Utilization of Aquatic Weeds Fibers for Handmade Papermaking

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Increasing global paper consumption has fostered the search for new alternative non-wood fiber sources. The aquatic weeds *Cyperus digitatus*, *Cyperus iria,* and *Scirpus grossus* were analysed for their fiber characteristics and chemical composition, and the processed fibers were transformed into handmade paper. The selected species yielded medium-length fibers (0.92 mm to 1.03 mm), which were thin-walled with a lumen diameter (3.37 µm to 5.26 µm) wider than cell wall thickness (2.73 µm to 2.97 µm). In terms of fiber derived values, the selected species possessed a slenderness ratio of 86.5 to 113.1 (favourable, > 30) and flexibility coefficient of 35.2 to 47.6 (favourable, within the range 50 to 70), which was classified as rigid fiber. The species also contained high cellulose, 42.1% to 44.8% (favourable, > 40%) and hemicellulose content, 42.8% to 45.6% (favourable, within the range of 30% to 50%), and low lignin content, 10.6% to 11.8% (favourable, < 12%). Handmade paper of *Cyperus digitatus* possessed relatively high tensile strength (2.61 ± 0.15 kN/m) and breaking length (1.20 ± 0.07 km) among studied species. Comparison with other non-wood fibers indicated that the studied plants fibers can be used for production of paper plates, paperboard, and decorative paper.

Keywords: Aquatic weeds; Fiber; Handmade papermaking

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INTRODUCTION

The pulp and paper industry has a huge impact in society, which is manifested in almost every human daily activity. It is undeniable that an excess consumption of paper has greatly reduced the forest area in recent decades, thus deteriorating environmental quality. In 2010, global paper consumption was estimated at approximately 400 million tonnes, and this figure is expected to increase by up to 25% by 2020 (WWF 2012). The increasing global consumption has resulted in constant market pressure to fulfil this demand, and thus leads to uncontrolled deforestation activity (Environmental Paper Network 2011). It becomes a great challenge to sustain the demand for paper in a way that does not endanger forests. Intensive forest management aims for short rotation of fiber production, but such efforts still require several years before the planted tree is ready for harvest (Dangerfield *et al.* 1998). This has prompted the search for an alternative fiber source that can help to support the demand when the production of fibers from wood source is low. Non-forest materials such as agriculture residues, *e.g.*, oil palm (Alriols *et al*. 2009; Daud and Law 2010), kenaf (Villar *et al*. 2009), canola (Ekhtera *et al*. 2008), and pineapple

(Tran 2006) and aquatic plants, *e.g.*, freshwater plants (Bidin *et al*. 2015) and seagrass (Syed *et al*. 2016) are also important sources of fiber that can be utilized in various paper products. Non-forest fibers obviously cannot replace the production of fibers from wood source on a large scale; however, they can become a good commodity to support the pulp and paper industry and reduce the dependence on forest fibers.

Rapid land development, intensive forestry and agricultural practices, and degradation of natural habitat have sped up the growth and invasion of aquatic weeds (Bakar 2004). In Malaysia, management of the noxious invasive aquatic weeds still relies heavily on the use of herbicides; however, prolonged used of such chemicals can lead to plant resistance to herbicides (Ruzmi *et al.* 2017). Infestation of aquatic weeds (*e.g.*, *Eichhornia crassipes*, *Salvinia molesta*, and *Pistia stratiotes*) in rice granaries of Peninsular Malaysia recorded a total of 8144 ha in 2002 (Baki and Mislamah 2003). The abundance of unwanted aquatic weeds make them a potential candidate as raw material for papermaking. Aquatic weeds or aquatic plants have some favourable characteristics, such as short growth cycles, modest irrigation and fertilization requirements, as well as low lignin content, which can minimize the use of energy and chemicals during pulping (Hurter and Riccio 1998). The management of aquatic plants is comparatively cost-effective and more environmentally friendly compared to wood sources (Reddy *et al*. 2012). In addition, recent studies also suggest that freshwater aquatic plants (Govindasamy *et al.* 2014; Bidin *et al*. 2015) and marine plants (seagrass) (Syed *et al*. 2016) can be used as raw materials for handmade papermaking. Meanwhile, the application of traditional Japanese handmade papermaking uses renewable resources and environmentally friendly techniques that preserve the environment (Taori 2002). The technique is practical and efficient in producing tougher and better quality fibers (Yamauchi and Usami 2005). It is also believed that the knowledge of handmade papermaking using minimal chemical and technology for paper arts and crafts can be easily transferred to people in the local community in helping them to improve their livelihood. The present study attempts to evaluate the suitability of aquatic plants' fibers in handmade papermaking.

EXPERIMENTAL

Materials

Collection of aquatic weeds

Stems of three species of marginal aquatic weeds were collected from the Aquaculture Research Station, Universiti Putra Malaysia, Puchong, Selangor, Malaysia. The species stem's length (l) and diameter (d) and specific coordinates were recorded for every species as follows; *Cyperus digitatus* Roxb. (0.64 ± 0.07 m (l), 1.1 ± 0.2 cm (d), 2° 59' 09.48" N, 101° 39' 00.07" E), *Cyperus iria* L. (1.20 ± 0.22 m (l), 1.2 ± 0.3 cm (d), 2° 59' 13.17" N, 101° 38' 51.99" E), and *Scirpus grossus* L. F. (1.18 ± 0.15 m (l), 1.3 ± 0.2 cm (d), 2° 59' 11.70" N, 101° 38' 45.80" E). The plants were washed under running tap water and cleaned of soil. Herbariums were prepared with voucher specimens (*Cyperus digitatus*: PGCD01-03, *Cyperus iria*: PGCIN01-03, and *Scirpus grossus*: PGSG01-04) and deposited at the Laboratory of Aquatic Botany, Department of Aquaculture, Faculty of Agriculture, UPM Serdang, Malaysia. The collected plants were divided into two portions. Fresh plants were used for the evaluation of fiber morphological characteristics. Dried plants were prepared by oven drying in a Memmert (Schwabach, Germany) airrecirculating oven at 60 °C until at constant weight, and they were then used for determination of chemical composition and as material for handmade papermaking.

Fiber dimension and derived values

Fresh stems were processed following the protocol of Ogbonnaya *et al.* (1997) for the determination of fiber dimensions [fiber length (mm), fiber diameter (µm), fiber lumen diameter (µm), cell wall thickness (µm)] and derived values [slenderness ratio, flexibility coefficient, and Runkel ratio]. First, 1 g of fresh stems were macerated with 50 mL of 32.5% nitric acid (HNO_3) in a water bath at 80 ± 0.5 °C for 2 h. Then the fibers were placed on 250-µm laboratory test sieve and washed under running tap water. The fiber suspension was obtained by mixing the washed fibers with distilled water in a 50-mL Falcon tube under a vortex mixer. One drop of fiber suspension was placed onto a glass microscope slide and stained with Safranin O solution. The stained fibers were measured for fiber length (FL) at 4× magnification, while the fiber diameter and fiber lumen diameter were measured at 40× magnification under a calibrated compound stereomicroscope (ZEISS Axioskop, Stemi SV11, New York, USA) attached with a Nikon DS-Fi1 camera (Nikon Instruments Inc., New York, USA). The measurements were taken using the Nikon NIS-Elements Documentation software (Nikon Instruments Inc., NIS-Elements D, New York, USA). Cell wall thickness (CWT) was calculated by the subtraction of fiber diameter from fiber lumen diameter and divided by two. The slenderness ratio (SR), flexibility coefficient (FC), and Runkel ratio (RR) (Ogbonnaya *et al*. 1997; Ververis *et al.* 2004) were calculated as below,

$$SR = FL/FD \tag{1}$$

$$FC = FLD/FD \times 100 \tag{2}$$

$$RR = CWT/FLD \times 2 \tag{3}$$

where *FL* is fiber length (mm), *FD* is fiber diameter (µm), *FLD* is fiber lumen diameter (µm), and *CWT* is cell wall thickness (µm).

Methods

Chemical composition

Dried stems of the selected aquatic plants were used for the determination of cellulose, hemicellulose, and lignin content using direct estimation methods following Moubasher *et al*. (1984). A total of 2 g of dried stem were pre-extracted with 500 mL of 1:2 ethanol-acetone solution for 4 h under Soxhlet extraction. Then, 1 g of extractive-free sample (fraction A) was placed in a conical flask and treated with 200 mL of 24% potassium hydroxide (KOH) solution in a water bath at 25 °C for 4 h. The mixture was filtered through a pre-weighed glass extraction thimble, washed with distilled water, and oven-dried at 105 °C for 7 h. The dry weight of the sample after being treated with KOH was recorded and labeled as fraction B. The treated sample was then further hydrolysed with 200 mL of 72% of sulphuric acid (H_2SO_4) for 3 h at room temperature, filtered through the glass thimble, and washed with distilled water. The sample was then boiled with 200 mL of 5% H_2SO_4 in a water bath at 90 °C for another 2 h. The mixture was filtered through a pre-weighted glass extraction thimble, washed with distilled water, and oven-dried at 105 °C for another 7 h. The dried mixture after being hydrolysed with KOH and H_2SO_4 was labeled as fraction C. The cellulose, hemicellulose, and lignin content with three replicates for each species were calculated based on the following formulas,

$$CC = (B - C) / A \times 100 \quad (4)$$

$$HC = (A - B) / A \times 100 \quad (5)$$

$$LC = C / A \times 100 \quad (6)$$

where CC is the cellulose content (%), A is the initial weight of the sample (g), B is the weight of the sample treated with KOH (g), C is the weight of the sample treated with KOH and H_2SO_4 (g), HC is the hemicellulose content (%), and LC is the lignin content (%).

Pulping and papermaking

Dried stems of selected aquatic plants were used for pulping and papermaking. A total of 300 g of the plants' dried stem was cooked with 6 L of 9.0 g/L sodium hydroxide (NaOH) solution in a stainless steel stock pot under an ELBA (Borso del Grappa, Italy) induction cooker at 100 °C for 2 h. The cooked mixture was transferred into a container, covered with an aluminum foil sheet and kept for 3 days to further soften the fibers. The fiber bundles were squeezed and separated by hand protected by rubber gloves. The mixture was drained and filtered using a 250-µm laboratory test sieve and washed with tap water until the black liquor was no longer present. The fibers were further separated by hand and beaten with a wooden mallet for 15 min to 30 min. The fibers were then treated with 6 L of 0.02% sodium hypochlorite twice before being washed under running tap water. Excess water was squeezed out from the fibers using a cotton cloth. The moisture content of the fibers was determined by using an AD-4715 moisture balance (Mettler Toledo, Greifensee, Switzerland) and used for the estimation of dry fiber yield.

Pulp suspension was obtained by mixing the pulp of 18 g of fibers to 1 L distilled water and 1 L of 1 g/L of cooked potato starch (to form a slurry suspension) in a 20-L food mixer GF-201 (Good Friend Food Machine Co., Taichung, Taiwan). Wet paper sheets were produced based on a still forming sheet technique (tamezuki) using a sugeta mould (Hiebert 2006) (21.0 cm × 29.5 cm × 1.2 cm dimension). The wet papers were stacked alternating with cotton cloth with wooden boards for the top and bottom cover, pressed, and then air-dried at room temperature.

Paper characteristics and mechanical property

The weight (g) and thickness (mm) of the produced papers were measured and recorded. The papersheet grammage (g/m^2) was calculated by dividing the weight (g) with area (m^2). Fiber distribution within the produced papersheet was observed under a stereomicroscope (ZEISS Axioskop, Stemi SV11, New York, USA), and images were captured using a Pixelink camera model PL-A662 (PixeLink, Ottawa, Japan). The moisture content of the paper was determined using an AD-4715 digital moisture balance (Mettler Toledo, Greifensee, Switzerland). The pH of the paper was determined using a previously developed method (Sithole 2005). Briefly, paper sheets were cut into smaller fragments (size 1 cm to 2 cm) and oven-dried at 105 °C overnight. Then, 5 g of the paper pieces were placed inside a 100-mL conical flask with 18 mL deionized water and 2 mL of 2 M sodium chloride (NaCl) solution. The mixture was placed inside a water bath at 65 ºC for 10 min, left to cool, and the water solution was extracted and tested using a Mettler Toledo SevenEasy pH meter (Darmstadt, Germany).

Paper strips of 17.8 ± 0.1 cm × 2.5 ± 0.1 cm dimensions were cut from the paper according to the TAPPI T404 cm-92 (2002) standard. Tensile strength and breaking length were determined according to TAPPI standard T404 cm-92 (2002). The paper strips used were free from watermarks, cut edges, and creases. The weight (g) of each paper strip was recorded. A total of 10 paper strips were tested using a 5 kN universal testing machine INSTRON 3365 (Instron, Norwood, MA, USA) with the rate of elongation of 5 mm/min. The maximum force (kN) used for breaking the paper strips apart was observed and recorded using BlueHill® Lite software (Instron, Bluehill 2, Norwood, MA, USA). The breaking force for breaking apart the paper strips was recorded only if the breaking point of the paper strips was within the acceptable area. Tensile strength and breaking length were calculated based on Eqs. 7 and 8,

$$TS = BF / W \tag{7}$$

$$BL = TS / G \times 102{,}000 \tag{8}$$

where TS is tensile strength (kN/m), BF is maximum breaking force (kN), W is width of the paper strip (m), BL is breaking length (km), and G is grammage (g/m^2).

Statistical analysis

The aquatic plants' fiber dimension and derived values, chemical composition, papersheet quality, and paper strip mechanical property were statistically analysed *via* a one-way analysis of variance (ANOVA; Duncan multiple range test, $p < 0.05$). Data obtained from the studied aquatic plants were compared with available published studies using principal component analysis (PCA, Pearson type) and agglomerative hierarchical clustering (AHC, similarities of Pearson correlation coefficient) using Addinsoft XLSTAT 2014 (XLSTAT, version 2014, New York, USA).

RESULTS AND DISCUSSION

Fiber Dimension and Derived Values

Based on IAWA (1989) classification, the studied aquatic plants were categorized under medium-sized fiber length (0.92 ± 0.03 mm to 1.11 ± 0.06 mm) and differed from other aquatic plants, *e.g.*, *Cyperus digitatus*, *C. rotundus*, *C. halpan*, *Scirpus grossus,* and *Typha angustifolia* that were categorized as having moderately short fibers (0.71 mm to 0.83 mm) (Bidin *et al*. 2015) (Table 1). Most agricultural crop residues possess a medium-sized fiber similar to the studied aquatic plants, except for *Gossypium hirsutum* (0.83 mm). Long-sized fiber length (> 2 mm) is undesirable, since such fibers tend to flocculate during pulping and papermaking process resulting in poor paper sheet formation (Ramezani and Nazhad 2004). Moreover, aquatic plant fiber consisted of a thin-walled fiber, based on Metcalfe (1971) fiber diameter classification with thick FLD compared to CWT (Table 1 and Fig. 1), similar with those reported by Bidin *et al.* (2015). Agricultural crop residues and inland weeds also have thin-walled fibers, except for *Saccharum officinerum* and *Solanum diphyllum* that have thick-walled fibers. Thin-walled fibers will give low coarseness values and is an important criteria for making printing paper (Yu *et al*. 1999). It also influenced positively on the bursting, tensile strength, and folding endurance of paper (Dutt and Tyagi 2011). A relatively high fiber slenderness ratio of > 60 (Veveris *et al.* 2004) has been recorded for *C. iria* and *C. digitatus* (113.08 ± 6.28 and 108.09 ± 4.87, respectively), followed by *S. grossus* (86.52 ± 2.94) (Table 2). The values were higher compared to previous reports of *C. digitatus* (76.8), *S. grossus* (73.8) (Bidin *et al.* 2015), and other aquatic plants. A compilation of the fiber derived values from various plant sources in Table 1 show that aquatic plants possessed a higher slenderness ratio than most agricultural crop residues and inland weed fibers, except for *Passiflora foetida* (120.0) (Brindha *et al*. 2012), *Solanum trilobatum* (167.5), and *S. diphyllum* (146.4) (Thongpukdee *et al*. 2013). Fibers with high slenderness ratio (> 33) are desirable for producing good quality of pulp and paper (Xu *et al.* 2006). In terms of the flexibility coefficient, the studied plants were in the rigid group (30 through 50) in Table 2 (Bektaş *et al.* 1999), and can vary from rigid fibers to elastic fibers (Bidin *et al.* 2015). The differences might be due to plant maturity, thus influencing the plants' fiber characteristics (Jawaid and Khalil 2011). Non-wood fibers with a slenderness ratio of > 60 and a Runkel ratio < 1 are the most desirable for papermaking (Ververis *et al*. 2004). Stem fibers of aquatic plants have a relatively high slenderness ratio (86.5 to 113.1, > 60) and Runkel ratio (1.22 to 2.02, > 1), with a lower flexibility coefficient (35.20 to 47.58) than the recommended range of 50 to 75 (Bektaş *et al.* 1999). High flexibility coefficient fibers can produce stronger paper, since the fibers provide a better fiber bonding in paper sheet (Ekhuemelo and Tor 2013). Fibers with Runkel ratio > 1 can form larger fibers bonding area and are more flexible, which make them desirable as papermaking materials (Sharma *et al*. 2011). Although the selected species in this study lack in flexibility and Runkel ratio criteria, they can still be used for the production of fiber plates and cardboard (Akgul and Tozluoglu 2009). The mechanical strength of the paper also can be improved by reinforcing it with suitable recycled fibers (Cappelletto *et al.* 2000).

Figure 2 shows the grouping of aquatic plants, agricultural crop residues, and inland weeds into four main clusters according to PCA variables and AHC analysis. A PCA factor of F1 (49.0%) and F2 (25.9%) accounted for 74.9% of the total variance. Fiber length, fiber diameter, fiber lumen diameter, cell wall thickness, and flexibility coefficients were positively correlated with F1, while the slenderness ratio and Runkel ratio were positively correlated with F2. Group A consisted of aquatic plants (Table 1, this present study (1 through 3), (4 through 8, Bidin *et al*. 2015), agricultural crop residues, *Oryza sativa* (Kiaei *et al.* 2011), and an inland weed, *P. foetida* (Brindha *et al*. 2012) that possessed narrow fiber diameter (9.13 µm to 12.11 µm) , fiber lumen diameter (3.37 µm to 7.30 µm), a thin cell wall, and a high Runkel ratio. Based on fiber morphological characteristics and correlation with other studies, the aquatic plants studied by Bidin *et al*. (2015) can be used as raw material for handmade production of paperboard, craft, and decorative paper. The studied aquatic weeds' fiber physical characteristics and derived values were different from Group B. Group B consisted of a majority of agricultural crop residues (9, 11 to 13, and 15 to 17) (Ververis *et al*. 2004; Goswami *et al*. 2008; Enayati *et al*. 2009; Kiaei *et al*. 2011; Shakles *et al*. 2011a, 2011b) and inland weeds (19 through 23, 26) (Sharma *et al*. 2013; Thongpukdee *et al*. 2013). Agricultural crop residues such as the *Gossypium hirsutum* stalk with high fiber flexibility coefficient (65.70) and low Runkel ratio (0.52) are suitable for making of writing and printing paper (Veveris *et al*. 2004), similar with *Musa paradisica* fibers which has elastic fiber (64.55) and low RR (0.77) suitable for producing greaseproof paper (Goswami *et al*. 2008). They were also different from Group C, which was a highly rigid fiber of *S. officinerum* (Agnihotri *et al.* 2010) and *S. diphyllum* (Thongpukdee *et al.* 2013), or Group D that consisted of *S. trilobatum* which possessed extremely long fiber length (4.10 mm) (Thongpukdee *et al*. 2013). Fiber from group D is less desirable because it will result in poor paper sheet formation (Ramezani and Nazhad 2004).

Table 1. Fiber Dimension of Aquatic Plants (1 through 8), Agricultural Residues (9 through 17), & Inland Weeds (18 through 26)

Plant Species		Part	Fiber Dimensions						Derived Values				References
			FL (mm)		FD (µm)	FLD (µm)	CWT (µm)		SR	FC		RR	
1	*Cyperus digitatus*	S	1.03 ± 0.06^{ab}	M	9.31 ± 0.20^{c}	3.37 ± 0.15^{c}	2.97 ± 0.05^{a}	T	108.09 ± 4.87^{a}	35.20 ± 0.91^{c}	R	2.02 ± 0.08^{a}	Present study
2	*Cyperus iria*	S	1.11 ± 0.06^{a}	M	10.00 ± 0.27^{b}	4.54 ± 0.27^{b}	2.73 ± 0.09^{b}	T	113.08 ± 6.28^{a}	43.60 ± 1.72^{b}	R	1.65 ± 0.11^{b}	Present study
3	*Scirpus grossus*	S	0.92 ± 0.03^{b}	M	10.94 ± 0.23^{a}	5.26 ± 0.19^{a}	2.84 ± 0.07^{ab}	T	86.52 ± 2.94^{b}	47.58 ± 1.15^{a}	R	1.22 ± 0.06^{c}	Present study
4	*Cyperus digitatus*	S	0.72	MS	9.64	5.15	2.25	T	76.85	52.91	E	1.06	Bidin *et al*. (2015)
5	*Cyperus rotundus*	S	0.71	MS	9.13	4.32	2.41	T	81.57	46.63	R	1.28	Bidin *et al*. (2015)
6	*Cyperus halpan*	S	0.73	MS	11.08	6.02	2.53	T	69.01	53.54	E	1.02	Bidin *et al*. (2015)
7	*Scirpus grossus*	S	0.83	MS	12.11	7.30	2.41	T	73.77	58.08	E	0.84	Bidin *et al*. (2015)
8	*Typha angustifolia*	S	0.83	MS	10.01	4.35	2.83	T	89.34	42.52	R	1.52	Bidin *et al*. (2015)
9	*Sorghum bicolor*	S	0.88	MS	20.12	10.92	4.59	T	44.08	54.27	E	0.84	Kiaei *et al*. (2011)
10	*Oryza sativa*	S	0.83	MS	10.89	4.57	3.16	T	76.58	41.96	R	1.38	Kiaei *et al*. (2011)
11	*Helianthus annus*	S	0.96	M	22.84	11.12	5.85	T	42.03	48.68	R	1.05	Kiaei *et al*. (2011)
12	*Nicotina tabacum*	St	1.23	M	24.31	15.38	8.93	T	50.59	63.26	E	1.16	Shakhes *et al*. (2011a)
13	*Hisbiscus cannabinus*	St	1.31	M	24.12	16.40	4.25	T	54.32	67.00	E	0.52	Shakhes *et al*. (2011b)
14	*Saccharum officinerum*	St	1.51	M	21.40	6.27	7.74	K	70.56	29.29	HR	2.46	Agnihotri *et al*. (2010)
15	*Brassica napus*	St	1.17	M	23.02	12.50	5.26	T	50.83	54.30	E	0.84	Enayati *et al*. (2009)
16	*Gossypium hirsutum*	St	0.84	M	18.07	12.32	3.18	T	44.60	65.70	E	0.52	Ververis *et al*. (2004)
17	*Musa paradisica*	S	1.55	MS	22.00	14.20	5.50	T	70.50	64.55	E	0.77	Goswami *et al*. (2008)
18	*Passiflora foetida*	S	1.20	M	10.00	6.00	2.00	T	120.00	60.00	E	0.66	Brindha *et al*. (2012)
19	*Scoparia dulcis*	S	0.93	M	27.14	16.81	5.17	T	34.27	61.89	E	0.62	Sharma *et al*. (2013)
20	*Urena lobate*	S	1.58	M	22.03	13.40	4.32	T	71.81	60.78	E	0.66	Sharma *et al*. (2013)
21	*Vernonia cinerea*	S	0.94	M	22.59	14.09	4.25	T	47.12	62.36	E	0.61	Sharma *et al.* (2013)
22	*Sida cordifolia*	S	1.30	M	22.74	14.00	4.37	T	58.22	61.83	E	0.69	Sharma *et al*. (2013)
23	*Bidens pilosa*	S	0.93	M	24.20	16.67	3.76	T	47.33	69.01	E	0.46	Sharma *et al*. (2013)
24	*Solanum trilobatum*	S	4.10	EL	24.48	17.04	3.44	T	167.48	54.15	E	0.81	Thongpukdee *et al*. (2013)
25	*Solanum diphyllum*	S	2.50	VL	17.08	4.76	5.60	K	146.37	24.32	HR	0.82	Thongpukdee *et al*. (2013)
26	*Solanum lasiocarpum*	S	1.61	ML	27.04	14.48	6.08	T	59.54	52.79	E	0.91	Thongpukdee *et al*. (2013)

Present study data are mean ± standard error; values in columns indicated with different superscript (a > b > c) are significantly different ($p < 0.05$, Duncan's Multiple Range Test (DMRT)); FS- fiber length, FD- fiber diameter, FLD- fiber lumen diameter, CWT- cell wall thickness, SR- slenderness ratio, FC- flexibility coefficient, RR- Runkel ratio, S- stem, St- stalk, M- medium, MS- moderately short, EL- extremely long, VL- very long, ML- moderately long, T- thin-walled, K- thick-walled, R- rigid, E- elastic, and HR- highly rigid

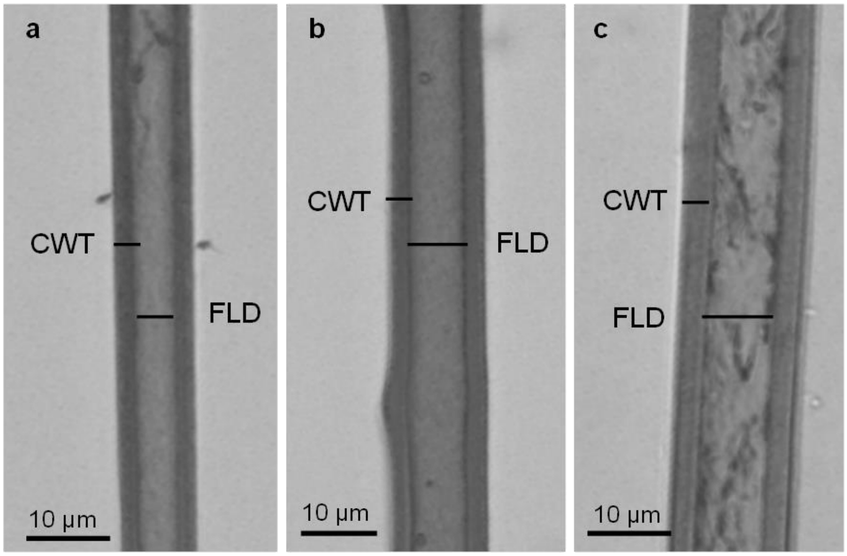

Fig. 1. Thin-wall stem fiber of (a) *Cyperus digitatus*, (b) *Cyperus iria,* and (c) *Scirpus grossus*; FLD- fiber lumen diameter, and CWT- cell wall thickness

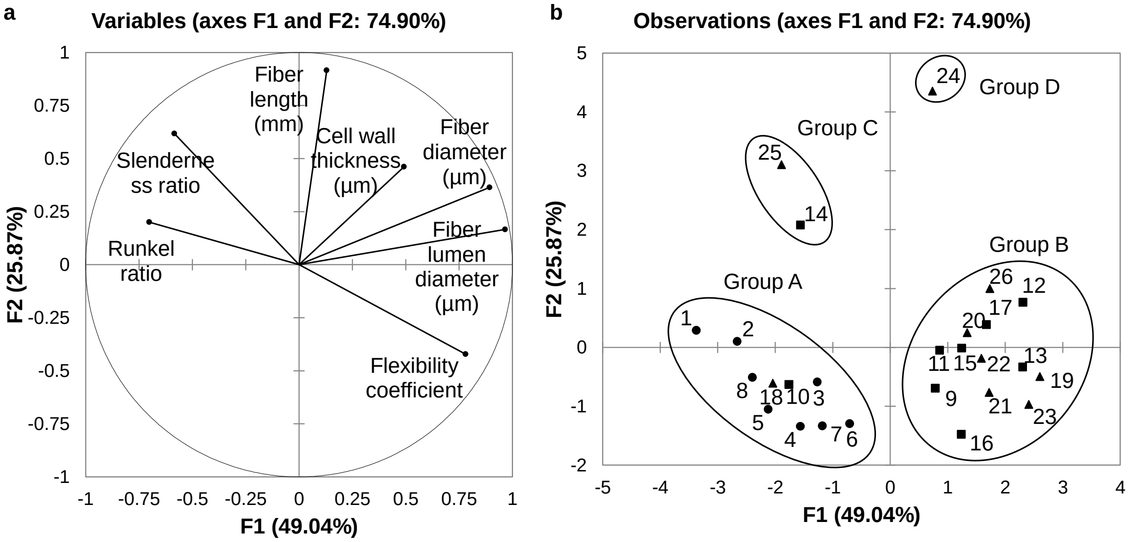

Fig. 2. (a) PCA variables of fiber dimension and derived value variables (fiber length, fiber diameter, fiber lumen diameter, cell wall thickness, slenderness ratio, flexibility coefficient, and Runkel ratio); (b) PCA score of fiber dimension and derived values according to species (●: aquatic plants (1 through 8), ■: agricultural crop residues (9 through 17), and ▲: inland weeds (18 through 26), with reference to Table 1)

Chemical Composition

The suitability of fibers for papermaking depends on a plant's cellulose, hemicellulose, and lignin contents. Differences in composition may require differences in treatment and processing for preparation of pulp. Japanese handmade papermaking uses minimal chemicals and technology (Hiebert 2006) in a way that plants with high cellulose and hemicellulose contents and low lignin are preferable. Screening of plants through this process can help to identify which raw materials meet these requirements and are suitable for pulping and papermaking. Cellulose content greatly influences paper strength (Ververis *et al*. 2004), because the pulp mechanical strength and tensile strength are directly proportional with cellulose content (Madakadze *et al.* 2010). The cellulose of studied plants showed a high content of more than 40%, which ranged from 42.13 ± 3.56% to 44.82 ± 0.29% (Table 2). Such levels of cellulose content have been recommended for paper production (Ververis *et al.* 2004; Mossello *et al.* 2010). Similarly with cellulose, the hemicellulose contents of aquatic plants was also more than 40% for *C. digitatus* (42.78 ± 0.29%), *C. iria* (43.44 ± 0.60%), and *S. grossus* (45.57 ± 0.51%). The hemicellulose content of the studied plants was similar to other aquatic plants (Bidin *et al*. 2015) and higher compared to agricultural crop residues and inland weeds (Table 2). A range of 30% to 50% of hemicellulose has been stated as adequate for papermaking (Knoshaug *et al*. 2013). The studied plants also showed a lignin content of < 12% for *C. digitatus* (11.81 ± 0.12%), *S. grossus* (11.44 ± 0.28%), and *C. iria* (10.63 ± 0.28%). Lignin is an undesirable polymer for papermaking (Saheb and Jog 1999), and should be < 12% to reduce the time, energy, and chemical usage during pulping (Hurter and Riccio 1998; Saheb and Jog 1999).

Table 2. Chemical Composition of Aquatic Plants (1 through 6), Agricultural Residues (7 through 10), and Inland Weeds (11 through 14)

Plants		Part	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	References
1	*Cyperus digitatus*	S	44.82 ± 0.29^{a}	42.78 ± 0.29^{a}	11.81 ± 0.12^{a}	Present study
2	*Cyperus iria*	S	44.60 ± 0.38^{a}	43.44 ± 0.60^{a}	10.63 ± 0.28^{b}	Present study
3	*Scirpus grossus*	S	42.13 ± 3.56^{a}	45.57 ± 0.51^{a}	11.44 ± 0.28^{ab}	Present study
4	*Cyperus rotundus*	S	42.58	45.64	9.54	Bidin *et al*. (2015)
5	*Scirpus grossus*	S	36.21	49.88	13.44	Bidin *et al*. (2015)
6	*Typha angustifolia*	S	44.05	54.84	20.04	Bidin *et al*. (2015)
7	*Zea mays*	S	39.00	42.00	7.30	Daud *et al*. (2014)
8	*Saccharum officinerum*	Bg	42.34	28.60	21.70	Agnihotri *et al*. (2010)
9	*Musa* sp.	Str	53.45	28.56	15.42	Silveira *et al*. (2008)
10	*Panicum virgatum*	S	43.40	35.90	21.80	Radiotis *et al*. (1999)
11	*Passiflora foetida*	S	40.00	36.00	24.00	Brindha *et al*. (2012)
12	*Cleome grandiflora*	S	56.53	21.74	21.73	Brindha *et al*. (2013)
13	*Cleome viscosa*	S	56.53	21.74	21.73	Brindha *et al*. (2013)
14	*Cleome burmannii*	S	49.25	31.88	18.84	Brindha *et al*. (2013)

Present study data are mean ± standard error in columns indicated with different superscripts (a > b) are significantly different ($p < 0.05$, DMRT); S- stem, Bg- Bagasse, and Str- straw

Figure 3 shows the grouping of aquatic plants, agricultural crop residues, and inland weeds in PCA based on chemical composition variables (Fig. 3). The PCA factor of F1 (71.1%) and F2 (21.5%) accounted for 92.6% of the total variance. The hemicellulose content was positively correlated with F1, while lignin and cellulose contents were positively correlated with F2. The studied aquatic plants, reported aquatic plants (Bidin *et al*. 2015), and *Zea mays* (Daud *et al.* 2014) were in group A, with high hemicellulose (42.0% to 54.8%) and low lignin content (7.3% to 20.0%) contents. Their high hemicellulose contents are suitable for handmade cardboard, paper, and paperboard (Bidin *et al.* 2015). It has been reported that the high contents of cellulose, hemicellulose, and hollocellulose in plant fibers provide high paper dimensional stability (Daud *et al*. 2014). None of the studied aquatic plants were in Group B, agricultural crop residues (*Musa* sp.) (Silveira *et al*. 2008), and inland weeds (*Cleome grandiflora*, *Cleome viscosa*, and *Cloeme burmanii*) (Brindha *et al*. 2013), which contained high cellulose (49.25 % to 56.53 %) with low hemicellulose (21.7% to 28.6%) composition that can be used for the production of filter paper (Brindha *et al*. 2013). Group C, which were the plants with high undesirable lignin content for papermaking (*S. officinerum*) (Agnihotri *et al.* 2010), *Panicum virgatum* (Radiotis *et al*. 1999), and *P. foetida* (Brindha *et al*. 2012). These species required more time, energy, and chemicals in the cooking and pulping process in order to remove the lignin from the fibers (Saheb and Jog 1999).

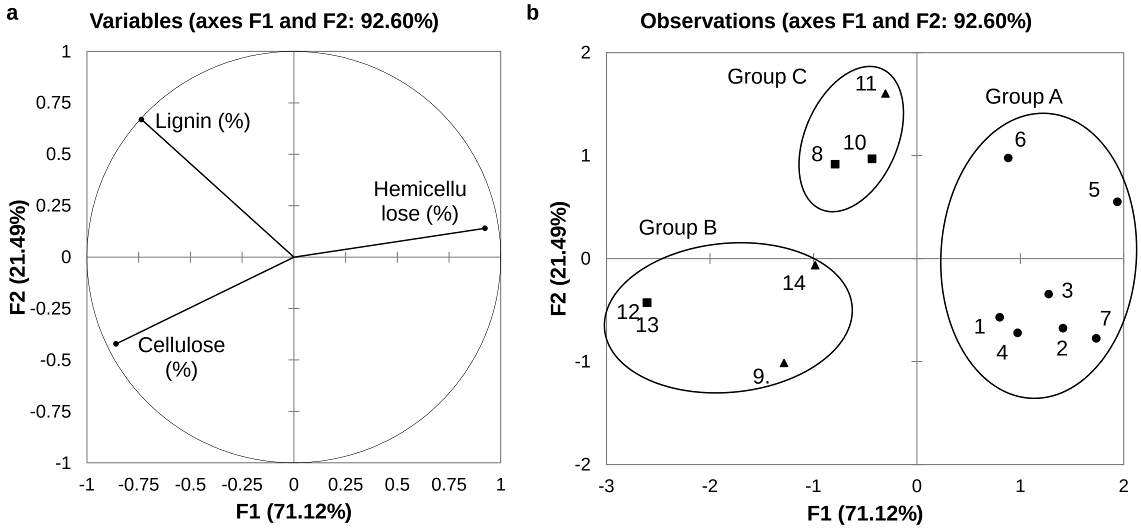

Fig. 3. (a) PCA variables of chemical composition variables (cellulose, hemicellulose, and lignin); (b) PCA score of chemical composition according to species (●: aquatic plants (1 through 6), ■: agricultural crop residues (7 through 10), and ▲: inland weeds (11 through 14), with reference to Table 2)

Pulp and Paper Quality

Pulp yield of *C. digitatus, C. iria*, and *S. grossus* were 22.0%, 22.6%, and 23.7%, respectively (Table 3). These values were much lower compared to *Cyperus papyrus*' pulp yield of 39.0% (Mollabashi *et al.* 2017). The moisture content of paper produced from the selected plants was between 13.00 ± 0.55% and 14.60 ± 0.12%, similar with the previous study of *T. angustifolia* (13.1%) and *S. grossus* (13.1%) (Bidin *et al*. 2015). The high moisture content of aquatic plants' paper was attributed to the high hemicellulose content (Madsen *et al.* 2004). Papers' pH values were slightly acidic with a pH range of 5.65 ± 0.05 to pH 6.47 ± 0.43. These values may be due to the degradation process initiated by atmospheric pollutants or by reactions from heat, light, and moisture (Slavin and Hanlan 1992). The presence of fiber bundles and plant cell debris in the paper sheets (Fig. 4) indicated that extra cooking and hand beating time were required to improve the paper quality. Japanese handmade papermaking techniques produced long fiber paper with a good mechanical strength; however, the technique requires a certain level of hand skill (Barret and Lutz 2005; Pietzcker 2012). Japanese handmade papermaking method uses a hand beating technique during pulping process, which helps to separate the fiber bundle and enhance the fiber fibrillation (Hiebert 2006).

Table 3. Pulp and Paper Characteristics

	Cyperus digitatus	*Cyperus iria*	*Scirpus grossus*
Pulp Yield (%)	22.04	22.55	23.70
No. of Paper Sheet Produced	4	4	4
Total Paper Weight (g)	63.00	63.25	65.13
Paper Weight Per Sheet (g)	15.75 ± 0.38^a	15.81 ± 0.44^a	16.28 ± 0.06^a
Area Per Sheet (cm^2)	619.50	619.50	619.50
Moisture (%)	13.00 ± 0.55^b	14.60 ± 0.12^a	13.80 ± 0.23^{ab}
pH	6.47 ± 0.43^a	5.65 ± 0.05^a	6.08 ± 0.11^a

Values are mean ± standard error, row values with different superscript (a > b) are significantly different ($p < 0.05$, DMRT)

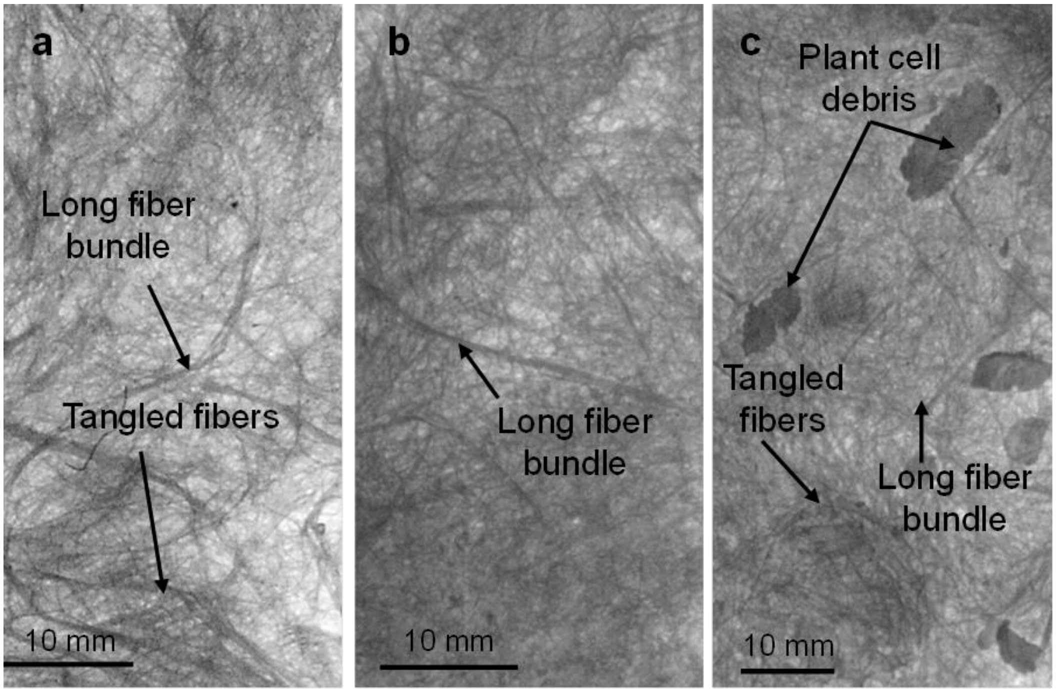

Fig. 4. Fiber distribution on produced paper as observed under 0.6× magnification: (a) *Cyperus digitatus*, (b) *Cyperus iria*, and (c) *Scirpus grossus*

External fibrillation through beating process increases bonding surface area of fibers and thus improves the paper strength (Laitinen *et al.* 2015). The Japanese handmade papermaking technique utilizes such a process to improve the fiber bonding ability as compared to a blending technique, which only breaks the fibers into small fragments (Kang and Paulapuro 2006). The technique also improves the tensile stress and modulus of elasticity of papersheets made from aquatic plants as compared to blended technique (Syed *et al*. 2016). Table 4 illustrates that *C. digitatus* paper strip possessed a high tensile strength (2.61 ± 0.15 kN/m) and breaking length (1.20 ± 0.07 km) compared to *C. iria* (1.82 ± 0.06 kN/m, 0.62 ± 0.05 km) and *S. grossus* (1.41 ± 0.10 kN/m, 0.65 ± 0.05 km). All selected aquatic plants have thin-walled fibers based on Metcalfe (1971), but fibers of *C. digitatus* had smaller fiber diameter (9.31 ± 0.20 µm) and fiber lumen diameter (3.37 ± 0.15 µm) compared to C. *iria* (10.00 ± 0.27 µm, 4.54 ± 0.27 µm) and *S. grossus* (10.94 ± 0.23 µm, 5.26 ± 0.19 µm), allowing a more compact arrangement of fibers in the papersheet without the need of extra beating times for the fibers to collapse and flatten.

Table 4. Mechanical Property of Paper Produced from Aquatic Plants (1 through 11) and Other Commercialized Paper Products (12 through 18)

Paper Products		Product Type	Grammage (g/m^2)	Tensile Strength (Kn/m)	Breaking Length (km)	References
1	*Cyperus digitatus*	Paper plate, paperboard, and decorative	237.15 ± 6.67^a	2.61 ± 0.15^a	1.20 ± 0.07^a	Present study
2	*Cyperus iria*	Paper plate, paperboard, and decorative	239.17 ± 9.40^a	1.82 ± 0.06^b	0.62 ± 0.05^b	Present study
3	*Scirpus grossus*	Paper plate, paperboard, and decorative	254.34 ± 10.08^a	1.41 ± 0.10^c	0.65 ± 0.05^b	Present study
4	*Cyperus rotundus*	Cardboard, paper, and paperboard	n/a	1.69	0.73	Bidin *et al*. (2015)
5	*Scirpus grossus*	Cardboard, paper, and paperboard	n/a	1.52	0.61	Bidin *et al*. (2015)
6	*Typha angustifolia*	Cardboard, paper, and paperboard	n/a	0.94	0.41	Bidin *et al*. (2015)
7	*Enhalus acoroides*	Paper plate and paperboard	170.93	3.46	2.49	Syed *et al*. (2016)
8	*Thalassia hemprichii*	Paper plate and paperboard	155.14	4.00	3.43	Syed *et al*. (2016)
9	*Cymodocea serrulata*	Paper plate and paperboard	114.67	1.24	2.24	Syed *et al*. (2016)
10	*Halophila ovalis*	Paper plate and paperboard	219.79	3.36	1.83	Syed *et al*. (2016)
11	*Halophila spinulosa*	Paper plate and paperboard	93.33	2.72	2.43	Syed *et al*. (2016)
12	Commercial paperboard	Paperboard	219.29-287.24	n/a	n/a	Huang *et al*. (2014)
13	Kitchen paper roll	Sanitary	17.9-48.8	0.16-0.47*	n/a	Green Seal (2013)
14	Paper napkin	Sanitary	14.6-46.4	0.16-0.43*	n/a	Green Seal (2013)
15	Bathroom tissue	Sanitary	13.0-35.8	0.05-0.35*	n/a	Green Seal (2013)
16	Facial tissue	Sanitary	13.0-30.9	0.10-0.29*	n/a	Green Seal (2013)
17	Release base paper	Wrapping and packaging	40.00-70.00	n/a	n/a	Yogi *et al*. (2002)
18	Decorative paper	Decorative	80.15-130.03	n/a	0.67-1.95	Bardak *et al*. (2011)

Present study data are mean ± standard error, in the columns values indicated with different superscripts (a > b > c) are significantly different ($p < 0.05$, DMRT); *Data are dry tensile strength (machine direction-MD) of papers using TAPPI T494 om-01 (2006) standards and the values are converted from 1 gf/in to 0.3886 N/m; n/a- not available

The collapsing of fibers into ribbons provides more surface contact area for bonding and has favourable effects on the bursting strength and tensile strength of paper produced (Dutt and Tyagi 2011). Tensile strength and breaking length of the produced papers were also higher than previously reported using blended technique for handmade paper from aquatic plants, *e.g.*, *C. rotundus* (1.69 kN/m, 0.73 km), *S. grossus* (1.52 kN/m, 0.61 km), and *T. angustifolia* (0.94 kN/m, 0.41 km) (Bidin *et al*. 2015). The *S. grossus* paper formed by the hand beaten technique also slightly improved in terms of the paper's tensile strength and breaking length as compared to the aforementioned study. In comparison, seagrass fibers with a better slenderness ratio (90.4 to 154.1) and high cellulose content (40.3% to 77.2%) demonstrated a higher tensile strength (1.24 kN/m to 4.00 kN/m) and breaking length (1.83 km to 3.43 km), with *Thalassia hemprichii* recording the highest for both variables (Syed *et al.* 2016). This is in accordance with Madakadze *et al.* (2010) who state that the cellulose content of plants is directly proportional to the mechanical property of paper produced. In addition, papers from seagrass, which have low grammage values (93.3 g/m^2 to 219.8 g/m^2) compared to the present study (237.15 ± 6.67 g/m^2 to 254.34 ± 10.08 g/m^2), showed better mechanical property. The difference might be due to the applied wet pressing force during the papermaking process. High grammage paper needed more pressure force to reduce the paper thickness and remove the remaining water. Without sufficient pressure, the paper density will be reduced and the fibers will not overlap uniformly in the sheet. An increase in density required more wet pressing pressure required during papermaking (Vainio and Paulapuro 2007). Increases in wet pressing pressure promote the fibers contact and bonding area, resulting in higher tensile strength (Pikulik *et al.* 1995; Vainio and Paulapuro 2007). This explains why the tensile strength and breaking length in the present study was much lower compared to the previous study. The present study also successfully produced paper with high grammage (> 200 g/m^2) that can be classified as paperboard (Huang *et al*. 2014). The tensile strength of the studied plants' paper (1.41 kN/m to 2.61 kN/m) was higher compared to that of a sanitary product (0.05 kN/m to 0.47 kN/m) (Green Seal 2013), which usually have a grammage of < 50 g/m^2. Release base papers (Yogi *et al.* 2002) that function as wrapping and packaging have a grammage of 40 g/m^2 to 70 g/m^2. Paper produced from the study also had similar breaking length with that of decorative paper (0.67 km to 1.95 km) (Bardak *et al.* 2011).

CONCLUSIONS

1. Based on the morphological characteristics of the selected species from this study, they can be considered as fiber sources for handmade papermaking, as they possessed medium fiber length (0.92 ± 0.03 mm to 1.11 ± 0.06 mm), thin wall fiber with fiber lumen diameter (3.37 ± 0.15 µm to 5.26 ± 0.19 µm) > cell wall thickness (2.73 ± 0.09 µm to 2.97 ± 0.05 µm), and good slenderness ratio (86.52 ± 2.94 to 113.08 ± 6.28 > 30).
2. Comparison with the selected plants which have rigid fibers with low flexibility coefficient of 35.20 ± 0.91 to 47.58 ± 1.15 < 50, and poor Runkel ratio (1.22 ± 0.06 to 2.02 ± 0.08 > 1) and other non-wood fiber sources indicate that these fibers can be used for the making of paper plates, paperboard, and decorative paper.
3. The high cellulose and hemicellulose contents, along with low lignin content of the studied plants can help reduce the use of bleaching agent during the pulping process.

4. Handmade papersheet produced from *C. digitatus* fibers with significantly smaller fiber diameter (9.31 ± 0.20 µm) and fiber lumen diameter (3.37 ± 0.15 µm) possessed a relatively high tensile strength (2.61 ± 0.15 kN/m) and breaking length (1.20 ± 0.07 km) compared to *C. iria* (1.82 ± 0.06 kN/m, 0.62 ± 0.05 km) and *S. grossus* (1.41 ± 0.10 kN/m, 0.65 ± 0.05 km).
5. Preparation of paperboard using handmade method required strong pressing pressure during the papermaking process in order to achieve a better paper tensile strength and breaking length.
6. Japanese handmade papermaking method uses minimal chemicals and technology and is applicable to local community practice.

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