP for wood and latex production and RFP for wood production only. Trees are tapped at eight years of planting and felled at fifteen years of planting[1]. Clear bole is harvested for sawn timber and large branches are processed for medium density fibre boards. Since *Hevea* species other than *H. brasiliensis* also presented high wood volume, but with

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negligible amount of latex[2], theoretically they serve as potential planting materials in RFP for wood production purposes only.

A highly potential area of exploitation in RFP is production of biofuel from unused plant parts. These include leaves, petioles, twigs, small branches and roots. The idea of complete utilisation of rubber plant parts originated from the solution to overcome root disease problems in replanting fields. These unused plant parts need to be cleared from the field to cease root disease pathogens from spreading aggressively due to abundance of nutrients[3]. However, the usual practice of placing the plant materials at a location further from the planting area while anticipating decay, leaves room for these pathogens to survive as the centre of infection consisting infected stumps remain active and constitute the primary inoculum[4]. Therefore, an ultimate solution to this problem would be to remove these plant materials beyond the field parameters and convert them into useful products such as ethanol.

Ethanol as biofuel is currently the most significant alternative fuel in terms of its volume and market value[5]. At present it is largely being produced from sugar and starch based materials such as sugarcane and corn. Over 11 gigalitres (three billion gallons) of ethanol are produced in Brazil annually from cane sugar. However, the high price of sugar is far too expensive to be a viable feedstock for ethanol production[6]. Therefore, about 3.8 GL (one billion gallons) of ethanol are produced from corn and other starch crops in the United States on a yearly basis. The cost of production however, still exceeds the value of the fuel. In view of this, second generation production of ethanol from lignocellulosic materials is now being tested in pilot plants[7]. Production of ethanol from renewable sources of lignocellulosic biomass can improve energy security, decrease urban air pollution and reduce accumulation of carbon dioxide in the atmosphere[8]. Ethanol is an excellent transportation fuel where it can be blended at about ten percent levels with gasoline as it is now in the U.S. or as 22 percent blends, as in Brazil[9].

Lignocellulosic materials consist of holocellulose, hemicellulose, alphacellulose and lignin[10]. Cellulose and hemicellulose can be broken down into sugars, which can then be fermented into ethanol. Lignin on the other hand has been a co-product in ethanol production from lignocellulosic material and has been used to produce heat that is required for the production process[11]. This material is frequently utilised as an energy source for power generation because there are few efficient chemical conversion processes available that can convert lignin into liquid fuels or higher value chemical substrates. However, higher technological advancements should be able to convert lignin into biofuel by using various pathways[12].

Holocellulose is the total polysaccharide fraction found in wood as well as any plant parts, composed of alphacellulose and all of the hemicelluloses. Holocellulose and lignin form the visible lignocellulosic structure of wood[13]. Lignin is an amorphous and insoluble organic polymer and molecular weights range from the low thousands to as high as 50,000. Lignin comprises 18 – 30% by weight of dry wood, most of it concentrated in the compound middle lamella and the layered cell wall. It imparts a woody, rigid structure to the cell walls and distinguishes wood from other fibrous plant materials of lesser lignin content.

Cellulose is the main component of the wood cell wall and its distribution represents 40 – 50% of dry wood. Alphacellulose is also known as chemical cellulose. It consists of a polymer of glucose residues linked by 1,4-β-glucosidic bonds. The degree of polymerisation (DP) is

variable and may range from 700 to 10,000 DP or more. A hemicellulose is any of several heteropolymers, matrix polysaccharides, such as arabinoxylans, present along with cellulose in almost all plant cell walls. Hemicelluloses include xylan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan. Hemicellulose has a random, amorphous structure with little strength and consists of a shorter chain. Hemicellulose content varies from 20 to 35%[14].

For this study, analysis of lignin and cellulose in all plant parts of the rubber tree is conducted to ascertain their concentration in all plant parts of seven *Hevea* species namely *H. brasiliensis* (RRIM 3001), *H. benthamiana, H. guianensis, H. pauciflora, H.spruceana, H. nitida* and *H. camargoana*. The plant parts include leaf, petiole, twig, small branch, large branch, various points of clear bole (bottom disc, disc at 150 cm from ground, disc at first branching, side point of discs and centre point of discs), bark and root. The information is used to determine the total amount of lignin and cellulose in all plant parts of *Hevea* species in order to make a projection of the amount of ethanol that can be produced.

MATERIALS AND METHODS

Sample Collection and Determination of Biomass

Samples were collected from seven *Hevea* species; *H. brasiliensis* (clone RRIM 3001), *H. spruceana, H. guianensis, H. pauciflora, H. benthamiana, H. nitida* and *H. camargoana* that were planted in Kota Tinggi, Johor. The age of planting was eight years. The trees selected for sampling were chosen based on the best representation of the whole sample in replication by assessment of clear bole height and girth at various points. Two trees for every species were uprooted and parts like leaves, petioles, small branches, large branches, trunks, barks and roots were separated and weighed. A small weighed portion of the sample was taken from every plant part for the assessment of moisture content, lignin and cellulose.

Sample Preparation and Determination of Moisture Content

Plant samples were cut into small pieces and left in the oven for drying for about a week at a temperature of 60ºC. After complete drying, sample weights were taken and the total content of water in the sample was calculated as a percentage.

Before carrying out the analysis of lignin and cellulose, all extractive components had been extracted out by dissolving the plant samples with alcohol-acetone solution using a soxhlet apparatus. The extractives were then separated from the alcohol-acetone solvent by using a rotary evaporator. The alcohol-acetone solvent was evaporated, leaving behind traces of extractives on the wall of a round bottom flask. The flask was weighed prior to the rotary evaporation process and re-weighed after it was oven-dried overnight. The extractives content was expressed as its concentration in the oven dried plant sample. The remaining sample was left at room temperature for air drying.

Determination of Lignin

One gram of air-dried, extractive free sample was placed in a 50 mL beaker and 10 mL of 72% sulphuric acid was added. The mixture was stirred occasionally at room temperature for 2 h and later transferred to a conical flask. The sample was diluted with distilled water until the final volume was 300 mL and then boiled under reflux for 3 hours. When the refluxing was complete, the

insoluble lignin was recovered by filtration through a pre-weighed crucible. The crucible containing lignin was then dried in the oven at a temperature of 110ºC, cooled in a desiccator and weighed. The lignin content was reported as a concentration of extractive free sample.

Determination of Holocellulose

Cellulose content can be determined by initially obtaining the total content of holocellulose in the plant sample through digestion with sodium chlorite and acetic acid. Holocellulose is a lignin-free fibrous material comprising hemicellulose and α-cellulose. The α-cellulose can be separated from hemicellulose by undergoing another digestion procedure using sodium hydroxide.

Two grams of air dried, extractive free plant sample was placed in a 250 mL conical flask and 100 mL of water was added, followed with 1.5 g sodium chlorite and 5 mL of 10% acetic acid. The flask was placed in a water bath at 70ºC and closed with a small inverted Erlenmeyer flask. The experiment was carried out in a fume hood. After 30 min, 5 mL of 10% acetic acid was added and after a further 30 min, 1.5 g sodium chlorite was added. This step was repeated at an interval of 30 minutes. After the final addition of sodium chlorite the mixture was left for 30 min and the remaining residue should be white and retain a woody structure.

The suspension was then cooled in an ice bath and filtered through a glass crucible. The residue was washed with iced distilled water and then with acetone. The crucible containing holocellulose was covered with a perforated aluminium foil and left at room temperature for air drying. The crucible was then weighed at daily intervals until the sample reached a constant weight. Holocellulose was calculated as concentration in extractive-free sample.

Determination of Alpha-cellulose

Two grams of air-dried holocellulose was placed in a 20 mL beaker inside a water bath at 20ºC. 15 mL of 17.5% NaOH was added and macerated gently for 1 minute. 10 mL of 17.5% NaOH was then added and mixed for 45 s before an additional 10 mL was added and mixed for 15 seconds. After this, another 10 mL of 17.5% NaOH was added and mixed for 2.5 min and this was repeated three times. The beaker was then covered with a watch glass and left in the water bath for 30 minutes.

Subsequently, 100 mL of distilled water at 20ºC was quickly added to the mixture and thoroughly mixed for 30 min in the water bath. The mixture was filtered through pre-weighed fritted glass crucible using aspirator pump. To finally transfer all the fibre, the beaker was rinsed with 25 mL of 8.3% NaOH at a temperature of 20ºC and passed through the crucible again. After all the fibre has settled onto the crucible, the aspirator pump was disconnected and the crucible was filled with 2N acetic acid at a temperature of 20ºC and left to soak for 5 minutes. The aspirator pump was reapplied afterwards to remove the acetic acid. The fibre was washed with distilled water until free of acid as indicated by litmus paper. The crucible was finally dried in the oven and weighed. Alphacellulose was calculated as concentration of holocellulose in the sample.

RESULTS

The summary of mean total fresh weight (TFW), total dry weight (TDW) and moisture content (MC) is shown in *Table 1*. Duncan's grouping is indicated in the table for every variable being measured by comparing the different plant

TABLE 1. TOTAL FRESH WEIGHT, TOTAL DRY WEIGHT AND MOISTURE CONTENT OF EIGHT YEAR OLD *HEVEA* SPECIES

Species	Plant part	Total fresh weight (kg) Mean ± s.e.	Total dry weight (kg) Mean ± s.e.	Moisture content (%) Mean ± s.e.
H. brasiliensis (RRIM 3001)	Leaf	16.74 ± 3.36^{C}	6.99 ± 3.28^{B}	60.59 ± 11.68^{A}
	Petiole	3.56 ± 1.02^{C}	1.26 ± 0.73^{B}	67.82 ± 11.39^{A}
	Twig	37.20 ± 15.66^{ABC}	13.55 ± 7.49^{B}	66.05 ± 5.85^{A}
	Small branch	59.54 ± 27.00^{ABC}	27.55 ± 13.05^{AB}	54.26 ± 1.17^{A}
	Large branch	194.54 ± 115.58^{A}	96.30 ± 57.20^{A}	50.50 ± 0.01^{A}
	Bole	178.54 ± 34.78^{AB}	91.90 ± 14.76^{A}	48.17 ± 1.83^{A}
	Bark	22.41 ± 2.29^{BC}	8.94 ± 3.41^{B}	61.24 ± 11.26^{A}
	Root	115.36 ± 21.38^{ABC}	54.30 ± 8.25^{AB}	2.63 ± 1.63^{A}
H. benthamiana	Leaf	25.95 ± 0.61^{DE}	12.03 ± 0.18^{CD}	53.64 ± 0.39^{AB}
	Petiole	4.44 ± 0.24^{E}	1.85 ± 0.15^{D}	58.30 ± 1.15^{AB}
	Twig	44.47 ± 0.73^{CDE}	13.95 ± 5.19^{CD}	68.43 ± 12.19^{A}
	Small branch	75.11 ± 14.23^{BCD}	32.08 ± 4.95^{BC}	57.00 ± 1.56^{AB}
	Large branch	146.86 ± 35.76^{A}	77.27 ± 19.21^{A}	47.46 ± 0.29^{B}
	Bole	113.10 ± 14.62^{AB}	67.73 ± 6.97^{A}	39.90 + 1.61^{B}
	Bark	12.12 ± 0.04^{E}	5.24 ± 1.33^{CD}	56.80 ± 10.81^{AB}
	Root	85.10 ± 3.20^{BC}	42.14 ± 2.95^{B}	50.54 ± 1.60^{AB}
H. guianensis	Leaf	21.59 ± 4.11^{D}	6.48 ± 1.03^{DE}	69.81 ± 0.99^{A}
	Petiole	4.38 ± 0.70^{D}	1.82 ± 0.53^{E}	55.30 ± 19.27^{A}
	Twig	48.94 ± 0.96^{C}	17.67 ± 4.89^{CD}	63.69 ± 10.69^{A}
	Small branch	49.35 ± 9.33^{C}	25.56 ± 4.60^{BC}	48.11 ± 0.49^{A}
	Large branch	71.87 ± 8.23^{B}	38.30 ± 4.25^{B}	46.68 ± 0.19^{A}
	Bole	96.59 ± 7.61^{A}	55.51 ± 3.74^{A}	42.48 ± 0.66^{A}
	Bark	10.88 ± 0.38^{D}	4.30 ± 0.29^{DE}	60.34 ± 4.03^{A}
	Root	62.50 ± 8.88^{BC}	30.73 ± 7.34^{BC}	51.52 ± 4.85^{A}
H. pauciflora	Leaf	20.97 ± 5.79^{BC}	7.76 ± 0.16^{B}	60.20 ± 10.21^{AB}
	Petiole	4.66 ± 0.74^{C}	1.27 ± 0.27^{B}	71.01 ± 10.44^{A}
	Twig	20.26 ± 5.18^{BC}	6.54 ± 0.24^{B}	65.16 ± 10.07^{AB}
	Small branch	78.42 ± 23.68^{AB}	41.86 ± 12.92^{AB}	46.73 ± 0.39^{B}
	Large branch	117.05 ± 28.59^{A}	61.30 ± 19.30^{A}	48.59 ± 3.93^{AB}
	Bole	134.04 ± 37.22^{A}	74.91 ± 22.43^{A}	44.48 ± 1.32^{B}
	Bark	15.72 ± 3.94^{BC}	4.94 ± 0.62^{B}	67.49 ± 4.20^{AB}
	Root	82.94 ± 13.69^{AB}	36.05 ± 2.60^{AB}	55.84 ± 4.16^{AB}

TABLE 1 (cont.). TOTAL FRESH WEIGHT, TOTAL DRY WEIGHT AND MOISTURE CONTENT OF EIGHT YEAR OLD *HEVEA* SPECIES

Species	Plant part	Total fresh weight (kg) Mean ± s.e.	Total dry weight (kg) Mean ± s.e.	Moisture content (%) Mean ± s.e.
H. spruceana	Leaf	27.97 ± 0.13^{D}	15.27 ± 1.65^{B}	45.38 ± 6.16^{A}
	Petiole	4.43 ± 0.21^{D}	1.80 ± 0.03^{B}	59.28 ± 2.65^{A}
	Twig	37.00 ± 3.10^{CD}	14.07 ± 0.84^{B}	61.50 ± 5.50^{A}
	Small branch	144.00 ± 51.94^{A}	67.30 ± 28.62^{A}	54.51 ± 3.47^{A}
	Large branch	117.21 ± 1.45^{AB}	57.12 ± 1.70^{A}	51.28 ± 0.85^{A}
	Bole	102.54 ± 13.96^{ABC}	56.54 ± 10.54^{A}	45.25 ± 2.82^{A}
	Bark	13.66 ± 2.06^{D}	7.04 ± 0.33^{B}	46.91 ± 10.45^{A}
	Root	70.89 ± 15.03^{BCD}	33.60 ± 11.94^{AB}	54.11 ± 7.11^{A}
H. nitida	Leaf	10.17 ± 6.75^{D}	4.78 ± 3.52^{DE}	57.14 ± 6.17^{AB}
	Petiole	2.33 ± 1.45^{D}	0.86 ± 0.64^{E}	67.93 ± 7.58^{A}
	Twig	34.54 ± 1.02^{CD}	10.56 ± 2.93^{CDE}	69.65 ± 7.58^{A}
	Small branch	85.85 ± 25.13^{A}	41.86 ± 12.85^{AB}	51.46 ± 0.76^{AB}
	Large branch	47.04 ± 6.86^{BC}	25.73 ± 4.10^{BC}	45.41 ± 0.75^{B}
	Bole	77.77 ± 8.95^{AB}	44.12 ± 3.97^{A}	43.11 ± 1.44^{B}
	Bark	9.67 ± 0.95^{D}	3.92 ± 0.99^{DE}	60.07 ± 6.30^{AB}
	Root	49.79 ± 4.79^{BC}	22.11 ± 0.39^{CD}	55.10 ± 5.10^{AB}
H. camargoana	Leaf	8.88 ± 8.00^{A}	2.92 ± 2.50^{AB}	60.15 ± 7.78^{A}
	Petiole	0.99 ± 0.77^{A}	0.21 ± 0.11^{B}	66.72 ± 15.26^{A}
	Twig	33.39 ± 31.45^{A}	10.78 ± 9.85^{AB}	60.22 ± 7.96^{A}
	Small branch	30.26 ± 12.56^{A}	16.10 ± 7.08^{AB}	47.46 ± 1.58^{A}
	Large branch	59.73 ± 35.87^{A}	31.90 ± 18.97^{A}	46.29 ± 0.50^{A}
	Bole	25.51 ± 12.97^{A}	15.20 ± 8.09^{AB}	41.37 ± 1.90^{A}
	Bark	3.45 ± 1.51^{A}	1.46 ± 0.38^{AB}	53.72 ± 9.28^{A}
	Root	31.68 ± 11.32^{A}	14.32 ± 3.95^{AB}	53.30 ± 4.20^{A}

parts for each species. For *H. brasiliensis,* the highest TFW and TDW were observed in the large branch. The highest MC was detected in the petiole. For *H. benthamiana,* the highest TFW and TDW were observed in the large branch. The highest MC was in the twig. For *H. guianensis,* the highest TFW and TDW were in the bole. The highest MC was in the leaf. For *H. pauciflora,* the highest TFW and TDW were observed in the bole. The highest MC was in the petiole. For *H. spruceana,* the highest TFW and TDW were observed in the small branch and the highest MC was in the twig. For *H. nitida,* the highest TFW and TDW were observed in the small branch and bole, respectively. The highest MC was in the twig. For *H. camargoana,* the highest TFW and TDW were observed in large branch. The highest MC was in the petiole. For all the seven species, the lowest TFW and TDW were in the petiole while the lowest MC was observed in the bole.

The sum of TFW per whole tree in the order of the highest to the lowest is *H. brasiliensis*, 627.89 kg, *H. spruceana*, 517.70 kg, *H. benthamiana*, 507.15 kg, *H. pauciflora*, 474.06 kg, *H. guianensis*, 366.10 kg, *H. nitida*, 317.16 kg and finally *H. camargoana*, 193.89 kg.

The summary of concentration of extractives, lignin, holocellulose and alphacellulose in plant parts of the *Hevea* species is shown in *Table 2*. Duncan's grouping is indicated in the table for every component's concentration, by comparing the different plant parts for each species. For *H. brasiliensis*, the highest figures were observed in the leaf (both extractives and lignin), centre point of bottom disc (holocellulose) and side point of bottom disc (alphacellulose). On the other hand, the lowest concentration was detected in the centre point of bottom disc (extractives), centre point of disc at 150 cm from ground (lignin), leaf (holocellulose) and bark (alphacellulose).

For *H. benthamiana*, the highest figures were observed in the leaf (both extractives and lignin), large branch (holocellulose) and side point of bottom disc (alphacellulose). On the other hand, the lowest concentration was detected in the bark (extractives), centre point of disc at 150 cm from ground (lignin), leaf (holocellulose) and root (alphacellulose).

For *H. guianensis*, the highest figures were observed in the leaf (both extractives and lignin), centre point of bottom disc (holocellulose) and centre point of disc at 150 cm from ground (alphacellulose). On the other hand, the lowest concentration was detected in the centre point of disc at 150 cm from ground (extractives), centre point of bottom disc (lignin), leaf (holocellulose) and bark (alphacellulose).

For *H. pauciflora*, the highest figures were observed in the leaf (both extractives and lignin), side point of bottom disc (holocellulose) and centre point of disc at first branching (alphacellulose). On the other hand, the lowest concentration was detected in the centre point of disc at 150 cm from ground (both extractives and lignin), leaf (holocellulose) and bark (alphacellulose).

For *H. spruceana*, the highest figures were observed in the leaf (both extractives and lignin), side point of disc at 150 cm from ground (holocellulose) and side point of bottom disc (alphacellulose). On the other hand, the lowest concentration was detected in the bark (extractives), side point of disc at first branching (lignin), leaf (holocellulose) and bark (alphacellulose).

For *H. nitida*, the highest figures were observed in the leaf (both extractives and lignin), centre point of disc at first branching

TABLE 2. CONCENTRATION OF EXTRACTIVES, LIGNIN, HOLOCELLULOSE AND ALPHACELLULOSE IN PLANT PARTS OF EIGHT YEAR OLD *HEVEA* SPECIES

Species	Plant part	Extractives (g/g) Mean + s.e.	Lignin (g/g) Mean ± s.e.	Holocellulose (g/g) Mean ± s.e.	Alphacellulose (g/g) Mean ± s.e.
H. brasiliensis (RRIM 3001)	Leaf	0.14354 ± 0.00030^{A}	0.43189 ± 0.00329^{A}	0.47987 ± 0.00495^{F}	$0.62964 \pm 0.00194^{BCD}$
	Petiole	0.05290 ± 0.00066^{B}	0.37781 ± 0.00751^{B}	0.51967 ± 0.03561^{E}	0.64324 ± 0.02140^{BC}
	Twig	0.04552 ± 0.00086^{C}	0.34164 ± 0.00887^{C}	0.59701 ± 0.00723^{D}	0.58874 ± 0.02173^{CD}
	Small branch	0.01731 ± 0.00038^{E}	0.23631 ± 0.00318^{EF}	0.48534 ± 0.00312^{EF}	0.76824 ± 0.00654^{A}
	Large branch	0.01832 ± 0.00027^{E}	0.22875 ± 0.00102^{F}	0.71682 ± 0.00646^{B}	0.59027 ± 0.00119^{CD}
	Bottom disc - side	0.01391 ± 0.00086^{GH}	0.19179 ± 0.00325^{G}	0.74142 ± 0.00177^{B}	0.79306 ± 0.00899^{A}
	Bottom disc - centre	0.01193 ± 0.00029^{I}	0.19319 ± 0.00017^{G}	0.81154 ± 0.00754^{A}	0.77501 ± 0.00676^{A}
	150 cm disc - side	0.01470 ± 0.00015^{FG}	0.18801 ± 0.00644^{G}	0.73643 ± 0.00381^{B}	0.66547 ± 0.02233^{B}
	150 cm disc - centre	0.01591 ± 0.00044^{F}	0.18364 ± 0.00022^{G}	0.73339 ± 0.00495^{B}	0.66265 ± 0.00173^{B}
	First branching disc - side	0.01483 ± 0.00031^{FG}	0.22130 ± 0.00958^{F}	0.67335 ± 0.00375^{C}	0.68542 ± 0.00834^{B}
	First branching disc - centre	0.01288 ± 0.00020^{HI}	0.25178 ± 0.01028^{DE}	0.71537 ± 0.00635^{B}	$0.62767 \pm 0.00191^{BCD}$
	Bark	0.01507 ± 0.00025^{FG}	0.39214 ± 0.00930^{B}	0.59417 ± 0.02482^{D}	0.57215 ± 0.04923^{D}
	Root	0.02519 ± 0.00069^{D}	0.25670 ± 0.00151^{D}	0.64383 ± 0.00942^{C}	0.64214 ± 0.03519^{BC}
H. benthamiana	Leaf	0.08676 ± 0.00115^{A}	0.58966 ± 0.00598^{A}	0.44783 ± 0.00351^{E}	0.65604 ± 0.00095^{AB}
	Petiole	0.03639 ± 0.00077^{C}	0.38978 ± 0.00559^{B}	0.57120 ± 0.01072^{D}	0.68162 ± 0.04809^{AB}
	Twig	0.04320 ± 0.00060^{B}	0.35607 ± 0.00220^{B}	0.44823 ± 0.01475^{E}	0.61483 ± 0.01379^{B}
	Small branch	0.02500 ± 0.00049^{D}	0.28822 ± 0.03629^{C}	0.73749 ± 0.00753^{A}	0.76558 ± 0.01763^{AB}
	Large branch	0.02224 ± 0.00050^{E}	0.22389 ± 0.00948^{E}	0.72286 ± 0.00383^{A}	0.77490 ± 0.00967^{AB}
	Bottom disc - side	0.01624 ± 0.00020^{H}	0.23773 ± 0.00628^{DE}	0.68410 ± 0.00506^{B}	0.81700 ± 0.16297^{A}
	Bottom disc - centre	0.01902 ± 0.00048^{F}	0.21966 ± 0.00819^{E}	0.68329 ± 0.00689^{B}	0.64182 ± 0.03096^{AB}
	150 cm disc - side	0.01888 ± 0.00022^{F}	$0.24637 \pm 0.02303^{CDE}$	0.68791 ± 0.00568^{B}	0.64659 ± 0.01797^{AB}
	150 cm disc - centre	0.01689 ± 0.00030^{H}	0.21093 ± 0.00198^{E}	0.64109 ± 0.00319^{C}	0.73611 ± 0.03321^{AB}
	First branching disc - side	0.01709 ± 0.00028^{GH}	0.23213 ± 0.00075^{DE}	0.69491 ± 0.01062^{B}	0.70604 ± 0.03619^{AB}
	First branching disc - centre	0.02223 ± 0.00042^{E}	0.23877 ± 0.00110DE	0.69587 ± 0.00885^{B}	0.63172 ± 0.04261^{B}
	Bark	0.01164 ± 0.00030^{I}	0.36942 ± 0.00404^{B}	0.64103 ± 0.01425^{C}	0.62868 ± 0.00131^{B}
	Root	0.01862 ± 0.00053^{FG}	$0.27425 \pm 0.01053C^{D}$	0.65594 ± 0.00862^{C}	0.61196 ± 0.02217^{B}

TABLE 2 (cont.). CONCENTRATION OF EXTRACTIVES, LIGNIN, HOLOCELLULOSE AND ALPHACELLULOSE IN PLANT PARTS OF EIGHT YEAR OLD *HEVEA* SPECIES

Species	Plant part	Extractives (g/g) Mean + s.e.	Lignin (g/g) Mean ± s.e.	Holocellulose (g/g) Mean ± s.e.	Alphacellulose (g/g) Mean ± s.e.
H. guianensis	Leaf	0.15461 ± 0.00158^{A}	0.61149 ± 0.00210^{A}	0.46394 ± 0.00568^{G}	0.58802 ± 0.00719^{BC}
	Petiole	0.07315 ± 0.00180^{B}	0.44241 ± 0.00605^{B}	0.53080 ± 0.01204^{F}	0.66451 ± 0.00544^{BC}
	Twig	0.04427 ± 0.00101^{C}	0.37876 ± 0.00339^{C}	0.62312 ± 0.01042^{E}	0.60811 ± 0.06118^{BC}
	Small branch	0.02753 ± 0.00124^{D}	$0.23931 \pm 0.01960^{DEF}$	0.79428 ± 0.02110^{AB}	0.56084 ± 0.02310^{C}
	Large branch	0.01919 ± 0.00077^{F}	0.26880 ± 0.01929^{DE}	0.74421 ± 0.01263^{C}	0.57871 ± 0.00786^{BC}
	Bottom disc - side	0.01786 ± 0.00053^{FG}	$0.21821 \pm 0.00469^{DEF}$	0.81830 ± 0.01161^{AB}	0.62371 ± 0.03201^{BC}
	Bottom disc - centre	0.01553 ± 0.00039^{GH}	0.19153 ± 0.04485^{F}	0.83268 ± 0.01308^{A}	0.65596 ± 0.00193^{BC}
	150 cm disc - side	0.01605 ± 0.00047^{GH}	$0.22210 \pm 0.02368^{DEF}$	0.78889 ± 0.01772^{B}	0.65360 ± 0.02632^{BC}
	150 cm disc - centre	0.01330 ± 0.00021^{H}	0.20713 ± 0.01418^{EF}	0.79509 ± 0.00462^{AB}	0.83848 ± 0.05976^{A}
	First branching disc - side	0.01716 ± 0.00063^{FG}	$0.24135 \pm 0.01159^{DEF}$	0.82786 ± 0.01275^{AB}	0.71526 ± 0.08492^{AB}
	First branching disc - centre	0.01712 ± 0.00034^{FG}	$0.24371 \pm 0.02653^{DEF}$	0.81541 ± 0.01246^{AB}	$0.64004 \pm 0.04172B^{C}$
	Bark	0.01676 ± 0.00019^{FG}	0.37337 ± 0.00676^{C}	0.69191 ± 0.00943^{D}	0.53410 ± 0.00077^{C}
	Root	0.02472 ± 0.00049^{E}	0.27693 ± 0.00411^{D}	0.66531 ± 0.01702^{D}	0.60145 ± 0.01452^{BC}
H. pauciflora	Leaf	0.12007 ± 0.00125^{A}	0.53703 ± 0.00016^{A}	0.46600 ± 0.01021^{E}	0.58667 ± 0.00049^{CD}
	Petiole	0.03059 ± 0.00138^{B}	0.32978 ± 0.00566^{B}	0.62323 ± 0.03398^{C}	0.60830 ± 0.01016^{CD}
	Twig	0.02048 ± 0.00035^{C}	0.33244 ± 0.01243^{B}	0.68858 ± 0.01213^{B}	0.59370 ± 0.00920^{CD}
	Small branch	0.01539 ± 0.00036^{D}	0.27895 ± 0.01315^{C}	0.77038 ± 0.00634^{A}	0.57555 ± 0.00943^{CD}
	Large branch	0.01107 ± 0.00052^{E}	0.19674 ± 0.01752^{EF}	0.80075 ± 0.01323^{A}	$0.69706 \pm 0.03509^{BCD}$
	Bottom disc - side	0.01467 ± 0.00035^{D}	0.21429 ± 0.00657^{EF}	0.80209 ± 0.00703^{A}	$0.70252 \pm 0.03115^{BCD}$
	Bottom disc - centre	0.01155 ± 0.00021^{E}	0.21820 ± 0.00841^{EF}	0.71031 ± 0.00240^{B}	$0.66909 \pm 0.01341^{BCD}$
	150 cm disc - side	0.01447 ± 0.00036^{D}	0.19979 ± 0.00479^{EF}	0.79485 ± 0.01659^{A}	$0.70591 \pm 0.09230^{BCD}$
	150 cm disc - centre	0.01025 ± 0.00027^{E}	0.18169 ± 0.00526^{F}	0.76150 ± 0.00892^{A}	$0.74808 \pm 0.09736^{ABC}$
	First branching disc - side	0.01183 ± 0.00032^{E}	0.23035 ± 0.02024^{DE}	0.77219 ± 0.00486^{A}	0.80042 ± 0.12301^{AB}
	First branching disc - centre	0.01608 ± 0.00036^{D}	0.25997 ± 0.01976^{CD}	0.79746 ± 0.00606^{A}	0.88664 ± 0.05612^{A}
	Bark	0.01034 ± 0.00018^{E}	0.33775 ± 0.00511^{B}	0.70464 ± 0.01468^{B}	0.55476 ± 0.00263^{D}
	Root	0.01895 ± 0.00058^{C}	0.25651 ± 0.00413^{CD}	0.57895 ± 0.00536^{D}	$0.67076 \pm 0.01791^{BCD}$

TABLE 2 (cont.). CONCENTRATION OF EXTRACTIVES, LIGNIN, HOLOCELLULOSE AND ALPHACELLULOSE IN PLANT PARTS OF EIGHT YEAR OLD HEVEA SPECIES

Species	Plant part	Extractives (g/g) Mean + s.e.	Lignin (g/g) Mean ± s.e.	Holocellulose (g/g) Mean ± s.e.	Alphacellulose (g/g) Mean ± s.e.
H. spruceana	Leaf	0.11290 ± 0.00145^{A}	0.54130 ± 0.00554^{A}	0.54153 ± 0.00606^{F}	0.57478 ± 0.00273^{CD}
	Petiole	0.03843 ± 0.00055^{B}	0.36524 ± 0.00065^{C}	0.59892 ± 0.00403^{E}	0.58177 ± 0.00086^{CD}
	Twig	0.01813 ± 0.00028^{C}	0.38301 ± 0.00380^{BC}	0.68499 ± 0.00712^{C}	0.52868 ± 0.02770^{EF}
	Small branch	0.01575 ± 0.00023^{DE}	0.29861 ± 0.00952^{D}	0.71104 ± 0.01032^{C}	0.54778 ± 0.01132^{DE}
	Large branch	0.01292 ± 0.00058^{FG}	$0.25673 \pm 0.00367^{FGH}$	0.79280 ± 0.01797^{AB}	0.58845 ± 0.00638^{BC}
	Bottom disc - side	0.01557 ± 0.00035^{DE}	0.25318 ± 0.00717^{GH}	0.79654 ± 0.00977^{AB}	0.62637 ± 0.00540^{A}
	Bottom disc - centre	0.01674 ± 0.00049^{CD}	0.26949 ± 0.00406^{FG}	0.80241 ± 0.01199^{AB}	0.57514 ± 0.00219^{CD}
	150 cm disc - side	0.01296 ± 0.00020^{FG}	0.29323 ± 0.00609^{DE}	0.81971 ± 0.01358^{A}	0.56625 ± 0.00952^{CD}
	150 cm disc - centre	0.01463 ± 0.00045^{EF}	0.27641 ± 0.00129^{EF}	0.71549 ± 0.00754^{C}	0.62057 ± 0.00936^{AB}
	First branching disc - side	0.01277 ± 0.00030^{G}	0.24448 ± 0.00188^{H}	$0.78335 \pm 0.01247_{B}$	0.56740 ± 0.00590^{CD}
	First branching disc - centre	$0.01430 \pm 0.00033^{EFG}$	$0.26707 \pm 0.01802F^{G}$	0.76964 ± 0.01738^{B}	0.56753 ± 0.00734^{CD}
	Bark	0.01072 ± 0.00023^{H}	0.38834 ± 0.00203^{B}	0.64886 ± 0.00971^{D}	0.51342 ± 0.00395^{F}
	Root	0.01520 ± 0.00090^{DE}	0.29727 ± 0.00070^{DE}	0.71229 ± 0.01099^{C}	$0.56228 \pm 0.01435^{CDE}$
H. nitida	Leaf	0.13904 ± 0.00101^{A}	0.61281 ± 0.00065^{A}	0.29021 ± 0.00868^{G}	0.65411 ± 0.00159^{BC}
	Petiole	0.05655 ± 0.00099^{B}	0.43523 ± 0.00136^{B}	0.44613 ± 0.01882^{F}	0.63464 ± 0.01095^{CD}
	Twig	0.04220 ± 0.00071^{C}	0.38222 ± 0.00141^{C}	0.57469 ± 0.03953^{E}	0.56446 ± 0.02554^{EF}
	Small branch	0.01502 ± 0.00011^{D}	0.23286 ± 0.00677^{E}	0.64018 ± 0.02716^{D}	0.63244 ± 0.00634^{CD}
	Large branch	$0.01322 \pm 0.00020^{EFG}$	0.18749 ± 0.01434^{FG}	0.58979 ± 0.01482^{C}	0.72848 ± 0.00721^{DE}
	Bottom disc - side	0.01082 ± 0.00024^{H}	0.17165 ± 0.00033^{G}	0.78421 ± 0.00754^{AB}	0.79179 ± 0.01674^{A}
	Bottom disc - centre	0.00806 ± 0.00011^{I}	0.20768 ± 0.00680^{EF}	$0.77848 \pm 0.01105^{ABC}$	0.63427 ± 0.00332^{CD}
	150 cm disc - side	0.01203 ± 0.00018^{GH}	0.21587 ± 0.00872^{EF}	0.76992 ± 0.01085^{BC}	0.66091 ± 0.01179^{BC}
	150 cm disc - centre	0.00835 ± 0.00015^{I}	0.18649 ± 0.01293^{FG}	0.78886 ± 0.01098^{AB}	0.68767 ± 0.00235^{B}
	First branching disc - side	0.01272 ± 0.00028^{FG}	0.22730 ± 0.01849^{E}	0.74773 ± 0.00807^{BC}	0.64975 ± 0.00956^{BC}
	First branching disc - centre	0.01256 ± 0.00029^{G}	0.16189 ± 0.02010^{G}	0.83106 ± 0.01181^{A}	0.67825 ± 0.01131^{BC}
	Bark	0.01433 ± 0.00014^{DE}	0.36637 ± 0.00048^{C}	0.66479 ± 0.01819^{D}	0.52197 ± 0.00274^{F}
	Root	$0.01411 \pm 0.00024^{DEF}$	0.29144 ± 0.00407^{D}	0.62981 ± 0.01192^{D}	0.65281 ± 0.03459^{BC}

TABLE 2 (cont.). CONCENTRATION OF EXTRACTIVES, LIGNIN, HOLOCELLULOSE AND ALPHACELLULOSE IN PLANT PARTS OF EIGHT YEAR OLD *HEVEA* SPECIES

Species	Plant part	Extractives (g/g) Mean + s.e.	Lignin (g/g) Mean ± s.e.	Holocellulose (g/g) Mean ± s.e.	Alphacellulose (g/g) Mean ± s.e.
H. camargoana	Leaf	0.10426 ± 0.00125^{A}	0.58691 ± 0.00194^{A}	0.42515 ± 0.00470^{E}	$0.62057 \pm 0.03906^{BCD}$
	Petiole	0.04173 ± 0.00074^{B}	0.46559 ± 0.00644^{B}	0.44741 ± 0.02134^{E}	0.72148 ± 0.05200^{A}
	Twig	0.04188 ± 0.00082^{B}	0.40616 ± 0.00428^{C}	0.53280 ± 0.00614^{D}	0.71702 ± 0.00010^{AB}
	Small branch	0.02965 ± 0.00103^{C}	$0.25728 \pm 0.00892^{DEF}$	0.70525 ± 0.01324^{AB}	0.58464 ± 0.00987^{D}
	Large branch	0.02622 ± 0.00079^{D}	0.26631 ± 0.01688^{DE}	0.71616 ± 0.04767^{AB}	0.69419 ± 0.00747^{AB}
	Bottom disc - side	0.02817 ± 0.00099^{CD}	$0.24562 \pm 0.00892^{DEF}$	0.66643 ± 0.00916^{BC}	$0.62062 \pm 0.02326^{BCD}$
	Bottom disc - centre	0.02221 ± 0.00080^{E}	0.21522 ± 0.00409^{G}	0.62683 ± 0.00934^{C}	$0.68416 \pm 0.00684^{ABC}$
	150 cm disc - side	0.02072 ± 0.00103^{EF}	0.23333 ± 0.00404^{FG}	0.71002 ± 0.00873^{AB}	$0.65309 \pm 0.00036^{ABCD}$
	150 cm disc - centre	0.02663 ± 0.00085^{D}	$0.25673 \pm 0.01271^{DEF}$	0.74661 ± 0.01855^{A}	$0.70404 \pm 0.02259^{ABC}$
	First branching disc - side	0.02199 ± 0.00089^{EF}	0.27560 ± 0.01224^{D}	0.74794 ± 0.01278^{A}	$0.64437 \pm 0.00943^{ABCD}$
	First branching disc - centre	0.02240 ± 0.00073^{E}	$0.24104 \pm 0.00804^{EFG}$	0.72697 ± 0.01685^{AB}	$0.68930 \pm 0.02181^{ABC}$
	Bark	0.01675 ± 0.00047^{G}	0.41570 ± 0.00221^{C}	0.44636 ± 0.09268^{E}	0.60126 ± 0.01036^{CD}
	Root	0.01929 ± 0.00092^{FG}	$0.25415 \pm 0.00048^{DEF}$	0.61079 ± 0.02233^{C}	$0.68871 \pm 0.02231^{ABC}$

Note:

1. Extractive concentration in oven dried sample, lignin concentration in extractives free air dried sample, holocellulose concentration in extractives free air dried sample, alphacellulose concentration in holocellulose air dried sample
2. Duncan's grouping is labeled by small uppercase alphabets to compare different plant parts for each species and is tabulated for every component measured. Values with the same alphabet means no statistical significant difference at $p<0.05$.

(holocellulose) and side point of bottom disc (alphacellulose). On the other hand, the lowest concentration was detected in the centre point of bottom disc (extractives), centre point of disc at first branching (lignin), leaf (holocellulose) and bark (alphacellulose).

For *H. camargoana*, the highest figures were observed in the leaf (both extractives and lignin), side point of disc at first branching (holocellulose) and petiole (alphacellulose). On the other hand, the lowest concentration was detected in the bark (extractives), centre point of bottom disc (lignin), leaf (holocellulose) and small branch (alphacellulose).

Summary of total content of extractives, lignin, holocellulose and alphacellulose is given in *Table 3*. Duncan's grouping is indicated in the table for every component's total content, by comparing the different plant parts for each species. For *H. brasiliensis*, the highest total content of extractives, lignin and holocellulose was observed in the large branch, while the highest total content of alphacellulose was observed in the bole. For *H. benthamiana*, the highest total content of extractives, lignin, holocellulose and alpha-cellulose was all observed in the large branch. For *H. guianensis* and *H. pauciflora*, the highest total content of extractives was found in the leaf, while the highest total content of lignin, holocellulose and alphacellulose was observed in the bole. For *H. spruceana*, the highest total content of extractives was found in the leaf, while the highest total content of lignin and holocellulose was detected in the small branch. The highest total content of alphacellulose was observed in the large branch. For *H. nitida*, the highest total content of extractives and lignin was detected in the leaf and small branch, respectively. The highest total content of holocellulose and alphacellulose was observed in the bole. For *H. camargoana*, the highest total content of extractives, lignin, holocellulose and alphacellulose was all observed in the large branch. The lowest total content of all components in all species was observed in petiole.

The sum of lignin per whole tree in the order of the highest to the lowest is *H. spruceana*, 75.45 kg, *H. brasiliensis*, 69.13 kg, *H. benthamiana*, 65.16 kg, *H. pauciflora*, 53.82 kg, *H. guianensis*, 47.76 kg, *H. nitida*, 37.03 kg and finally *H. camargoana*, 26.08 kg. The sum of holocellulose per whole tree in the order of the highest to the lowest is *H. brasiliensis*, 197.75 kg, *H. spruceana*, 177.59 kg, *H. pauciflora*, 169.04 kg, *H. benthamiana*, 162.74 kg, *H. guianensis*, 128.08 kg, *H. nitida*, 99.55 kg and finally *H. camargoana*, 60.23 kg.

Calculation of total lignin and holocellulose that can be extracted from unused plant parts during timber harvesting is shown in *Table 4*. The highest amount of total lignin and holocellulose from unused plant parts is observed in *H. spruceana*. The energy combustion from 45.58 kg of lignin is 1.16 MJ and ethanol production from 93.03 kg holocellulose is 47.25 kg.

DISCUSSION

The seven *Hevea* species were analysed for their lignin and cellulose distribution for possible incorporation into rubber forest plantations. Currently in Malaysia, only *H. brasiliensis* is commercially planted in rubber plantations for the purpose of latex and wood harvesting. In rubber forest plantations, latex timber clones (LTC) of *H. brasiliensis* such as RRIM 2001, RRIM 2002, RRIM 2023, RRIM 2024, RRIM 2025, RRIM 2026, RRIM 3001, PB 260 and PB 350 are planted where suitable. Therefore, the comparative study with other *Hevea* species using RRIM 3001 as a model system of *H. brasiliensis* is to evaluate if any other species could match up to the performance of RRIM 3001 in terms of

TABLE 3. TOTAL CONTENT OF EXTRACTIVES, LIGNIN, HOLOCELLULOSE AND ALPHACELLULOSE IN PLANT PARTS OF EIGHT YEAR OLD *HEVEA* SPECIES

Species	Plant part	Extractives (kg) Mean + s.e.	Lignin (kg) Mean ± s.e.	Holocellulose (kg) Mean ± s.e.	Alphacellulose (kg) Mean ± s.e.
H. brasiliensis (RRIM 3001)	Leaf	1.00 ± 0.47^{AB}	2.59 ± 1.21^{B}	2.87 ± 1.35^{B}	1.81 ± 0.85^{C}
	Petiole	0.07 ± 0.04^{B}	0.45 ± 0.26^{B}	0.62 ± 0.36^{B}	0.40 ± 0.23^{C}
	Twig	0.62 ± 0.34^{AB}	4.42 ± 2.44^{AB}	7.72 ± 4.27^{B}	4.54 ± 2.51^{C}
	Small branch	0.48 ± 0.23^{AB}	6.40 ± 3.03^{AB}	13.14 ± 6.22^{B}	10.09 ± 4.78^{BC}
	Large branch	1.76 ± 1.05^{A}	21.62 ± 12.84^{A}	67.76 ± 40.25^{A}	40.00 ± 23.76^{AB}
	Bole	1.46 ± 0.23^{AB}	16.61 ± 2.67^{AB}	66.33 ± 10.65^{A}	43.95 ± 7.06^{A}
	Bark	0.13 ± 0.05^{B}	3.45 ± 1.32^{B}	5.23 ± 2.00^{B}	2.99 ± 1.14^{C}
	Root	1.37 ± 0.21^{AB}	13.59 ± 2.06^{AB}	34.08 ± 5.18^{AB}	21.88 ± 3.33^{ABC}
H. benthamiana	Leaf	1.04 ± 0.02^{B}	6.48 ± 0.10^{CDE}	4.92 ± 0.07^{E}	3.23 ± 0.05^{CD}
	Petiole	0.07 ± 0.01^{C}	0.70 ± 0.06^{E}	1.02 ± 0.08^{E}	0.70 ± 0.06^{D}
	Twig	0.60 ± 0.22^{BC}	4.75 ± 1.77^{DE}	5.98 ± 2.23^{DE}	3.68 ± 1.37^{CD}
	Small branch	0.80 ± 0.12^{B}	9.01 ± 1.39^{BCD}	23.07 ± 3.56^{CD}	17.66 ± 2.72^{B}
	Large branch	1.72 ± 0.43^{A}	16.91 ± 4.21^{A}	54.61 ± 13.58^{A}	42.32 ± 10.52^{A}
	Bole	1.14 ± 0.12^{AB}	14.05 ± 1.45^{AB}	42.69 ± 4.39^{AB}	31.42 ± 3.23^{A}
	Bark	0.06 ± 0.02^{C}	1.91 ± 0.48^{E}	3.32 ± 0.84^{E}	2.09 ± 0.53^{D}
	Root	0.78 ± 0.05^{B}	11.34 ± 0.79^{ABC}	27.13 ± 1.90^{BC}	16.60 ± 1.16^{BC}
H. guianensis	Leaf	1.00 ± 0.16^{A}	3.35 ± 0.53^{DE}	2.54 ± 0.40^{D}	1.49 ± 0.24^{D}
	Petiole	0.13 ± 0.04^{B}	0.75 ± 0.22^{E}	0.90 ± 0.26^{D}	0.60 ± 0.17^{D}
	Twig	0.78 ± 0.22^{A}	6.40 ± 1.77^{BCD}	10.52 ± 2.91^{CD}	6.40 ± 1.77^{CD}
	Small branch	0.70 ± 0.13^{A}	5.95 ± 1.07^{CD}	19.74 ± 3.55^{BC}	11.07 ± 1.99^{BC}
	Large branch	0.73 ± 0.08^{A}	10.10 ± 1.12^{AB}	27.96 ± 3.10^{B}	16.18 ± 1.80^{B}
	Bole	0.74 ± 0.05^{A}	11.34 ± 0.76^{A}	43.55 ± 2.93^{A}	36.51 ± 2.46^{A}
	Bark	0.07 ± 0.00^{B}	1.58 ± 0.11^{E}	2.93 ± 0.20^{D}	1.56 ± 0.10^{D}
	Root	0.76 ± 0.18^{A}	8.30 ± 1.98^{ABC}	19.94 ± 4.76^{BC}	11.99 ± 2.86^{BC}
H. pauciflora	Leaf	0.93 ± 0.02^{A}	3.66 ± 0.08^{BCD}	3.18 ± 0.07^{C}	1.87 ± 0.04^{C}
	Petiole	0.04 ± 0.01^{B}	0.41 ± 0.09^{D}	0.77 ± 0.16^{C}	0.47 ± 0.10^{C}
	Twig	0.13 ± 0.00^{B}	2.13 ± 0.08^{CD}	4.41 ± 0.16^{C}	2.62 ± 0.09^{C}
	Small branch	0.64 ± 0.20^{A}	11.50 ± 3.55^{AB}	31.75 ± 9.80^{ABC}	18.28 ± 5.64^{BC}
	Large branch	0.68 ± 0.21^{A}	11.93 ± 3.76^{A}	48.54 ± 15.28^{AB}	33.83 ± 10.65^{AB}
	Bole	0.77 ± 0.23^{A}	13.47 ± 4.03^{A}	56.46 ± 16.91^{A}	42.24 ± 12.65^{A}
	Bark	0.05 ± 0.01^{B}	1.65 ± 0.21^{CD}	3.45 ± 0.43^{C}	1.91 ± 0.24^{C}
	Root	0.68 ± 0.05^{A}	9.07 ± 0.65^{ABC}	20.48 ± 1.47^{BC}	13.74 ± 0.99^{BC}

TABLE 3 (cont.). TOTAL CONTENT OF EXTRACTIVES, LIGNIN, HOLOCELLULOSE AND ALPHACELLULOSE IN PLANT PARTS OF EIGHT YEAR OLD *HEVEA* SPECIES

Species	Plant part	Extractives (kg) Mean + s.e.	Lignin (kg) Mean ± s.e.	Holocellulose (kg) Mean ± s.e.	Alphacellulose (kg) Mean ± s.e.
H. spruceana	Leaf	1.72 ± 0.19^{A}	7.33 ± 0.79^{BCD}	7.34 ± 0.79^{B}	4.22 ± 0.46^{B}
	Petiole	0.07 ± 0.00^{D}	0.63 ± 0.01^{D}	1.04 ± 0.02^{B}	0.60 ± 0.01^{B}
	Twig	0.26 ± 0.02^{CD}	5.29 ± 0.32^{BCD}	9.47 ± 0.57^{B}	5.00 ± 0.30^{B}
	Small branch	1.06 ± 0.45^{B}	19.78 ± 8.41^{A}	47.10 ± 20.03^{A}	25.80 ± 10.97^{A}
	Large branch	0.74 ± 0.02^{BCD}	14.47 ± 0.43^{ABC}	44.70 ± 1.33^{A}	26.30 ± 0.78^{A}
	Bole	0.83 ± 0.15^{BC}	15.40 ± 2.87^{AB}	39.86 ± 7.43^{A}	24.74 ± 4.61^{A}
	Bark	0.08 ± 0.00^{D}	2.70 ± 0.13^{CD}	4.52 ± 0.21^{B}	2.32 ± 0.11^{B}
	Root	0.51 ± 0.18^{BCD}	9.84 ± 3.50^{ABCD}	23.57 ± 8.37^{AB}	13.25 ± 4.71^{AB}
H. nitida	Leaf	0.66 ± 0.49^{A}	2.52 ± 1.86^{CD}	1.19 ± 0.88^{C}	0.78 ± 0.58^{E}
	Petiole	0.05 ± 0.04^{A}	0.35 ± 0.26^{D}	0.36 ± 0.27^{C}	0.23 ± 0.17^{E}
	Twig	0.45 ± 0.12^{A}	3.87 ± 1.07^{BCD}	5.81 ± 1.61^{BC}	3.28 ± 0.91D^{E}
	Small branch	0.63 ± 0.19^{A}	9.60 ± 2.95^{A}	26.40 ± 8.10^{A}	16.69 ± 5.12^{B}
	Large branch	0.34 ± 0.05^{A}	4.76 ± 0.76^{BCD}	14.98 ± 2.38^{B}	10.91 ± 1.74^{BC}
	Bole	0.37 ± 0.03^{A}	8.16 ± 0.73^{AB}	34.51 ± 3.11^{A}	23.73 ± 2.14^{A}
	Bark	0.06 ± 0.01^{A}	1.42 ± 0.36^{D}	2.57 ± 0.65^{C}	1.34 ± 0.34^{E}
	Root	0.31 ± 0.01^{A}	6.35 ± 0.11^{ABC}	13.73 ± 0.24^{B}	8.96 ± 0.16^{CD}
H. camargoana	Leaf	0.30 ± 0.26^{A}	1.53 ± 1.31^{A}	1.11 ± 0.95^{B}	0.69 ± 0.59^{B}
	Petiole	0.01 ± 0.00^{A}	0.09 ± 0.05^{A}	0.09 ± 0.05^{B}	0.07 ± 0.03^{B}
	Twig	0.45 ± 0.41^{A}	4.19 ± 3.83^{A}	5.50 ± 5.03^{AB}	3.95 ± 3.61^{AB}
	Small branch	0.48 ± 0.21^{A}	4.02 ± 1.77^{A}	11.02 ± 4.84^{AB}	6.44 ± 2.83^{AB}
	Large branch	0.84 ± 0.50^{A}	8.27 ± 4.92^{A}	22.25 ± 13.23^{A}	15.44 ± 9.18^{A}
	Bole	0.40 ± 0.22^{A}	3.80 ± 2.02^{A}	11.05 ± 5.88^{AB}	7.78 ± 4.14^{AB}
	Bark	0.02 ± 0.01^{A}	0.60 ± 0.15^{A}	0.64 ± 0.17^{B}	0.38 ± 0.10^{B}
	Root	0.28 ± 0.08^{A}	3.57 ± 0.99^{A}	8.58 ± 2.37^{AB}	5.91 ± 1.63^{AB}

Note: Duncan's grouping is labelled by small uppercase alphabets to compare different plant parts for each species and is tabulated for every component measured. Values with the same alphabet means no statistical significant difference at $p<0.05$.

TABLE 4. CALCULATION OF TOTAL LIGNIN AND HOLOCELLULOSE FROM UNUSED PLANT PARTS OF EIGHT YEAR OLD *HEVEA* SPECIES AND PROJECTION OF COMBUSTION ENERGY AND ETHANOL PRODUCTION

Species	Lignin (kg)	Holocellulose (kg)	Energy combustion of lignin (kJ)	Ethanol production from holocellulose (kg)
H. brasiliensis	30.89	63.67	784187.02	32.34
H. benthamiana	34.20	65.44	868020.10	33.24
H. guianensis	26.32	56.57	668093.32	28.74
H. pauciflora	28.42	64.04	721481.33	32.53
H. spruceana	45.58	93.03	1156913.12	47.25
H. nitida	24.11	50.06	611935.33	25.43
H. camargoana	14.01	26.94	355518.77	13.68

Note: Gross heat of combustion of lignin is 25383 kJ/kg[18] and estimated ethanol production is 0.32 g per 0.63 g holocellulose[17].

producing abundant amounts of lignocellulosic materials for ethanol production.

Biomass weight data analysed in the study was intended for the calculation of total content of lignin and cellulose as well as preliminary observation of the species with high biomass. Based on findings, the species that contained highest biomass was *H. brasiliensis*, with total fresh weight (TFW) of 627.89 kg. The species that ranked second was *H. spruceana*, with TFW of 517.70 kg, followed by *H. benthamiana*, with TFW of 507.15 kg. For these three species, the high value of TFW was contributed by their large branch, bole, small branch and root biomass weight. The information on moisture content indicates the amount of water in each plant sample and thus estimates duration of the drying process during the early steps of biofuel processing. For example if plant samples were found to contain about the same amount of water, they can be dried simultaneously at the same temperature and drying period. From the results of Duncan's grouping, most species plant parts do not differ significantly while some were divided into two groups. This indicated that the drying process can be conducted concurrently for all parts of plants, thus shortening the total period of the drying process.

Presence of alphacellulose, hemicelluloses and lignin in the cell wall contributes to cell wall thickness and subsequently cell density, contributing to mechanical properties such as strength and hardness[15] in wood or any plant parts. Comparison of concentration of lignin and holocellulose (which consists of both hemicelluloses and alphacellulose) in plant parts of the *Hevea* species could give an indication of plant parts with better strength. Lignin was significantly highest in the leaf for all species. However, the concentration of holocellulose in the leaf was significantly the lowest for all species, indicating that the low holocellulose concentration was brought by the dominant presence of lignin in the dried leaf sample. On the other hand, the highest holocellulose concentration was mainly observed in the clear bole sample at various points such as the centre point of bottom disc, side point of bottom disc, side point of disc at 150 cm from ground, centre point of

disc at first branching and side point of disc at first branching, while the lowest lignin was also observed in these samples of plant parts. These findings suggested that the high amount of holocelluloses gave wood its fibrous property while the high amount of lignin in the leaf provides mechanical support for the leaves[16].

The centre point of disc samples differ from the side point of disc samples in that the centre part is called heartwood while the side part is called sapwood. Sapwood is the younger, softer outer portion of a tree that lies between the heartwood and the cambium (formative layer just under the bark). As comparatively new wood, sapwood is less durable and more permeable than heartwood. Heartwood is the older, harder central portion of a tree. It usually contains deposits of various materials that frequently give it a darker colour than sapwood. Heartwood is denser and more durable than sapwood and is found primarily in aged trees. However, when comparing the concentration of extractives, lignin and holocellulose between centre and side point of disc samples, there was no visible pattern as to whether heartwood contains more lignin and cellulose. When comparing the concentration of lignin and holocellulose among discs at the bottom, disc at 150 cm from ground and disc at first branching, there was also no clear pattern of whether the disc near the ground has higher concentration of holocellulose and lignin than the disc near the tree's first branching. Perhaps with mechanical testing methods, the strength of the wood could be differentiated clearer for these various points of the clear bole.

Calculation of total content of lignin and cellulose in plant parts gives information on the amount of lignocellulosic materials that could be extracted from unused plant parts during timber harvesting. Unused plant parts include the leaf, petiole, twig, small branch, bark and root. From the results, it was found that the plant part with the highest amount of lignin and holocellulose was the large branch, followed by the bole, small branch and root. This finding suggested that parts like the small branch and root could be excellent sources for lignocellulosic materials during the replanting programme. When comparing total amount of lignin and holocellulose that could be extracted from unused plant parts among the seven *Hevea* species, it was observed that *H. spruceana* contained the highest amount with 45.58 kg lignin and 93.03 kg holocellulose. The species that ranked second was *H. benthamiana* with 34.20 kg lignin and 65.44 kg holocellulose. Next in order was *H. brasiliensis* with 30.89 kg lignin and 63.67 holocellulose. The results showed that the former two species are potentials to be suggested for planting in rubber forest plantations for the purpose of ethanol production.

Both *H. spruceana* and *H. benthamiana* had been described in literature to produce natural hybrids with *H. brasiliensis. H. benthamiana* and *H. brasiliensis* hybrids were found in a very small area west of Manaos, while *H. spruceana* and *H. brasiliensis* hybrids have been studied and proved promising for disease resistance in the South America. In the East, the hybrids of *H. spruceana* and *H. brasiliensis* were reported to have greatly increased the yields of high-yielding clones when the hybrids were used as rootstocks[2]. These findings suggested that both species could possibly be utilised in the rubber breeding programme for inter-specific hybridisation with *H. brasiliensis*, with an objective to breed for high latex and lignocellulosic content rubber trees.

Based on an average of 42% cellulose and 21% hemicelluloses in wood, the maximum theoretical yield of ethanol can be calculated to be 0.32 grams of ethanol per gram of wood or per 0.63 gram of pure holocellulose[17]. This calculation is based on a full conversion

of cellulose and hemicelluloses to sugars and conversion of sugars to ethanol at the theoretical yield of 0.51 g/g. Gross heat of combustion of lignin on the other hand is about 25,383 kJ/kg[18]. It can be argued that the annual rubber replanting in Malaysia which involves 20,000 ha area of rubber plantation (RP) and 25,000 ha area of rubber forest plantation (RFP) represents a good resource of lignocellulosic materials. Using the calculation values for total lignin and holocellulose in *H. spruceana*, the projected amount of lignin and holocellulose that could be extracted from unused plant parts of an eight year old rubber tree with 524 tree stands per hectare is 23,884 kg of lignin and 48,748 kg of holocellulose. For a 15 year planting cycle in RFP, the number increases by ten percent to 26,272 kg of lignin and 53,623 kg of holocellulose, while for a 25 year planting cycle in RP, the number increases by fifteen percent to 27,467 kg of lignin and 56,060 kg of holocellulose. These shall produce 0.67 GJ of combustion energy from lignin and 27,237 kg of ethanol from RFP cycle as well as 0.70 GJ of combustion energy from lignin and 28,475 kg of ethanol from RP cycle. For 25,000 ha in the RFP replanting programme, the estimated amount of lignin combustion energy and ethanol production are 13.34 TJ and 545 million kg, respectively, while for the replanting programme in RP which involves a 20,000 ha area, the estimated amount of lignin combustion energy and ethanol production are 13.94 TJ and 570 million kg, respectively.

Embarking on the idea of converting unused plant materials into biofuel for major scale production clearly needs detailed planning of work activities. For example, trees are usually felled for replanting between the months of June to August. During this period, only the raw materials are supplied in enormous amounts and therefore, warehouses with sufficient capacity are needed to store and dry the feedstock. The factories needed to carry out pretreatment, hydrolysis and fermentation, should be built with an installed capacity lower than that of the warehouses since feedstock is transferred from the warehouses in smaller amounts but in a continuous manner throughout the year. This will ensure that the factories operate on a daily basis eliminating shortage of raw materials. For example, the storage capacity for the warehouse should be about three million tonnes of fresh raw materials while installed capacity of the factory needs to be about 3,800 tonnes of dry raw materials. The expected ethanol production from daily operation is about 1,500 tonnes.

CONCLUSION

This study has successfully analysed the different distribution patterns among plant parts of *Hevea* trees, where lignin was found to be of highest concentration in the leaf while holocellulose concentration was highest in the clear bole samples. The investigation did not indicate any relationship between concentration of lignin and holocellulose in sapwood and heartwood as well as among clear bole samples at different levels. The study has also successfully recognised two species other than *H. brasiliensis* which indicates potential as planting material in rubber forest plantations for wood harvesting. These are *H. spruceana* and *H. benthamiana*, since both species have comparable high biomass weight and high amounts of lignin and holocellulose in unused plant parts.

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