Journla of Molecular Pharmaceutics & Organic Process Research

Abe et al., J Mol Pharm Org Process Res 2014, 2:3
DOI: 10.4172/2329-9053.1000118

Research Article Open Access

Decortication of *Eichhornia Crassipes* (Pontederiaceae) Fibers for the Production of Multifilament Non-Absorbable Surgical Suture

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Abstract

Exhibiting fibrous and antimicrobial characteristics, *Eichhornia crassipes* (water hyacinth) presents an edge to be used in medicine. The study aims to provide a new source of multifilament surgical sutures from decorticated petiole fibers of water hyacinth. The fibers were braided and autoclaved for sterilization. After which, diameter measurement, sterility test, and heavy metal test were done. Tensile strength, knot strength of the sutures and wound tensile strength were measured using a tension meter. Surgical incisions done under anesthesia on dorsolateral areas of six male albino rabbits (*Oryctolagus cuniculus*) were sutured. Silk suture was used as the positive control. Observation was done for 7 days to grade skin reactions. Skin samples were then excised after euthanasia was done. The samples were then subjected to histopathological examination. Statistical analyses comparing the sutures were done using independent t-tests. Differences in diameter and tensile strength of both sutures were statistically significant ($p<0.05$). Differences in knot tensile strength and wound tensile strength of both sutures were not statistically significant ($p>0.05$). Edema, erythema and wicking were absent in both sutures. Dehiscence was seen in 2 wounds with silk suture; 1 wound with water hyacinth suture. Histopathology showed varied degrees of tissue reaction. However, presence of non-representative samples made the test inconclusive. Conclusively, sutures from *Eichhornia crassipes* are physically comparable to silk.

Keywords: *Eichhornia crassipes;* Fibers; Decortication

Background of the Study

Water hyacinth (*Eichhornia crassipes)* is one of the worst aquatic weeds in the Philippines [1]. Its rapid infestation is due to its ability to double its number in as fast as 12 days. It has a detrimental impact on agriculture, economy, environment, recreational activities, water quality and quantity, and human health.

Despite it being one of the world's most destructive weed, it exhibits antibacterial, antifungal and anti-algal properties [2]. Crude extracts of the plant showed growth inhibition of Gram-positive bacteria, Gram-negative bacteria, yeast, green microalgae, and cyanobacteria. Moreover, the Philippine Research Textile Institute (PTRI) is developing its use in domestic textile to trim the industry's import of synthetic-based fibers [3]. The plant's fibers are used as raw materials for the production of handicrafts, furniture's, and tableware's.

Due to its relatively strong and flexible fibers, this study aims to produce a natural multifilament non-absorbable surgical thread out of water hyacinth.

Methodology

Materials and equipment: *Eichhornia crassipes* with 20-inch petioles were collected. After formal plant identification, the leaf blades and roots of the plants were removed, leaving the petioles behind for the decorticating process via a decorticating machine. After decortication, the fibers were then air-dried and manually braided to produce the suture. Samples were categorized according to quantitative composition: 15 strands of fiber and 30 strands of fiber; according to relative size: Size 1 Silk Suture, Size 4-0 Silk Suture.

The materials: 15-strand and 30-strand braided water hyacinth threads, silk sizes 1 and 4-0, needle holder, surgical needle, thumb forceps, mayo scissors and iris scissors, were packed in aluminum foil and sterilized using an autoclave (121°C at 15 psi). On the other hand, wooden clamps, metal hooks, and the weight receptacle composed the improvised tension meter.

Six adult male albino rabbits (*Oryctolagus cuniculus*), each weighing approximately 2 kg, were utilized in the experiment. The animals were kept in a room at 23-27°C with 75% relative humidity. Ventilation rate was maintained at 10-20 air changes/hr by mechanical means. The fluorescent lighting provided followed a diurnal cycle. An acclimatization period of at least 5 days was allotted.

With respect to the animal welfare policies, evaluation of the scientific use, handling and care of the adult male albino rabbits was conducted by the Institutional Animal Care and Use Committee of the University of Santo Tomas. All experimental methods, reagents, and equipment to be used on the rabbits were assessed by the committee before proceeding with *in-vivo* scientific tests.

Physical properties of the water hyacinth threads

Diameter: The number of fibers of each braided suture was estimated to match the diameter of the silk sutures. A microscope with an ocular micrometer was used to measure the diameter of the braided plant fibers.

Tensile strength test: Six inches of both the water hyacinth sutures and silk sutures were tested using the improvised tension meter. Three trials were done for each sample to determine the mean tensile strength.

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Received August 06, 2014; **Accepted** August 27, 2014; **Published** August 30, 2014

Citation: Abe MDJA, Cariño CF, Gabrito CJN, Gironella RN, Sadhwani AJV, et al. (2014) Decortication of *Eichhornia Crassipes* (Pontederiaceae) Fibers for the Production of Multifilament Non-Absorbable Surgical Suture. J Mol Pharm Org Process Res 2: 118. doi: 10.4172/2329-9053.1000118

Knot tensile strength test: Six inches of both the water hyacinth sutures and silk sutures were tested using the improvised tension meter. Samples contained four throws of square knot in the middle to determine knot tensile strength. Re-tests were done when suture breakage did not occur beside the square knot. Three trials were done for each sample to determine mean knot tensile strength

Heavy metal content determination: Atomic absorption spectrophotometry was used to determine the presence of heavy metals. The test portions were dried and then incinerated and turned to ash at 450°C under a gradual increase (<50°C/hr) in temperature. Then, 6 M hydrochloric acid (HCl) was added and the solution was evaporated to dryness. It was digested completely until dissolved using 0.1 M nitric acid (HNO_3) and filtered out. Distilled water was added. After which, atomic absorption spectrophotometer was used to analyze the analyte (AOAC Official Methods of Analysis).

Pharmacological properties of the water hyacinth threads

Sterility test (Direct transfer method): The autoclaved water hyacinth suture samples were tested for their sterility by direct transfer method (USP 30/NF 25 2007). Threads measured 30 cm each. Eight sutures of the 15-strand and eight sutures of the 30-strand were required to complete one run of the test.

Test for aerobic and anaerobic bacteria: The sample sutures were transferred directly into the fluid thioglycollate broth. Other tubes of fluid thioglycollate broth inoculated separately with *Staphylococcus aureus* and *Pseudomonas aeruginosa* served as the positive controls. A tube of non inoculated fluid thioglycollate broth served as the negative control. All tubes were incubated at a temperature of 37°C then observed for turbidity for 14 days (USP 30/NF 25 2007).

Test for fungi: The sample sutures were transferred directly into the Trypticase soy broth. Another tube of Trypticase soy broth inoculated with *Candida albicans* served as the positive control. A tube of non-inoculated Trypticase soy broth served as the negative control. All tubes were incubated at 37°C and observed for turbidity for 14 days (USP 30/NF 25 2007).

Growth promotion test for aerobes

The sample sutures were directly transferred into four tubes of fluid thioglycollate broth. Two tubes were inoculated with *S. aureus* (<100 cfu). The remaining two tubes were incoluated with *P. aeruginosa*. Another two tubes of fluid thioglycollate broth inoculated separately with *S. aureus* and *P. aeruginosa* served as the positive controls. A tube of non-inoculated fluid thioglycollate broth served as the negative control. The tubes were incubated for not more than 3 days (USP 30/NF 25 2007).

Growth promotion test for fungi

The sample sutures were directly transferred into two test tubes of Trypticase soy broth. These were inoculated with *C. albicans* (<100 cfu). Another test tube of Trypticase soy broth was inoculated with *C. albicans* and served as the positive control. A tube of non-inoculated Trypticase soy broth served as the negative control. The tubes were incubated for not more than 5 days.

Surgery and skin irritation test

Prior to surgery, the rabbits were divided into two groups. Skin integrity was checked. The fur was shaved from the dorsal area of the trunk to expose the skin. Caution was observed to avoid abrasion. Before incision, 15 mg/kg ketamine 2 mg/kg midazolam was administered intramuscularly to the test animals. After onset of the drug through physical inspection, the skin on the dorsal area of each rabbit was incised twice, each having a 6 cm incision parallel to each other. Group A of the test animals was sutured with 15 strand water hyacinth suture and silk 4-0. On the other hand, Group B was sutured with 30 strand water hyacinth suture and silk 1. A triangular-tipped surgical needle was used to employ a simple interrupted pattern of stitching having a 4 mm suturing interval. Incision wound was treated with povidone-iodine solution and secured with sterile gauze. Daily inspection with gauze replacement and wound cleaning was done for two weeks.

Inspection included grading of erythema and edema, detection of pus discharge and wound dehiscence [4]. After 7 days, euthanasia by cranial concussion was done to harvest the pelt and subject it to wound tensile strength measurement and histopathology. (Tables 1 and 2)

Euthanasia

Cranial concussion (Stunning): The rabbit was suspended by its hind legs, grasping around both hocks with the left hand. A cudgel was used to deliver a single, heavy, sharp blow to the back of the skull. Cessation of respiration, heartbeat, and reflexes were noted to indicate irreversible death.

Removal of the suture: The suture was cut under the knot, close to the skin surface. When pulled from the wound, the previously exposed and contaminated portion of the suture does not travel back through the wound.

Wound tensile strength test: Twelve square centimeters of excised skin length containing the sutured incision was subjected to wound tensile strength test. Tensile strength was determined when the first sign of wound opening was seen. Weight of sand used in the procedure was measured using weighing balances with 1 g and 5 g. calibration marks.

Histopathology

Preparation of specimens for tissue processing: Out of the 6-cm incision made on each rabbit, 2 cm were taken for tissue processing. After cutting the pelt into its appropriate size, it was further divided into 2-mm strips then immersed into 10% neutral buffered formalin solution. The specimens were then submitted to a diagnostics laboratory for tissue processing.

Histopathologic analysis: After tissue processing, the specimens which were mounted onto microscope slides were then submitted to a histopathologist for examination of antigenicity, extent of wound healing and the formation of granulation tissue were included.

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness)	4
to eschar formation	

Table 1: Criteria for Erythema and Eschar Formation (OECD Guidelines 404,[4]).

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

Table 2: Criteria for Edema Formation (OECD Guidelines 404, [4]).

Results and Discussion

Physical properties of the water hyacinth threads diameter

Diameter of the 15-strand water hyacinth suture and silk size 4-0 are considered statistically different (t-value=0.000). Also, diameter of 30-strand water hyacinth suture and silk size 1 are considered statistically different (t-value=0.000). Diameter means, standard deviations, and t-values are shown in Table 3.

Tensile strength test

Tensile strength of the 15-strand water hyacinth suture and silk size 4-0 are considered statistically different (t-value=0.002). Also, tensile strength of 30 strand water hyacinth suture and silk size 1 are considered statistically different (t- value=0.004). Tensile strength means, standard deviations, and t-values are shown in Table 4.

Knot tensile strength

Knot tensile strength of the 15-strand water hyacinth suture and silk size 4-0 are considered not statistically different (t-value=0.324). Also, knot tensile strength of 30-strand water hyacinth suture and silk size 1 are considered not statistically different (t-value=0.065). The knot tensile strength means, standard deviations, and t-values are shown in Table 5.

Pharmacologic properties of water hyacinth fibers sterility test

As shown in Table 6, both samples are positive on the growth promotion for aerobes and fungi. On the other hand, one 30-strand water hyacinth suture is positive on the test for the presence of fungi.

Wound tensile strength

Wound tensile strength of the 15-strand water hyacinth sutured wound and silk size 4-0 sutured wound are considered not statistically different (t-value=0.095). Also, wound tensile strength of 30 -strand water hyacinth sutured wound and silk size 1 sutured wound are considered not statistically different (t-value=0.647). The means, standard deviations, and t-values are displayed in Table 7.

Skin irritation and drag test: Skin irritation was not observed in all rabbits after surgery. During surgery, it was noted that the 15-strand water hyacinth thread had moderate drag compared to the silk sutures having no drag. The 30-strand water hyacinth thread had the most drag but yielded a better wound closure compared to the silk control. Strand breakage for all threads was not observed along the course of surgery procedure (Table 8).

Presence of wicking and wound dehiscence

Wound exudates from both threads were absent. Hence, wicking did not occur. Wound dehiscence was noted on two incisions sutured with Silk 4-0 and one incision with 15-strand (Table 9).

Histopathology

Examination revealed that wounds sutured with 15-strand water hyacinth underwent moderate to severe inflammation while wounds sutured with silk 4-0 had shown mild to moderate inflammation.

	Mean (μm)	Standard Deviation (SD)	t-value
15-Strand	839.9460	66.25177	0.000
Silk 4-0	150.0000	0.00000	
30-Strand	1269.8830	77.67583	0.000
Silk 1	400.0000	0.00000	

Table 3: Diameter Measurement Means, Standard Deviations, and t-value.

	Mean	SD	t-value
15-Strand	9.241400	1.1126777	0.002
Silk 4-0	14.687000	0.8010749	
30-Strand	21.383600	0.6640178	0.004
Silk 1	28.224000	1.8738967	

Table 4: Tensile Strength Means, Standard Deviations, and t-value.

	Mean	SD	t-value
15 Strand	10.107067	2.3433342	0.324
Silk 4-0	11.658733	0.4855040	
30 Strand	21.223533	2.9092028	0.065
Silk 1	16.970333	0.1337739	

Table 5: Knot Tensile Strength Means, Standard Deviations, and t-value.

Samples	Test for Aerobes/ Anaerobes	Test for Fungi	Growth Promotion for Aerobes	Growth Promotion for Fungi
30-strand	Negative	Positive	Positive	Positive
15-strand	Negative	Negative	Positive	Positive

Table 6: Sterility Test Results of Autoclaved Water Hyacinth Sutures.

	Mean	SD	t-value
15-Strand	0.167067	0.0765194	0.095
Silk 4-0	0.286900	0.0566707	
30-Strand	0.208067	0.0889500	0.647
Silk 1	0.235500	0.0364000	

Table 7: Wound Tensile Strength Means, Standard Deviations, and t-value.

Rabbit No.	Surgical Thread	Erythema	Edema
1	15-strand	0	0
	Silk 4-0	0	0
2	15-strand	0	0
	Silk 4-0	0	0
3	15-strand	0	0
	Silk 4-0	0	0
4	30-strand	0	0
	Silk 1	0	0
5	30-strand	0	0
	Silk 1	0	0
6	30-strand	0	0
	Silk 1	0	0

Table 8: Scoring of Erythema and Edema for Six Male Albino Rabbits.

Rabbit No.	Surgical Thread	Wound Dehiscence
1	15-strand	-
	Silk 4-0	+
2	15-strand	+
	Silk 4-0	+
3	15-strand	-
	Silk 4-0	-
4	30-strand	-
	Silk 1	-
5	30-strand	-
	Silk 1	-
6	30-strand	-
	Silk 1	-

Table 9: Rabbits that exhibited with wound dehiscence.

Examination on wounds sutured with 30-strand water hyacinth and silk 1 were inconclusive.

One wound sutured with 15-strand water hyacinth and all three wounds sutured with silk 4-0 exhibited neovascularization. However, neovascularization was present on one wound sutured with 30-strand and another wound sutured with silk 1.

Excessive formation of fibrous connective tissue were observed on one wound sutured with 15-strand water hyacinth, three wounds with silk 4-0, and one wound with silk 1.

Conclusion

Diameter and tensile strength data showed that the differences of the means of water hyacinth sutures and silk sutures were statistically significant ($t<0.05$). However, knot tensile strength and wound tensile strength data showed that the differences of the means of water hyacinth sutures and silk sutures were not statistically significant ($t>0.05$). Also, cadmium, silver, mercury, lead, and copper were considered undetected in plant fiber. This passes the water hyacinth suture in the physical aspect of the study. Edema, erythema, and wicking were absent on both silk and water hyacinth sutures. Wound dehiscence was seen in 3 sutured wounds; 2 of which were with silk suture and the latter with the water hyacinth fiber suture. Conclusively, the decorticated fibers from *E. crassipes* can be utilized to produce multifilament non-absorbable sutures with physical properties comparable to silk.

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