Fiber Characteristics and Papermaking of Seagrass Using Hand-beaten and Blended Pulp

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Marine angiosperms could inevitably offer considerable potential resources for their fiber, yet little research has been conducted, especially in Malaysia. Fiber characteristics of five species of seagrass – *Enhalus acoroides, Cymodocea serrulata, Thalassia hemprichii, Halophila ovalis,* and *Halophila spinulosa* – were evaluated. Fiber dimensions were studied to determine slenderness ratio, flexibility coefficient, Runkel's ratio, and Luce's shape factor species selection. The seagrass species have the potential in papermaking production as they possessed slenderness ratio >33 (98.12 to 154.08) and high Luce's shape factor (0.77 to 0.83); however the species exhibited low flexibility coefficient <50 (30.07 to 35.18) and >1 Runkel's ratio (1.11 to 1.60), which indicate rigid fiber. The five seagrass species have high cellulose >34% (40.30 to 77.18%) and low lignin content <15% (5.02 to 11.20%), which are similar to those encountered in non-wood plant species. Handmade paper sheet of *Enhalus acoroides* using pulp subjected to mechanical blending exhibited the highest tensile strength (4.16 kN/m) compared to hand-beaten pulp (3.46 kN/m). The highest breaking length (3.43 km) was achieved by a paper sheet of *Thalassia hemprichii* using hand-beaten pulp. Based on their physical and chemical composition properties, seagrass have potential as sources of fibrous material for handmade papermaking.

Keywords: Seagrass; Fiber dimension; Chemical composition; Papermaking; Handmade paper; Mechanical strength

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INTRODUCTION

In ancient times, humans used non-wood plants as surfaces for writing. *Cyperus papyrus* L. was widely used as writing material by Egyptians since 3000 BC (Saijonkari-Pahkala 2008). The first recorded practitioner of the papermaking technique, Ts'ai Lun, also used non-wood plants, such as hemp, rags, and mulberry to produce paper sheets. There is an increasing trend of pulp production from non-wood sources worldwide, *e.g*., China and India, mainly because of the shortage of hardwood fiber raw material (Oinonen and Koskivirta 1999; Saijonkari-Pahkala 2008). Han James (1998) reported that certain non-wood plants such as *Gossypium* sp. have high cellulose content (85% to 90%) compared to the wood of *Pinus sylvestris* (35% to 49% cellulose). Hemp, *Cannabis* sp. also has a low lignin content (3%), which suggests that these non-wood fibers are suitable for papermaking. Photosynthetic aquatic species such as seagrass also contain cellulose or other fibrous materials potentially suitable for paper production, with the added advantage of faster propagation and maturation than terrestrial plants.

Fiber characteristics vary between plants, and analysis of physical properties and chemical composition of the fiber is important in order to provide an indication of the papermaking potential of various species (Saijonkari-Pahkala 2008). Fiber morphology such as fiber length and width are important in determining pulp fibers quality (Wood 1981). One of the most important physical properties of fibers for papermaking is fiber length, which basically influences the tearing strength of the paper (Marques *et al*. 2010). Plant fibers consist of three structural polymers, which are the polysaccharides cellulose and hemicellulose, plus the aromatic polymer lignin, as well as some minor non-structural components such as proteins, extractives, and minerals (Marques *et al*. 2010). Some non-wood fibers contain more pentosans (>20%), holocellulose (>70%), and less lignin (≃15%) compared to hardwoods (Hunsigi 1989). In addition, the higher hot water solubility characteristic possessed by non-wood plants ease the cooking liquors accessibility (Saijonkari-Pahkala 2008).

Photosynthetic aquatic species such as seagrass also contains cellulose or other fibrous materials potentially suitable for paper production. Seagrasses are aquatic angiosperms, which are restricted to the marine environment (Kuo and den Hartog 2001). In Malaysia, the seagrass can be found mostly in the shallow inter-tidal, semi-enclosed lagoons, sub-tidal zones, coral reef, and mangrove ecosystems (Japar Sidik and Muta Harah 2003). Peoples in Ria de Aveiro have been collecting more than 100,000 tons of aquatic vegetation (*e.g.*, *Potamogeton pectinatus*, *Ruppia cirrhosa*, and *Zostera noltii*) per year including seagrass (Silva *et al*. 2004; Cunha *et al*. 2013). In Germany, seagrass fiber was substituted for cotton in the manufacture of nitrocellulose during the Second World War, (Milchakova *et al*. 2014). Based on the above information, although seagrass contain fiber which can be used for papermaking, the pulping method suitability as raw materials for papermaking were not fully explored during the 1980s (Cunning 1989). With this prospective, an attempt was made to evaluate the suitability of seagrasses in papermaking based on their fiber morphology and chemical composition. Tested parameters on the paper properties including tensile strength, breaking length, stress-strain curve, and modulus elasticity were compared and recorded based on seagrass species fibers and pulping method.

EXPERIMENTAL

Raw Materials

Enhalus acoroides, Thalassia hemprichii, *Cymodocea serrulata, Halophila ovalis* and *Halophila spinulosa* were collected along the sub tidal shoals of Tanjung Adang-Merambong shoals (01° 19' N, 103° 36' E). Entire seagrass plants were collected, except for *Enhalus acoroides*, where only the blades were used as raw materials for handmade papermaking. The samples were cleaned and placed in an ice chest before being transported to the laboratory for further processing as described below.

Physical Properties Identification

Cleaned fresh plants were fragmented into small pieces (2 cm) and macerated in 10 ml of 67% nitric acid (HNO_3) in a test tube and boiled in a water bath at 100±2 °C for 10 min. The samples were then washed with over flowing tap water before placing in a centrifuge tube containing 10 mL of distilled water (Ververis *et al*. 2004). The macerated fiber suspensions in the tube was mixed using vortex before being examined under calibrated compound microscope, ZEISS Germany Axioskop attached with camera Nikon model DS-Fi1 to measure the fiber length (L), fiber diameter (D), lumen diameter (d), and cell wall thickness (W) (Fig. 1). The derived values; slenderness ratio (L/D), Runkel's ratio ($2W/d$), flexibility ratio [$d/(D \times 100)$], and Luce's shape factor [$(D^2-d^2)/(D^2+d^2)$] of each fiber dimensions were calculated in order to assess the fiber quality for paper production (Ohshima *et al*. 2005).

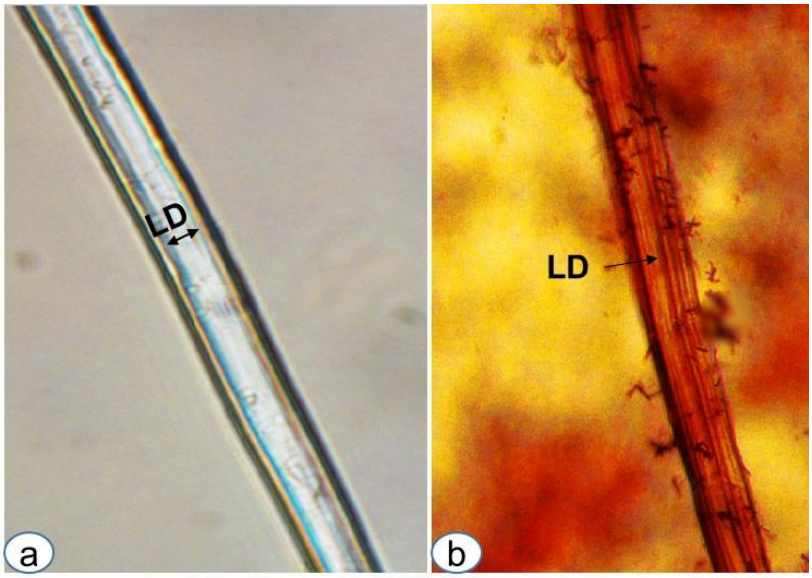

Fig. 1. Fiber wall of seagrass (20X magnification). (a) Thin walled *Halophila spinulosa* fiber (b) Thick walled *Enhalus acoroides* fiber. LD represents lumen diameter.

Chemical Composition Identification

Air dried seagrass plants were ground and passed through a 70 µm mesh screen. Direct estimation of cellulose, hemicellulose, and lignin were carried out using the method as described by Moubasher *et al*. (1982). Two grams of ground sample were boiled in 2:1 ethanol-toluene solvent for 4 h using Soxhlet extraction and washed thoroughly and dried (105 °C) in an air circulation oven overnight. The dry samples were weighed and divided into 2 parts. The first part was labeled as fraction A, while the second part was treated with 24% potassium hydroxide (KOH) solution for 4 h at 25 °C, then filtered using a weighed glass extraction thimble, labeled as fraction B. The fraction B was further treated with 72% sulphuric acid (H_2SO_4) for 3 h and filtered using the glass thimble and refluxed with 5% H_2SO_4 at 90 °C for 2 h. The residues were then filtered and washed to remove the H_2SO_4 and later dried at 80 °C for 24 h. The dry samples were weighed as fraction C.

Cellulose content = B – C
Hemicellulose content = A – B
Lignin content = C

Pulp and Papermaking

Dried seagrass plants were chopped into small fragments (2 to 5 cm length) and completely soaked in water overnight to soften the fibers. Samples were cooked in a solution having the ratio of 1 water to 2 sodium hydroxide (NaOH) using an ELBA induction cooker at a constant temperature of 160 °C for 2 to 6 h as shown in Table 1. The cooking durations varied with seagrass plant species; for example *Enhalus acoroides* need longer cooked time and with additional water and NaOH for the pulp to soften. Fibers were then washed with water to remove the residue.

Each pulp derived from seagrass plant species were processed using two different methods; (i) beaten using wooden mallet for 20 min until the fiber spread out and became soft, (ii) and blended for 10 min. The next stage was sheet formation, in which the processed pulps were diluted with water in a vat using mould and deckle dipped them under the fibers. The deckles containing the pulp were lifted, shaken slightly to help distribute the fibers evenly, before being laid down on the felt, and the deckle was carefully removed. Excessed water was removed by air drying at room temperature (25 to 27 °C), pressed, and oven dried at 60 °C for 3 days.

Table 1. Yield and Pulp Weight of Seagrass during Cooking Process

Species	Yield (dry weight) (g)	Cooking duration (hr)	Water (L) to NaOH (g) (1:2 ratio)	Dry Pulp weight (g)
Enhalus acoroides	200	6	10:20	443.52
Thalassia hemprichii	200	4	8:16	403.20
Cymodocea serrulata	200	4	5:10	510.21
Halophila ovalis	200	2	4:8	397.21
Halophila spinulosa	200	3	4:8	382.41

Mechanical Strength of Paper

Fiber distribution of paper sheets produced was observed under ZEISS Stemi SVII with camera PixeLINK model PL-A662. Tensile strength of each paper was measured by following TAPPI Method T-404 (1992). The handmade paper was cut into 5 strips, each with a dimension of 1 cm wide x 5 cm long. The strips were weighed, and the thickness was measured using the micrometer (Mitutoyo, Japan) before being tested using Universal Testing Machine ISNTRON 3365 (5kN) at the rate of elongation of 5 mm per minute. All the data (tensile stress, strain and modulus elasticity) were recorded using the Bluehill® software. The tensile strength and breaking length were calculated using the formula as followed:

Tensile strength (kN/m) = maximum breaking force (kN)/ paper width (m)
Breaking length (km) = 120,000 (tensile strength/ grammage)

Statistical Analysis

Comparison for fiber dimension, derived values and chemical composition between seagrass species were using 1-way ANOVA and if result is significant, this was followed by a post-hoc Tukey's Test (p<0.05) using Statistical Package for the Social Science (SPSS) software. Data comparison for paper produced using hand-beaten and blended pulp were analyzed using Independent Sample T-Test (p<0.05). Principal component analysis (PCA) based on the Pearson method was carried out using XLSTAT software version 2013 (Addinsoft, New York, USA) to obtain the relationship between variables obtained for seagrass species compared with other non-wood plant species from other studies.

RESULTS AND DISCUSSION

Fiber Dimensions and Derived Valued

Table 2 shows the fiber dimensions of seagrass. Fiber length of *Enhalus acoroides* (2.04 mm) was significantly longer (p<0.05) compared with other species (ranged 1.51 to 1.55 mm). Based on the standard published by International Association of Wood Anatomy (IAWA 1937), among the species tested, *Enhalus acoroides* is categorized as moderately long fiber (1600 to 2200 µm), while the rest as medium-sized fiber (1200 to 1500 µm).

The strong fibers of *Enhalus* plants are also being utilized for construction of nets by people in Yap Island (Falanruw 1992). Longer fiber tends to be less uniformly distributed in a paper sheet and can cause malformation. The proportion of fiber length to diameter which is suitable for paper production is about 100:1, while for textiles a typical fiber length is >1000:1 (Saijonkari-Pahkala 2008). In this study, *Enhalus acoroides* with 128:1 ratio indicated its suitability in papermaking, and the value is slightly higher compared to 125:1 for *Cyperus papyrus* (Hurter 1990; Saijonkari-Pahkala 2008). In contrast, *Halophila ovalis* has wider fiber diameter (20.87 µm), lumen diameter (6.91 µm), and cell wall thickness (7.81 µm) compared to others. Lumen size is important for fiber dimensions because it will effect on the rigidity and strength of paper (Akpakpan *et al*. 2012). However, greater value of fiber diameter increases the void volume and forms a coarse-surfaced paper sheet (Kaur and Dutt 2013). Large fibers with thin walls also give a positive effect as they tend to form non-porous tightly bonded paper sheet that is easily collapse and flexible. In contrast, the less flexible thick walled fiber will produced less bounded and bulkier paper sheet resulting in a lower burst (Akpakpan *et al*. 2012).

Derived values from the fiber dimensions are important to determine the suitability of material for paper production. There were significant differences in slenderness ratio values (p<0.05) between *Enhalus acoroides* (154.08) with the rest of seagrass species (Table 3). It is invaluable for pulp and paper quality if the slenderness ratio of fibrous material is <70 (Young 1981). According to Sharma *et al*. (2013) and Xu *et al*. (2006) slenderness ratio of >33 is considered good for pulp and paper production. All the seagrass species in this study exceeded the satisfactory slenderness ratios of 33. A higher slenderness ratio in fibers will produce higher rate of paper tear resistance (Akpakpan *et al*. 2012). Based on PCA, the seagrass species and other non-woods plants were clustered into five main groups (Fig. 2). The first two factors (F) accounted for 86.55% of total variance. F1 explained the high percentage of total variance (66.21%) compared to F2 (20.34%). Fiber diameter, lumen diameter, and flexibility coefficient were positively correlated to F1, while fiber length, cell wall thickness, slenderness ratio, and Runkel ratio were positively correlated to F2. Based on their slenderness ratio, all the seagrass species were in Group 2, clustered with Kenaf bark (*Hibiscus cannabinus*). Kenaf bark comprises 35% of fibrous part (Manzanares *et al*. 1997) and contains two fiber components that are different in morphology and chemical properties, and both are suitable for paper and paperboards (Shakhes *et al*. 2011; Touzinski *et al.* 1973), writing, printing, packaging and wrapping purposes (Ververis *et al*. 2004). Group 1 have the highest number of non-wood plants, which shared similar value of fiber diameter, lumen diameter, and flexibility coefficient. The members of Group 3, which comprised grass, lemon grass, and cane, were clustered together, as they have high value of Runkel ratio. *Pinus kesiya* and *Gmelina arborea* were together in Group 4, having a high value of lumen diameter. *Camellia sinensis* plant had the highest value in all fiber morphology variables and was clustered in Group 5.

Table 2. Fiber Morphology of Seagrass and Other Non-wood Plants

Species	Fiber length (mm)	Fiber diameter (µm)	Lumen diameter(µm)	Cell wall thickness (µm)	References
Seagrasses					
1. *Enhalus acoroides*	2.04±0.13[a]	15.96±1.18[b]	5.31±0.57[b]	5.43±0.44[b]	Present study
2. *Thalassia hemprichii*	1.53±0.05[b]	16.13±0.69[ab]	4.91±0.24[b]	6.31±0.29[ab]	Present study
3. *Cymodocea serrulata*	1.51±0.07[b]	16.82±0.69[ab]	5.15±0.23[b]	6.53±0.29[ab]	Present study
4. *Halophila ovalis*	1.55±0.07[b]	20.87±2.07[b]	6.91±0.50[a]	7.81±1.07[a]	Present study
5. *Halophila spinulosa*	1.54±0.07[b]	17.44±0.84[ab]	5.50±0.30[ab]	6.43±0.32[ab]	Present study
Non-wood plants					
6. *Ageratum conyzoides*	0.74	26.33	19.6	3.36	(Sharma *et al.* 2013)
7. *Bidens pilosa*	0.93	24.2	16.67	3.76	(Sharma *et al.* 2013)
8. *Crotalaria pallida*	1.24	25.15	15.86	4.64	(Sharma *et al.* 2013)
9. *Eupatorium odoratum*	1.08	25.85	16.81	4.37	(Sharma *et al.* 2013)
10. *Scorpia dulcis*	0.93	27.14	16.81	5.17	(Sharma *et al.* 2013)
11. *Sida cordifolia*	1.30	22.74	14	4.37	(Sharma *et al.* 2013)
12. *Solanum torvum*	0.85	34.79	27.99	3.39	(Sharma *et al.* 2013)
13. *Urena lobata*	1.58	22.03	13.4	4.32	(Sharma *et al.* 2013)
14. *Vernonia cinerea*	0.94	22.59	14.09	4.25	(Sharma *et al.* 2013)
15. *Gmelina arborea*	1.48	37.96	28.86	4.55	(Sharma *et al.* 2013)
16. *Pinus kesiya*	3.12	63.61	52	5.81	(Sharma *et al.* 2013)
17. *Cymbopogon citratus*	1.09	16.3	6.73	4.62	(Kaur and Dutt 2013)
18. *Cymbopogon martini*	0.87	14.7	5.07	3.86	(Kaur and Dutt 2013)
19. *Hibiscus cannabinus* (bark)	2.32	21.9	11.9	4.2	(Ververis *et al*. 2004)
20. *Hibiscus cannabinus* (core)	0.74	22.2	13.2	4.3	(Ververis *et al*. 2004)

21. *Hibiscus cannabinus* (whole)	1.29	22.1	12.7	4.3	(Ververis *et al*. 2004)
22. *Arundo donax* (internodes)	1.22	17.3	8.5	4.4	(Ververis *et al*. 2004)
23. *Arundo donax* (nodes)	1.18	18.8	8.6	5.6	(Ververis *et al*. 2004)
24. *Miscanthus giganteus*	0.97	14.2	5.9	4.1	(Ververis *et al*. 2004)
25. *Panicum virgatum*	1.15	13.1	5.8	4.6	(Ververis *et al*. 2004)
26. *Gossypium hirsutum*	0.83	19.6	12.8	3.4	(Ververis *et al*. 2004)
27. *Camellia sinensis*	3.7	870.9	291.8	289.5	(Tutus *et al*. 2015)

Means ± standard error with the same alphabet in the column is not significant different at $p<0.05$ by Tukey's Test

There are four groups of fibers based on the flexibility coefficient (Bektas and Tutus 1999; Hemmasi *et al*. 2011); (a) high elastic fibers with elasticity ratio >75, (b) elastic fibers with elasticity ratio between 50 and 75, (c) rigid fibers with elasticity ratio between 30 and 75, and (d) highly rigid fibers with ratio <30. In this present study all the seagrass have rigid fiber with flexibility coefficient (30.07 to 35.18). As the flexibility coefficient increase, the suppleness of the fibers also increase, resulting in a well-bonded sheet (Akpakpan *et al*. 2012). During the papermaking process, the rigid fibers will not conform in the paper sheet, hence decreasing the fiber bonding and elasticity. Rigid fibers are usually used more on fiber plate, rigid cardboard, and cardboard production (Akgul and Tozluoglu 2009).

All seagrass species in this present study have Runkel's ratio >1, which corresponds to their rigid fiber characteristic. Eroglu (1980) has classified the most suitable Runkel's ratio as <1 where the cell wall is thin as the fibers are more flexible and form paper with large bonding. Runkel's ratio equal to 1 is also suitable for paper production but less preferable. The fiber properties with this Runkel value is hard and stiff, resulting in poor bonding ability and therefore reduced paper quality (Wahab *et al*. 2006). This value can be improved by using a suitable screening technique which needs more cost (Ververis *et al*. 2004).

The Luce's shape factor indices did not vary to an important degree between the five seagrass species (0.77 to 0.83) and comparable to the reed species (*Arundo donax*). This showed that the mechanical and structural properties of seagrass plants were in range with reed, compared to the other non-wood species (Table 3). The Luce's shape factor is derived from circular shape of both fiber diameter and fiber lumen diameter, which give the indicator to paper sheet density and the breaking length of paper (Ona *et al*. 2001).

Chemical Composition

Chemical composition of all species showed good percentage value (40.30 to 77.18%) for cellulose, which is the main component of plant fibers used during pulping (Table 4). *Enhalus acoroides* has the highest cellulose content with 77.18% (p<0.05) compared to others. According to Nieschlag (1960), plant fibers with cellulose >34% would be preferable for pulp and paper manufacture. Higher cellulose content of plant material will provide stability and high tensile strength for the paper produced (Agu *et al*. 2012). *Enhalus acoroides* and *Zostera marina* were clustered in Group 4 (Fig. 3) due to similarity in hemicellulose and lignin content. Good mechanical properties of marine eelgrass, *Z. marina* fibers which contain high polysaccharides and pectin but low lignin are comparable to sisal and jute (Davies *et al*. 2007). Other previous study showed that *Z. marina* and green algae, *Cladophora* sp. produce a similar strength as ground wood, however due to its low brightness, these plants are only useful as filler material up to 20% in papermaking (Knoshaug *et al*. 2009; Leopold and Marton 1974).

The hemicellulose content of the seagrass ranged between the lowest (14.33%) in *Thalassia hemprichii* to the highest (28.31%) in *Enhalus acoroides*. This value is within the range of wheat straw (23 to 30), a widely used non-wood plants papermaking in China (Ogunwusi 2014). Higher hemicellulose values result in higher strength of paper (especially tensile, burst, and fold) and the yield of pulp (Biermann 1996); however, it may have a negative effect during pulping, because hemicellulose is the cell wall polymer component having the highest water sorption (Madsen *et al.* 2004).

Table 3. Derived Value of Seagrass and Other Non-wood Plants

Plants/Species	Slenderness ratio	Flexibility coefficient	Runkel's ratio	Luce's shape factor	References
Seagrasses					
1. *Enhalus acoroides*	154.08±12.82[a]	31.79±1.78[ab]	1.60±0.19[a]	0.80±0.02[ab]	Present study
2. *Thalassia hemprichii*	104.89±5.59[b]	30.07±0.58[b]	1.43±0.10[ab]	0.83±0.01[a]	Present study
3. *Cymodocea serrulata*	100.23±7.06[b]	30.44±0.45[b]	1.33±0.05[ab]	0.83±0.01[a]	Present study
4. *Halophila ovalis*	90.38±6.09[b]	35.18±1.55[a]	1.11±0.06[b]	0.77±0.02[b]	Present study
5. *Halophila spinulosa*	98.12±5.78[b]	31.36±0.59[ab]	1.26±0.07[ab]	0.82±0.01[ab]	Present study
Non-wood					
6. *Ageratum conyzoides*	27.97	74.41	0.35	0.27	(Sharma *et al*. 2013)
7. *Biden spilosa*	47.33	69.01	0.46	0.33	(Sharma *et al*. 2013)
8. *Crotolaria pallida*	50.65	63.56	0.6	0.4	(Sharma *et al*. 2013)
9. *Eupatorium odoratum*	42.3	65.81	0.52	0.5	(Sharma *et al*. 2013)
10. *Scorpia dulcis*	34.27	61.89	0.62	0.41	(Sharma *et al*. 2013)
11. *Sida cordifolia*	58.22	61.83	0.69	0.41	(Sharma *et al*. 2013)
12. *Solanum torvum*	24.62	80.3	0.25	0.19	(Sharma *et al*. 2013)
13. *Urena lobata*	71.81	60.78	0.66	0.41	(Sharma *et al*. 2013)
14. *Vernonia cinerea*	47.12	62.36	0.61	0.39	(Sharma *et al*. 2013)
15. *Gmelina arborea*	39.09	76.03	0.32	0.26	(Sharma *et al*. 2013)
16. *Pinus kesiya*	49.04	81.74	0.22	0.19	(Sharma *et al*. 2013)
17. *Cymbopogon citratus*	66.9	31.1	1.45	0.71	(Kaur and Dutt 2013
18. *Cymbopogon martini*	59.2	30	1.52	0.79	(Kaur and Dutt 2013)
19. *Hibiscus cannabinus* (bark)	105.9	54.3	0.7	0.75	(Ververis *et al*. 2004)
20. *Hibiscus cannabinus* (core)	33.3	59.5	0.5	0.72	(Ververis *et al*. 2004)
21. *Hibiscus cannabinus* (whole)	58.3	57.5	0.67	0.73	(Ververis *et al*. 2004)
22. *Arundo donax* (internodes)	70.5	49.2	1	0.78	(Ververis *et al*. 2004)

23. *Arundo donax* (nodes)	60	46	1.3	0.81	(Ververis *et al*. 2004)
24. *Miscanthus giganteus*	68.3	41.5	1.3	0.83	(Ververis *et al*. 2004)
25. *Panicum virgatum*	87.7	44.2	1.5	0.81	(Ververis *et al*. 2004)
26. *Gossypium hirsutum*	42.3	65.3	0.5	0.68	(Ververis *et al*. 2004)
27. *Camellia sinensis*	42.49	33.52	2.0	0.80	(Tutus *et al*. 2015)

Means ± standard error with the same letter designation in a column is not significantly different at $p<0.05$ by Tukey's Test

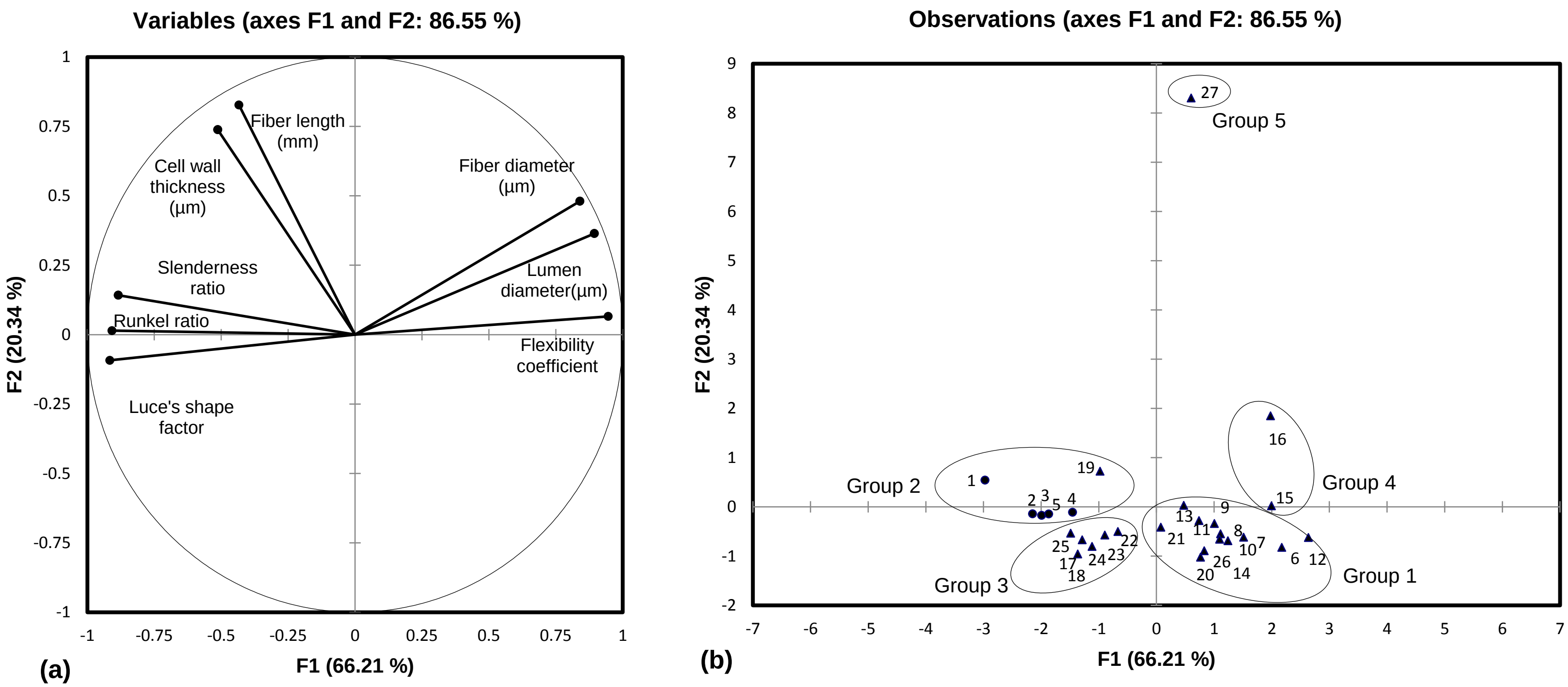

Fig. 2. (a) Plot of fiber characteristics (fiber length, fiber diameter, lumen diameter, cell wall thickness, slenderness ratio, Runkel's ratio, Luce's shape factor, and flexibility coefficient). (b) Position of PCA score of seagrass species (●) and other non-wood plants (▲)

A low content of hemicellulose decreases the capacity of water absorbing (Bakker and Elbersen 2005), thus minimizing the duration of pulping activity. A review by Knoshaug *et al*. (2013) reported that hemicellulose is not typically required and as useful as wet end additives or fillers; thus levels in the range 30 to 50% hemicellulose of the biomass materials would be adequate in papermaking using aquatic species.

Thalassia hemprichii possessed relatively low lignin content (5.02%), while a higher content was observed in *Cymodocea serrulata* (11.20%). The pulp and paper industry would prefer low lignin (<15%) content because the fibers are more porous and flexible and they give a brighter colour during bleaching. The PCA in Fig. 3 showed that the two factors accounted for 92.26% of total variance, with high percentage of F1 (60.67%) compared to F2 (31.59%). Three seagrass species *Cymodocea serrulata*, *Halophila ovalis*, and *Halophila spinulosa* were clustered with reed canary grass (*Phalaris arundinacea*) and banana (*Musa* sp.) pseudo-stem in Group 2 based on their lignin content. As reported in banana pseudo-stem, high cellulose and low lignin content give a high potential in papermaking, boards and composite materials production (Cordeiro *et al*. 2004). *Enhalus acoroides* shared a similar chemical composition with *Zostera marina* (Group 4) which studied to be particularly suitable for use in bio-degradable structures. *Thalassia hemprichii* in the present study was clustered together with *Halophila ovalis* and *Posidonia australis* (Group 3) due to their low cellulose and hemicellulose content.

Posidonia oceanica was clustered in Group 1 with 11 non-wood plants that possessed moderate values of all the variables. *Thalassia testudinum* was clustered in Group 5 as this seagrass was reported to have the highest content of cellulose (86.22%) and hemicellulose (65.13%).

Paper Characteristics and Mechanical Properties

The pulp from long fibers of *Enhalus acoroides* with higher cellulose content produced more paper sheets when compared to other species. The distribution and formation of fiber was hard to observe under the microscope in some of the produced paper sheets due to the thickness. Pressing techniques during the papermaking process also reduced the paper thickness, in which sufficient energy was needed to remove the remaining free water as much as possible in order to obtain fine paper sheets. Sirvio and Nurminen (2004) reported that, as the density is constant, the fiber network thickness is a linear function of network grammage. Thus, these three factors influenced each other, as the thickness increased, paper sheet weight for a given area increased as well. The grammage value >120 g/m^2 was recorded in *E. acoroides*, *T. hemprichii*, and *H. ovalis*, indicating that all these species are within the range of paperboard standard (Table 5). The thinner paper sheet produced from *C. serrulata* and *H. spinulosa* resulted in lower grammage. No specific values of grammage were targeted in this work. Fiber fractionation processes can reduce the grammage variations of the paper in order to increase paper strength (Nazhad *et al*. 2000), cut costs, and achieve savings in raw materials (Sood *et al*. 2005).

Based on Fig. 4, the fiber of blended and hand-beaten can be distinguished based on their fiber network. Fibers strands can be seen on most of the blended paper (Fig. 4d-f), while fiber bundle were detected on hand-beaten paper (Fig. 4a-c). The longer beating process was required in order to improve the fiber bonding ability to shorten and formed fine fibers (Jahan and Rawshan 2009).

Table 4. Chemical Composition of Seagrass and Other Non-wood Plants

Species	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference (s)
Seagrass				
1. *Enhalus acoroides*	77.18 ± 3.65[a]	28.31 ± 0.70[a]	6.46 ± 1.32[bc]	Present study
2. *Thalassia hemprichii*	40.30 ± 1.15[b]	14.33 ± 0.70[c]	5.02 ± 0.08[c]	Present study
3. *Cymodocea serrulata*	47.33 ± 2.83[b]	25.92 ± 0.62[ab]	11.20 ± 0.36[a]	Present study
4. *Halophila ovalis*	53.18 ± 4.58[b]	27.62 ± 0.45[a]	9.05 ± 0.55[ab]	Present study
5. *Halophila spinulosa*	54.77 ± 3.30[b]	22.89 ± 1.58[b]	10.83 ± 0.29[a]	Present study
6. *Thalassia testudinum*	86.22	65.13	-	(Bjorndal 1980)
7. *Halophila ovalis*	33.4	-	0.125	(Baydoun and Brett 1985)
8. *Posidonia australis*	20.2	11.7	14.5	(Torbatinejad and Sabin 2010)
9. *Posidonia oceanica*	40.0	20.8	29.8	(Khiari *et al*. 2010)
10. *Zostera marina*	57	38	5	(Davies *et al*. 2007)
Non-wood				
11. *Cymbopogon citratus*	44.16	29.07	17.39	(Kaur and Dutt 2013)
12. *Cymbopogon martini*	45.55	28.12	17.04	(Kaur and Dutt 2013)
13. *Phalaris arundinacea*	35	35	9	(Hatakka *et al*. 1996)
14. *Festuca arundinacea*	34	29	19	(Janson *et al*. 1996)
15. *Triticum* spp. (Wheat straw)	37	23-30	20	(Paavilainen and Torgilson 1994)
16. *Prosopis alba*	42	22	19.3	(Jiménez *et al*. 2008)
17. *Chamaecytisus* sp.	44	31.7	14.8	(Jiménez *et al*. 2008)
18. *Phragmites* sp.	40	24.4	23.6	(Jiménez *et al*. 2008)
19. *Retama monosperma*	43	29	21.5	(Jiménez *et al*. 2008)
20. *Arundo donax*	29.2	32.1	20.9	(Shatalov *et al*. 2001)
21. *Musa* sp. pseudo-stem	40	25.2	12.7	(Cordeiro *et al*. 2004)
22. *Paulownia fortuna*	37	33.3	22.4	(Jiménez *et al*. 1993; Jiménez *et al*. 2008)
23. *Camellia sinensis*	29.42±0.57	31.39	36.94±0.34	(Tutus *et al.* 2015)

Means ± standard error with the same letter designation in the column is not significantly different at $p<0.05$ by Tukey's Test

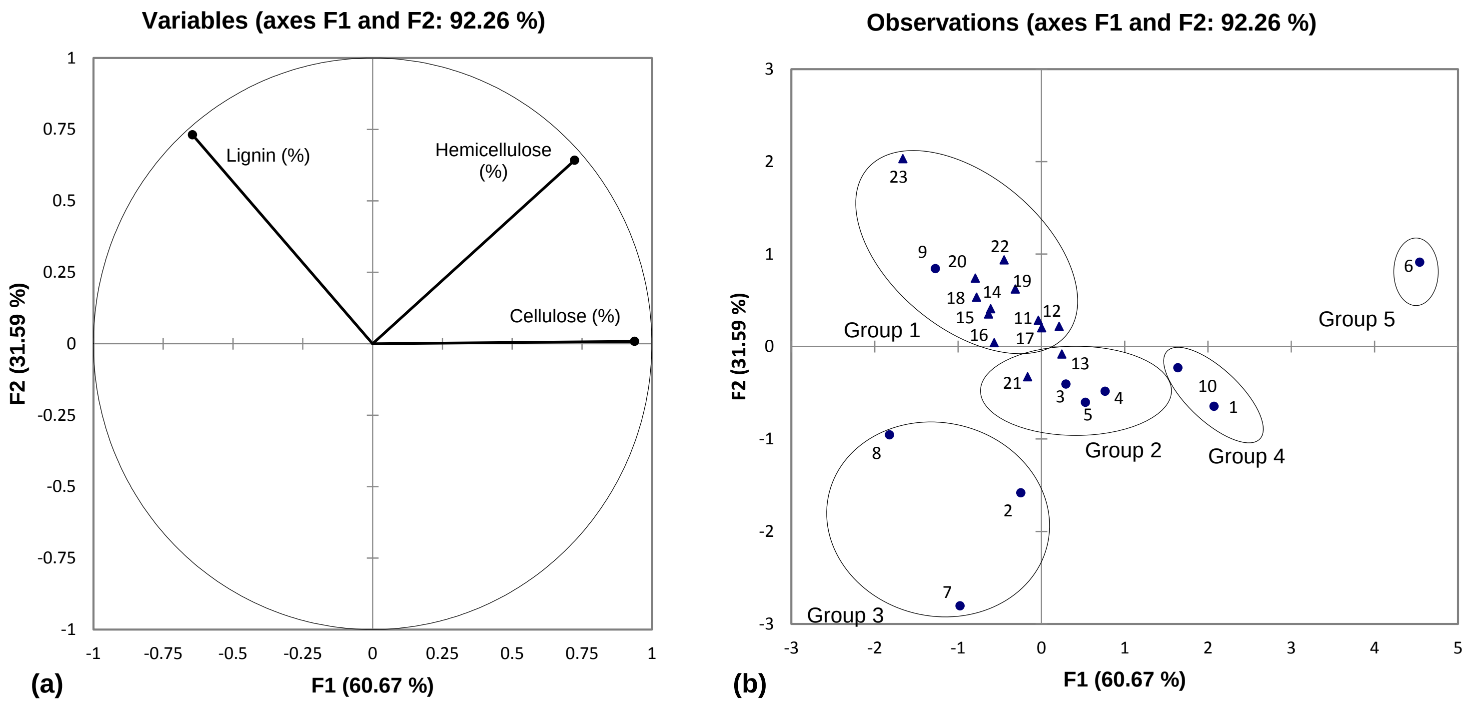

Fig. 3. (a) Plot of chemical composition (cellulose, hemicellulose and lignin). (b) Position of PCA score of seagrass species (●) and other non-wood plants (▲

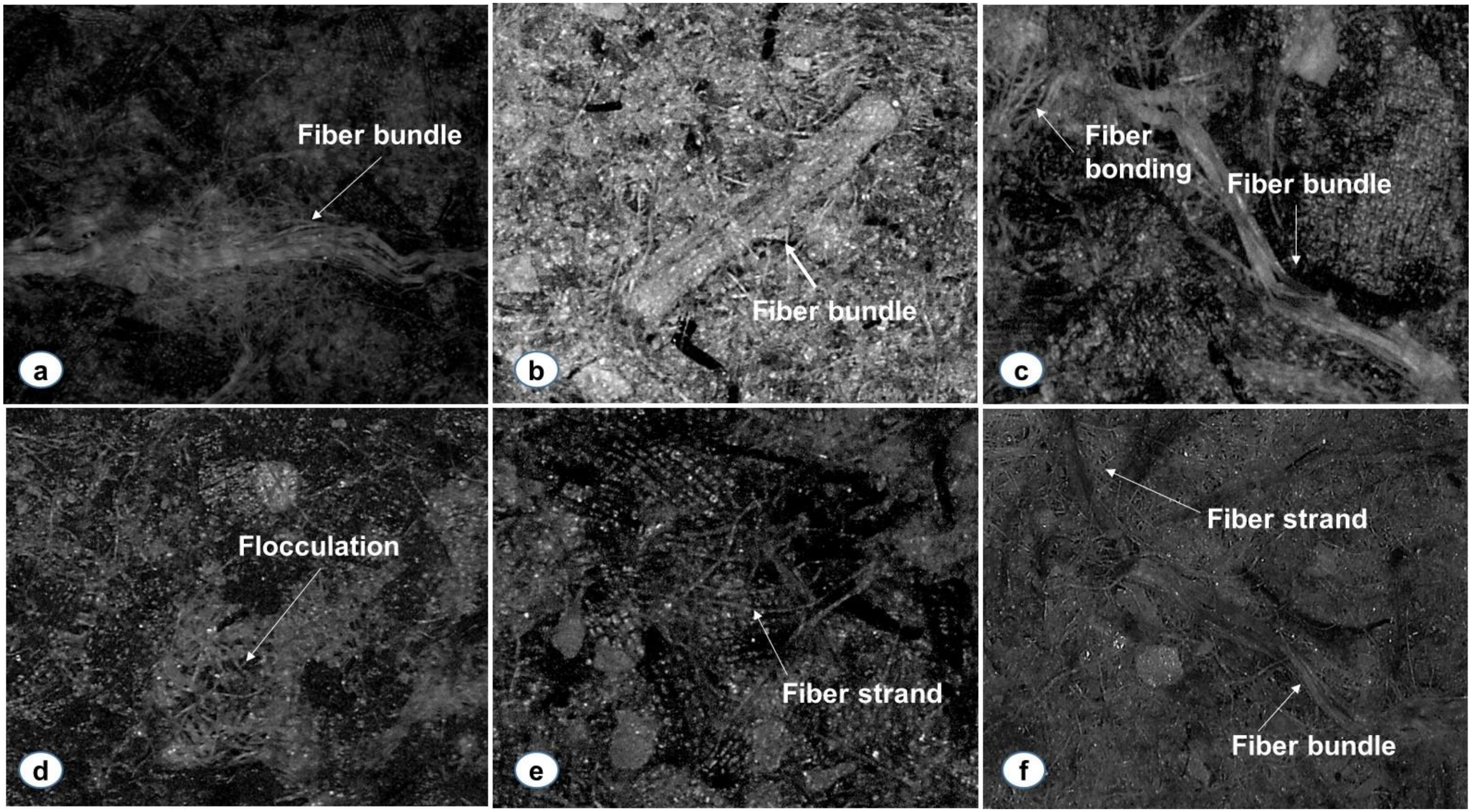

Fig 4. Fiber distribution of the seagrass paper using hand-beaten (a - c) and blended pulp (d - f). (a) *Enhalus acoroides* with 4.0 magnification, (b) *Halophila spinulosa* with 4.0 magnification, (c) *Halophila ovalis* with 3.2 magnification, (d) *Enhalus acoroides* with 3.2 magnification, (e) *Halophila spinulosa* with 3.2 magnification and (f) *Halophila ovalis* with 3.2 magnification

Table 5. Paper Sheet Properties from Seagrass Species

Species	Method	Paper weight per sheet (g)	Area per sheet (cm^2)	Grammage per sheet (g/m^2)
Enhalus acoroides	Japanese	1.28±0.09	75	170.93±37.63
	Western	1.20±0.16	75	160.00±21.15
Thalassia hemprichii	Japanese	1.16±0.63	75	155.14±34.09
	Western	0.99±0.15	75	132.58±35.01
Cymodocea serrulata	Japanese	0.86±0.03	75	114.67±4.00
	Western	0.74±0.01	75	98.67±1.33
Halophila ovalis	Japanese	1.65±0.19	75	219.79±24.71
	Western	1.05±0.30	75	141.09±40.64
Halophila spinulosa	Japanese	0.70±0.02	75	93.33±2.78
	Western	0.56±0.02	75	75.00±2.57

Studies showed that good fiber distributions are difficult to obtain using a hand-beating process, which requires a specific technique practiced by traditional Japanese papermakers (Barrett 1983). This explained why paper produced using blended pulp tended to have a fine fiber resulting in smoother surface (Fig. 4e) compared to the hand-beaten pulp. Flocculation was also detected, meaning that there was a non-uniform fiber distribution at some areas of the paper (Fig. 4f). Flocculation can be reduced by using shortened fibers, but this will affect the paper strength, because shorter fiber length will decrease tensile strength (Biermann 1996).

Typical stress-strain curves of paper sheets of different seagrass species by using hand-beaten and blended pulp are shown in Figs. 5 and 6, respectively. For the blended pulp paper, the highest tensile stress was 283.8 MPa from *Enhalus acoroides* with a strain of 0.35, where the fibers can support heavy load before being ruptured compared to the others seagrass fibers. *Halophila spinulosa* had the lowest tensile stress of 67.43 MPa and managed to hold a tensile strain of 0.02 when compared to *Halophila ovalis* (0.01).

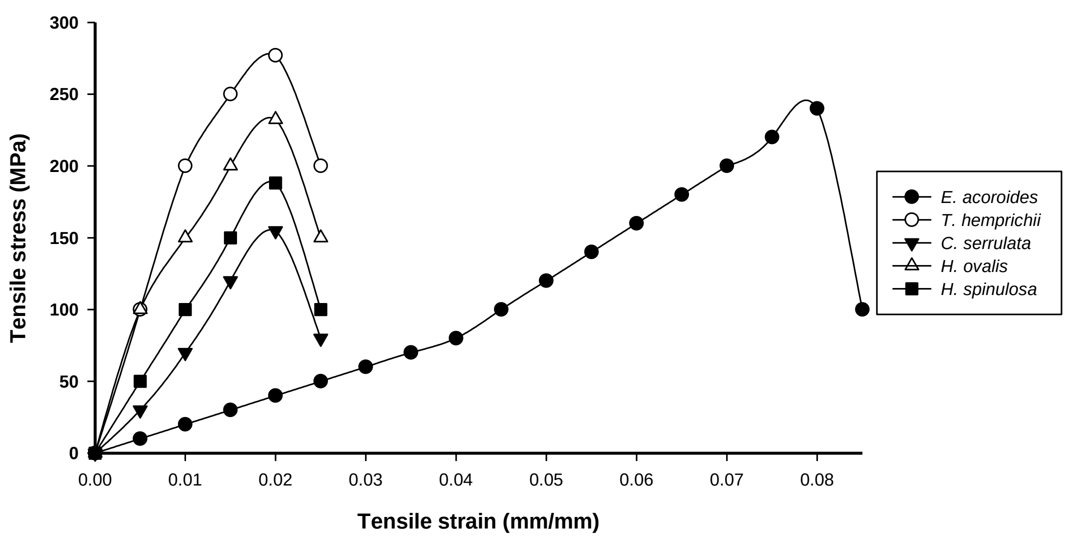

Fig. 5. Stress-strain curve of paper using hand-beaten pulp

For the hand-beaten paper, *Thalassia hemprichii* depicted the highest tensile stress of 277 MPa, and tensile strain of 0.02 followed by *Enhalus acoroides*, 240 MPa with tensile strain of 0.08. The stress increased with the strain until it attained its maximum value before the paper starts to tear apart. All four species except *Enhalus acoroides* have similar elongation before ruptured.

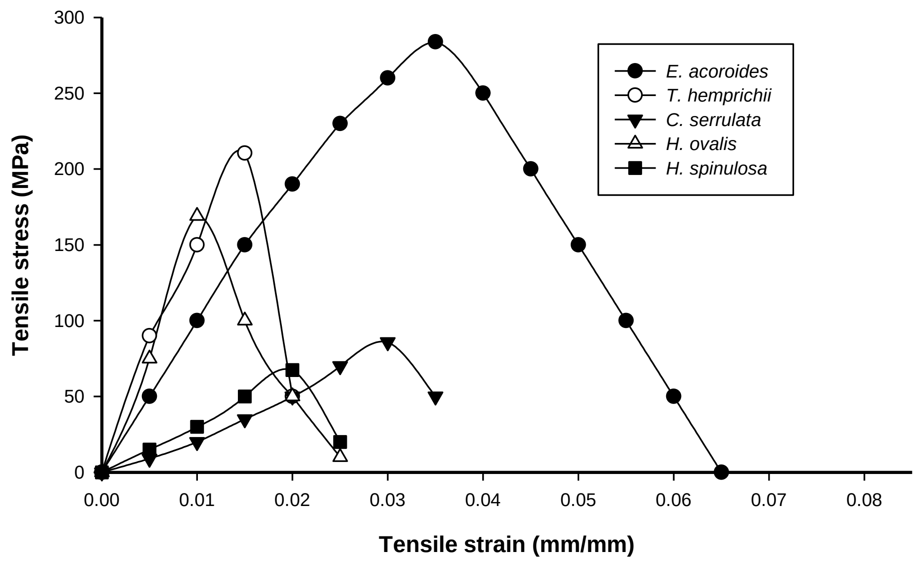

Fig. 6. Stress-strain curve of paper using blended pulp

Young's modulus or modulus of elasticity is a measure of stiffness when the fibers are stretched or compressed. Hand-beaten paper of *Enhalus acoroides* possessed the highest elasticity (502.9 MPa) compared to the paper made by blended method (Fig. 7).

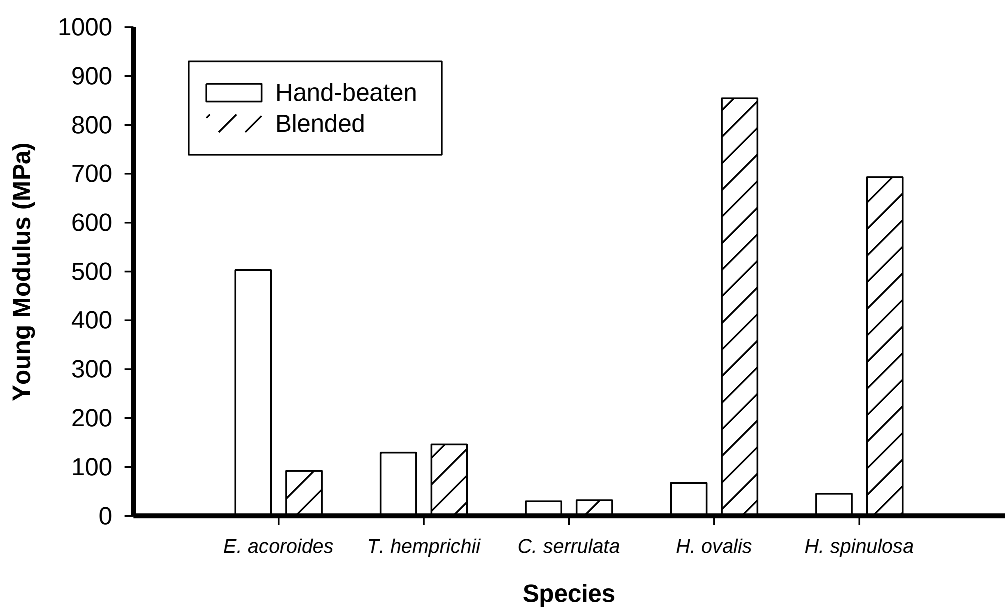

Fig 7. Young's modulus for paper of different seagrass species using hand-beaten and blended pulp

The blended pulp of *Halophila ovalis* produced the highest modulus elasticity paper of 854.24 MPa due to its high fiber diameter, followed by *Halophila spinulosa* with 692.93 MPa. Thick wall fiber is more advantageous in tearing strength as it is stronger, flexible, and does not flatten during sheet forming compared to thin wall fiber. The higher fiber stiffness will cause the fiber to be in contact with their neighbors in the thickness direction instead of bending (Alava and Niskanen 2006).

An independent samples t-test was conducted to compare the mechanical effect of paper between hand-beaten and blended pulp. The highest tensile strength was blended pulp of *Enhalus acoroides* (4.10 kN/m, Table 6), while hand-beaten pulp of *Thalassia hemprichii* had the highest breaking length (3.43 km, Table 7). The tensile strength, fracture toughness, and breaking strain of paper were influenced by fiber length (Wathen 2006). As the fiber length increased together with high hemicellulose (Spiegelberg 1966), the tensile strength also increased which explained highest tensile strength in *Enhalus acoroides*. Biermann (1996) stated that typical paper has a breaking length in the range of 2.5 to 12 km, and it can be increased by the addition of a certain amount of starch (Shen *et al*. 2012) during the pulping process. A previous study on red algae, *Gelidium corneum* (2.29 kN/m, 3.84 km) and *Gelidium amansii* (1.86 kN/m, 3.01 km) indicated comparable tensile strength and breaking length value, respectively, with seagrass species of this present study. Thus, both materials may be suitable for many types of paper such as printing paper, filter paper, and edible paper (Seo *et al*. 2010).

Based on Table 5, there were significant differences when comparing tensile strength in hand-beaten pulp (M= 2.24, SD= 0.23) and blended pulp (M= 1.24, SD= 0.7) of *Cymodocea serrulata*, t(8)= 3.04, p= 0.016. A highly significant difference was detected in hand-beaten pulp (M= 2.22, SD= 0.5) and blended pulp (M= 0.802, SD= 0.414) of *Halophila spinulosa*, t(8)= 4.901, p= 0.001. High beating of pulp can improve the mechanical strength of paper (Seo *et al*. 2010). For breaking length (Table 6), there was a highly significant difference in beaten pulp (M= 2.43, SD= 0.22) and blended pulp (M= 1.09, SD= 0.54) of *Halophila spinulosa*, t(8)= 4.068, p= 0.004. Blending only cut the fiber into small fragments, while beating caused changes in fiber simultaneously including fibrillation, fiber shortening, or cutting and fines formation that improved the fiber's bonding ability (Kang and Paulapuro 2006).

Table 6. Tensile Strength of Paper Strips of the Seagrass Species Using Two Different Methods

Species	Tensile strength (kN/m)					
	Mean ± s.e		T-test			
	Beating pulp	Blended pulp	Mean dif.	t	df	f
E. acoroides	3.46±0.40^{ab}	4.10±0.60^{a}	0.64	0.89	8	0.399
T. hemprichii	4.00±0.43^{ab}	3.24±0.73^{abc}	0.76	0.898	8	0.395
C. serrulata	1.24±0.70^{bcd}	2.24±0.10^{cd}	-1.00	-3.04	8	0.030*
H. ovalis	3.36±0.19^{ab}	2.44±0.36^{abcd}	0.92	2.257	8	0.054
H. spinulosa	2.72±0.57^{bcd}	0.80±0.18^{d}	1.92	3.225	8	0.012*

Mean ± standard error in a column with different superscript (a>b>c>d) are significantly different (p<0.05 Tukey multiple range test) * Significant at level p<0.05 for t-test

Table 7. Breaking Length of Paper Strips of the Seagrass Species Using Two Different Methods

Species	Breaking length (km)					
	Mean ± s.e		T-test			
	Beating pulp	Blended pulp	Mean dif.	t	df	f
E. acoroides	2.49±0.23^{bc}	2.12±0.51^{bc}	-0.37	-0.651	8	0.533
T. hemprichii	3.43±0.25^{ab}	2.64±0.58^{bc}	0.79	1.254	8	0.245
C. serrulata	2.24±0.21^{bc}	1.11±0.24^{c}	-1.3	-4.34	8	0.007*
H. ovalis	1.83±0.41^{bc}	2.07±0.41^{bc}	-0.24	-0.409	8	0.693
H. spinulosa	2.43±0.22^{bc}	1.09±0.24^{c}	1.34	4.068	8	0.004*

Mean ± standard error in a column with different superscript (a>b>c>d) are significantly different (p<0.05 Tukey multiple range test) * Significant at level p<0.05 for t-test

CONCLUSIONS

1. This study showed that seagrass plant species' fibers can be considered as raw materials in handmade papermaking production as they possessed moderate long (*e.g.*, *Enhalus acoroides*) to medium-sized fibers (*T. hemprichii*, *C. serrulata*, *H. ovalis* and *H. spinulosa*), good slenderness ratio (>33), great value of Luce's shape factor, higher cellulose (>35%), and less than 15% lignin content.
2. The derived low flexibility coefficient and Runkel's ratio resulted in rigid fibers, where seagrasses have more potential for paperboard and paper plate production.
3. Of the seagrass species assessed, *Enhalus acoroides* is the most preferable seagrass for papermaking, as it possesses the longest fiber and highest cellulose content compared to other species.
4. The low lignin content of the seagrass gives an advantage in paper strength and also in reducing the bleaching cost.
5. Fiber strands produced by beating and blending required longer duration in order to obtain better fiber formation and distribution in produced paper sheets.
6. It is suggested that to obtain the uniform paper sheet thickness, the grammage value must be fixed prior to paper production.
7. Tensile strength and breaking length of the produced paper showed that beating method resulted in more flexible fiber compared to the blending method hence increasing the bonding strength.

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