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Impact of Different Pre-treatments of *Agave sisalana* Leaves on Yield and Anatomical Traits of Fibre

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Abstract: ***The study presented was aimed to investigate the effect of different pre-treatments of Agave sisalana leaves on yield, physicochemical and anatomical characteristics of fibre. A. sisalana leaves were treated with acid, alkali and biomaterial. Fibres obtained through different pre-treatment conditions were examined for their physicochemical and anatomical characteristics. Results indicated a notable influence of the three treatments on yield and physical properties of fibres. Total yield (%) and number of fibre per leave was recorded from the biomaterial treated leaves followed by alkali treated leaves. Physical properties including fibre length and fibre diameter were also maximum for the fibres obtained from biomaterial treated leaves. Results of solubility and other chemical examination of fibres from all the treatments were same. Microscopic study of fibre indicated their cellular structure and needle shape with sharp apex and lumen. The study thus established the biomaterial pretreatment as an ecofrienly, efficient and productive way for extraction of sisal fibre.***

Keywords: *Agave sisalana*, Pre-treatment, Fibre extraction, Physicochemical Properties

1. Introduction

Growing environmental concerns and astringent legislative regulations have led manufacturers to develop new materials from renewable resources such as natural fibers. Natural fibers have gained considerable attention and have successfully proven their qualities when also taking into account an ecological view. The introduction of natural fibers in polymer matrix as a reinforcement material can provide significant advantages compared to synthetic fibers **[1]**. Natural fibers have many advantages over the synthetic fiber such as wide availability, renewability, biodegradability, low density, ecofriendliness and low cost which leads to the considerable attention as an alternative to synthetic fiber **[2-8]**. Studies on these natural materials are continually increasing and their development has become an important issue. Sisal fibre is one such natural material that has lately recorded a rapid increase in demand. An increase in demand invariably leads to improved commodity prices, therefore making Sisal fibre production a very profitable venture. However, variability in yield and quality of fiber is an important issue that deserves scientific intervention. In fact, the botanical origin and maturity of source plant and method of extraction involve variations in production and structural properties of the fibers **[9, 10]**.

Agave sisalana Perrine ex Engelm (Agavaceae), commonly known as Sisal is an herbaceous monocotyledonous plant is one of the major source of natural fibre apart from jute. In addition, the plant is highly praised for diverse bioactive chemical constituents and medicinal property **[11]**. The genus Agave represents an important group of plant with immense socioeconomic value. Agave is largely cultivated for its fiber in various Asian countries. As per estimate, Sisal constitutes about 70% of world's supply of hard natural fibers **[12-14].** Fibre obtained from leaves of the plant is very strong and is well recognized for its varied industrial and commercial significance. Conventionally Sisal fibre has been extracted by decortications process, where leaves are crushed and beaten by a rotating wheel set with blunt knives. Water is used to wash away the waste parts of the leaf followed by drying, brushing and balding. The mechanical process of fibre extraction leads to low fibre yield and poor quality owing to deformation on the fibre surface as well as it is expensive and poor villagers could not by the machine. Fibre extraction by water and chemical retting of sisal leaves and subsequent washing and cleaning is much time consuming, water intensive, unhygienic and eco-unfriendly. In view of the fact that the properties of plant fibres depend largely on the extraction method used, facilitating fibre separation from pith through pretreatment of Sisal leaves was thought to be an efficient and feasible way for extraction of good quality fibre. The present work was aimed to study the impact of pretreatment of Sisal leaves with chemical and biological materials on yield and quality of fibre in terms of certain physicochemical and anatomical characteristics.

2. Material and Method

2.1 Plant material

Leaves of *Agave sisalana* (Figure: 1) were collected from Chakarata Tehsil which is situated at an elevation of about 7000-7250 feet amid the Tons and Yamuna Rivers adjoining the state capital of Uttarakhand state, India (Figure:1a). The collected plant material was identified by Systemic Botany Division, FRI, Dehradun.

Figure 1: *Agave sisalana* leaf sheath **Figure 1a:** District map and satellite image of the plant collection site

2.2 Chemicals

Sodium hydroxide pellets of 98% strength used for alkali pre-treatment and sulphuric acid with 99% strength for acid pre-treatments were of laboratory grade (Sd finechem). Chemicals were diluted to the stipulated concentrations. Cow dung collected locally was used for biological pre-treatment.

2.3 Fiber extraction

For chemical pre-treatment, 1 kg of sisal leaves of uniform maturity and size were separately drenched in 2% solution of sodium hydroxide and sulphuric acid for 10 days in large vessel. Biological pretreatment was done by soaking 1 kg of leaves in solution of biological material (BM) i.e. cowdung-water mixture (1:3) for 15 days. After chemical and biological treatment all the leaves were taken out from the treatment solutions and rinsed with water several times and then therefore fibre was extracted by mild hand rubbing and further cleaned with water. Bundles of fibers so obtained were dried to the ambient air for 48 h. After drying, the bonded fibers were subjected to brushing and combing foe separation of individual fibre. All treatment experiments were replicated in triplicate.

2.4 Determination of Yield

Total weight of dried fibres obtained from each of the three treatments was taken and percentage yield was calculated with the help of the formula **[15]**:
Yield (%) =Weight of the fibre X 100 / weight of the plant material
Number of fibre separated from each of the leaves was counted separately and average count was calculated for each of the three treatments.

2.5 Determination of Solubility & Chemical traits

Solubility of fibre samples derived out the three treatments were examined in 5% KOH, Conc. HCl, Conc. H_2SO_4 and 10% Phenol. Fiber samples were subjected to ignition test, Molisch's test and Fluoroglucinol+HCl test to find out the chemical framework of the fibres **[16].**

2.6 Fiber anatomy

Standard laboratory methods were followed for the determination of fiber length, fiber diameter and wall thickness. The whole fiber length was measured in 25 randomly sampled fibers from each of the three treatments with the help of a measurement tape. The fiber samples were fragmented into small pieces and taken in test tubes. The fiber material was then macerated with 50% HNO_3 and a pinch of $KClO_3$. The macerated fiber elements were thoroughly mixed and spread on a glass slide and the observations were recorded with the compound microscope. Twenty five unbroken cells were sampled for the measurement of each parameter **[17, 18]**. The fiber samples derived from all the three treatments were observed under compound microscope (Leitz Wetzlar Research Microscope equipped with Nikon DS-Fi1 Camera) for the structural studies.

3. Results and Discussion

3.1. Fiber yield

The results of fibre yield are presented in Table 1. Results clearly indicated a remarkable influence of the treatments on yield of fibres.

Table1: Yield of Fibre from Sisal Leaves under different extraction treatments

Treatments	*Fiber yield (%)*	*No. of fiber/leaf*
Alkali treated	4.42±0.14	833±64
Acid treated	2.75±0.36	730±57
Biomaterial Treated	4.65±0.45	935±69

The highest total yield (%) of fibre and number of fibre per leaf obtained from biomaterial treated leaves was found to be 4.65±0.45 and 935±69 respectively. Yield values in case of alkali treated leaves were almost at par with biomaterial treated leaves; however these values were significantly low for acid treated leaves.

3.2 Solubility and general chemical determination of type of fibre

Solubility of fibres obtained from all the three treatments were found soluble in conc. H_2SO_4 and insoluble in KOH (5%), conc. HCl and Phenol (10%) (Table 2).

Table 2: Solubility Analysis of the Sisal fiber

Solubility	**Alkali treated**	**Acid treated**	**BM treated**
KOH (5%)	Insoluble	Insoluble	Insoluble
HCl (conc.)	Insoluble	Insoluble	Insoluble
H_2SO_4 (conc)	Soluble	Soluble	Soluble
Phenol (10%)	Insoluble	Insoluble	Insoluble

Response of all fibre samples obtained from leaves under the three treatments towards ignition test, Molisch's test and Fluoroglucinol+HCl test was same (Table 3).

Table 3: Chemical characterization of Fibres

Treatment	*Test*	*Observation*	*Inference*
Alkali treated	Ignition test	Rapid burning with flame, no foul odour, white ash	Carbohydrate fiber
Acid treated	Ignition test	Rapid burning with flame, no foul odour, white ash	Carbohydrate fiber
BM Treated	Ignition test	Rapid burning with flame, no foul odour, white ash	Carbohydrate fiber
Alkali treated	Molisch's test	Violet colour	Carbohydrate fiber
Acid treated	Molisch's test	Violet colour	Carbohydrate fiber
BM Treated	Molisch's test	Violet colour	Carbohydrate fiber
Alkali treated	Fluoroglu-cinol +HCl	Pink colour	Lignified cells
Acid treated	Fluoroglu-cinol +HCl	Pink colour	Lignified cells
BM treated	Fluoroglucinol +HCl	Pink colour	Lignified cells

Ignition test of fibres obtained from the three treatments showed rapid burning with flame without foul odour and resultant white ash that indicated the fibres as carbohydrate fibre which was further supported by appearance of violet colouration when the fibres were subjected to Molisch's test. Fibre sample when tested with Fluoroglucinol in presence of hydrochloric acid resulted in pink colouration indicative of lignified cells.

3.3 Anatomical studies

The anatomical studies of the differently extracted plant fiber were carried out as described above and the results are given in Table: 4. The fiber length, diameter and wall thickness of fibres extracted from all the treatments was found in the range of 99.06 - 108.08 cm, 3.4 - 4.7 µm and 0.7 - 1.0 µm respectively.

Table 4: Anatomical studies of Sisal fiber

Treatments	*Fiber length (cm)*	*Fiber diameter (µm)*	*Wall thickness (µm)*
Alkali treated	106.68± 13	4.7±0.45	1.00±0.08
Acid treated	99.06±24	3.4± 0.43	0.7±0.02
BM Treated	108.08±23	4.2±0.38	0.7±0.32

Length of fibre was recorded to be highest for the fibres obtained from biomaterial treated Sisal leaves followed by that from alkali treated leaves. Values of anatomical properties as given in table 4 clearly suggest that fibre diameter was the highest for the fibres obtained from biomaterial treated leaves whereas wall thickness was found maximum for the fibre derived from alkali treated leaves (figure:2).

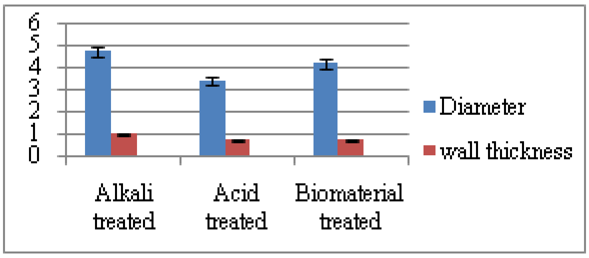

Figure 2: Anatomical studies of different Sisal fiber

The microscopic views of fibers from different pretreated leaves were showed in figure: 3. The microscpical studies suggested the presence of cellular structure of fiber. Fibers were found to be present as needle shape with sharp apex and lumen. It was also revealed that accumulation of the cells formed a typical cellular structure as shown in Fig 3.

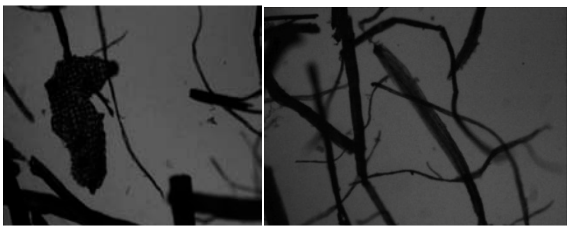

Figure 3: Microscopy of the fiber

The fibers were seemed as flattened fiber and sub branches of fiber were also presented.

4. Conclusion

The study led to the conclusion that pre-treatment of *A. sisalana* (Sisal) leaves facilitate smooth and efficient extraction of fibre. Based on the highest yield and comparatively better anatomical properties, it is evidently clear that treatment of Sisal leaves with biomaterial would a cost-effective, ecofriendly, efficient and productive way of fibre extraction from the plant. The presence of lignified fibre cells are indicative of the high strength of sisal fibre that supports good mechanical properties and use in strong composite materials and other applications requiring high strength and firmness. However, further the studies are needed to reveal the fine structural features of the fibre derived through various pretreatment of sisal leaves to ascertain the fibre quality and efficiently of the process as well.

Environmental concerns have led to promote renewable resources of as natural fibers new materials from. Introduction of natural fibers in polymer matrix can provide significant advantages in terms of good mechanical properties, biodegradability and low cost. The number of studies on these new materials is continually increasing and their development is an important issue. In this context, optimal extraction of good quality fibre is an important aspect for commercial production of natural fibre while addressing the environmental concerns at the same time.

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