Studies on the Preparation of Soft Leathers from *Himantura* (Family *Dasyatidae*) Stingray Skins and their Applications in Foot and Hand Reflexology

R. Karthikeyan[1], N.K. Chandra Babu[2], A.B. Mandal[3] and P.K. Sehgal*[1]

[1.] Bioproducts Laboratory, Central Leather Research Institute, Council of Scientific and Industrial Research, Adyar, Chennai 600 020, India.
[2.] Tannery Division, Central Leather Research Institute, Council of Scientific and Industrial Research, Adyar, Chennai 600 020, India.
[3.] Central Leather Research Institute, Council of Scientific and Industrial Research, Adyar, Chennai 600 020, India.

Abstract: Conversion of fish skins into leather has gained interest among tanners due to their attractive and unique grain structure possessing high market value. The stingray fish belongs to cartilaginous family found in both salt and fresh coastal waters as well as some rivers around the world. Instead of scales the dorsal portion of the stingray skins are having denticles imparts very attractive and beautiful appearance to the final leather. Due to calcification of the skeleton, the stingray skin is very hard and tough and it is difficult to produce soft leathers by conventional leather processing. Hence, to aim at the preparation of soft leathers from stingray skins two different process technologies has been developed in the present study. Initially, the calcium content present in the raw stingray skin has been determined by atomic absorption spectroscopy. To minimize the calcium content and to impart fibre splitting a suitable modification has been made in the pickling and chrome tanning process. Histological and SEM investigations have been carried out to reveal the effect of tanning system on fibre separation resulting in softness. The proteoglycan content in stingray skin is comparatively more due to the presence of cartilaginous skeleton. Xylanase enzyme has been effectively used to acting upon this linear carbohydrate polymer thereby open up the rigid fibre structure. The stingray leathers with denticles have been used for the preparation of massage footwear where the denticles are used to stimulate the reflex points present in our soles during walking. Efforts have been made to utilize the leather with unique grain structure for the preparation of outer covers for steering wheel, motor bike handle, tennis racket and cricket bat handles where the encased stingray leather imparts more grip to the user and these products could also used to stimulate the reflex points present in our palms. Thus this paper will discuss about the process technologies for the preparation of soft leathers from stingray skins and their novel applications.
Keywords: stingray; denticles; reflexology; fish leather

1 Introduction

Fish skins have gaining interest among tanners as an additional source of raw material for making leathers due to their attractive and unique grain structure possessing high market value. But fish skins are generally considered weak in strength and it is difficult to get raw material of uniform sizes. Unlike other fishes, the stingray fish belongs to cartilaginous family, they are found both in salt and fresh coastal waters including some rivers around the world. The thickness of the raw skin after green fleshing ranges from 2-5 mm with beautiful grain structure, and it is traditionally used for the production of decorative leather for ornamental goods. Stingray skins have denticles instead of scales[1] , it imparts attractive appearance to the finished leather.

*Corresponding author. Phone 91-44-24420709. Mailto:sehgal_pk@yahoo.co.in

The stingray reaches a maximum size of just over 6 feet wide, 4 feet long, and about 100 pounds in weight, with the females being larger than the males[2]. The stingray skin is composed of various types of cartilage surrounded by a fibrous perichondrium. Calcified cartilage made by the calcification of Type II collagen, is the stiff material present in the skin of many chondrichthyean fishes[3]. Species of *Himantura* family have developmental stages of dorsal denticles, increasing gradually in size with age. Denticles are scattered in two distinct sizes, smaller denticles are intermingled between larger denticles[4]. Apart from producing ornamental goods from stingray leathers an attempt has been made in Central Leather Research Institute (CLRI), Chennai to utilize the stingray leathers for the preparation of massage footwear, where the stingray leather is used as insole/in-sock material. Successful attempts have been made to utilize the leather with unique grain structure for the preparation of outer covers for steering wheel, motor bike handle, tennis racket and cricket bat handles where the stingray leather imparts more grip to the user and these products could also be used to stimulate the reflex points on our palms.

Reflexology or acupressure is an ancient healing art developed in Asia about 5,000 years ago and this deals with the human body and the flow of natural energy within the body. This medical science has been practiced in China for more than 2000 years[5]. Various organs of our body are connected by a network of nerves and end with certain spots or zones called as reflex zones. By stimulating the foot and hand reflex zones, functions of the target organs can be controlled by more intensive blood circulation flowing to the target organs, thereby enhancing the body energy and immunity. Applying pressure into these points can relieve imbalances by either stimulating or easing the energy flow[6]. Traditionally fingers or thumbs are used to apply pressure to regulate qi. Qi flows through the 12 major energy pathways called meridians, each linked to specific internal organs or organ systems and 365–2000 acupoints[7]. Reflexology is a safe and effective technique to manage fatigue and decrease adverse health outcomes to improve their quality of life. Reflexology has been studied in improving the quality of sleep in the gerontology population.

Foot massage was used as a form of therapy even 5000 years ago which is evident from the pictures of Egyptian tombs[8]. Foot massage and reflexology has been proved on decreasing the anxiety, pain and nausea in patients with cancer[9]. Nowadays foot reflexology has been practiced using acupressure shoes and with specially designed insoles. Conventional shoes are simply designed to protect the feet during walking, whereas an acupressure shoe can improve the wearer's health by stimulating the foot reflex zones[10]. Turucz has prepared shoe insoles having convex protuberances for providing a rhythmic pressure on the soles of the feet during walking[11]. In the present study, the stingray skins with mineralized denticles chemically linked to the collagen fibres have been converted into leather and the leather is used to prepare various products which are used to stimulate the reflex points present in our palms and soles.

2 Experimental

2.1 Materials and Methods

Stingray fish skins were collected from local fish markets in Chennai, Hydrochloric acid was purchased from SD Fine chemicals, Chennai, Xyalanase and Microbate enzymes were purchased from Tex Biosciences (P) Ltd, Chennai and other chemicals were purchased from standard dealers in Chennai.

2.2 Determination of Calcium Content

The stingray skins were green fleshed, washed and samples were collected from middle and side portion of the skin. The calcium content in the raw stingray skin was determined by atomic absorption spectrophotometer (AAS). The sample was prepared as per the AOAC method[12]. Initially a known quantity of sample has been ashed at 550^{0}C and the ash was treated with 15ml of 1:1 HCl, filtered through

Whatmann no.1 filter paper. The filtrate was washed with hot water, cooled and made up to 100ml with distilled water for AAS analysis.

2.3 Scanning Electron Microscopy (SEM) and Energy Dispersive X- Ray (EDX) Analysis of Stingray Denticle

A piece of denticle was cut from the stingray and subjected to SEM and EDX analysis. The study was carried out on Hitachi S- 3400 N instrument. The sample was coated with gold using Hitachi E-1010 ion sputter coating unit. The micrographs for the denticle surface were taken at different magnifications by operating the instrument at accelerating voltage.

2.4 Conversion of Stingray Fish Skins into Leather

After collecting the meat, the stingray skins were brought from the fish market for leather processing. The process details are given below.

Process 1: Sulphide Depigmentation + HCl Pickling + Chrome tanning + Fatliqour

Process 2: Xyalanse Depigmentation and fibre opening + Chrome tanning

2.4.1 Process 1

Raw Material: Fresh stingray skins

Green Fleshing: The fresh skins were green fleshed and rinsed with water for 15'.

Depigmentation: Dark brown coloured pigment adhered to the entire dorsal portion of the skin may not be removed by using lime. A sulphide rich paste is applied to remove the adhered pigments. Sodium sulphide flakes 4% (percentage based on green fleshed weight) Water 5%. Left for 12 h or over night. Next day, the skins were rubbed with gunny bag or brush to remove the pigments.

Fibre Opening: Lime 10%, Water 250% Left for 3 days in a pit. Handled twice a day. The skins were fleshed and the weight was noted. After weighing, the skins were piled grain to grain and secured with staples or stitched along the outside edge to protect the denticles of stingray from the mechanical action of drum during processing. This method of processing also avoids the folding of the skin and protects the beautiful grain structure from damage.

Deliming and Bating: Water 100%, Ammonium chloride 1.0% drummed for 30', Microbate 2% 45', Fleshed and washed thoroughly.

Pickling: Water 100%, Salt 10% 10', Hydrochloric acid 1% (in 10% water) 4 x10'+60', Leave over night. Next day, Hydrochloric acid 1% (in 10% water) 4 x10'+60', PH 2.0. Leave over night in the bath. Next day the pelts were taken and piled for 10 days.

Repickling: Water 80%, Salt 8% 10', Hydrochloric acid 1% (in 10% water) 3x10'+60'

Chrome Tanning: Pickle water 50%, BCS powder 4%, Cationic fatliquor 1%, Nonionic fatliquor 1%, Run the drum for 60', BCS powder 4%, Cationic fatliquor 1%, Nonionic fatliquor 1%, Run the drum for 60', Water 50% 30', Sodium formate 1%(in 10% water), Sodium bicarbonate 1% 3x10' + 60' (in 10% water), pH 3.8 drain, rinsed and piled overnight.

2.4.2 Process 2

Raw Material: Fresh stingray skins.

Green Fleshing: The fresh skins were green fleshed and rinsed with water for 15'.

Depigmentation & Fibre Opening: Xyalanase enzyme 4% (percentage based on green fleshed weight), Water 30%, Run the drum for 5 h. The skins were rubbed with brush to remove the pigments, fleshed and the weight was noted.

Pickling: Water 100%, Salt 10% 10', Sulphuric acid 1% (in 10% water) 4 x10'+60', Leave over night. Next day adjust the pH to 2.8 and drain 50% pickle water.

Chrome Tanning: Pickle water 50%, BCS powder 4%, Cationic fatliquor 1%, Nonionic fatliquor 1%, Run the drum for 60', BCS powder 4%, Cationic fatliquor 1%, Nonionic fatliquor 1%, Run the drum for 60', Water 50% 30', Sodium formate 1%(in 10% water), Sodium bicarbonate 1% 3x10' + 60' (in 10% water), pH 3.8 drain, rinsed and piled overnight.

2.5 Histological Studies

The histological studies for the soaked, limed and xyalanase treated stingray skins were carried out. After the completion of the above mentioned processes, the samples were cut and preserved in 10% formalin for 48 h. The fixed samples were dehydrated in a series of solutions of alcohol of different concentrations (50 to 100%) and then cleared in xylene. They were finally embedded in paraffin wax into moulds. The moulds were labeled and stored until use. Thin sections (10µm thick) were cut on a microtome, mounted on glass slides and stained with Van Gieson.

2.6 Post Tanning

The tanned leathers from the above two experiments were shaved using single width shaving machine to remove the excess fibre bundles and then neutralized to pH 5.5. The neutralized leathers were dyed with 2% acid dye, subsequently fatliquored, retanned, dried and buffed.

2.7 SEM Analysis of Stingray Leather

Scanning electron microscope analysis has been carried out to reveal the effect of different processes on the structural characteristics of the stingray fish leathers. The samples measuring 5mm x 2mm were cut from the crust leather and they were mounted both vertically and horizontally on aluminium stubs using an adhesive. These were then coated with gold using an Edwards E-306 sputter coater. The stubs were introduced into the specimen chamber of FEI-Quanta 200 scanning electron microscope. The stubs mounted on the stage could be tilted, rotated, and moved to the desired position and orientation. The micrographs for the cross-section were obtained by operating the microscope at an accelerating voltage.

2.8 Physical Testing and Visual Assessment of the Leathers

The samples for physical testing were cut from the tanned stingray crust leathers. The samples were conditioned at 20±2° C and 65±4% relative humidity for 48 h. The tensile strength, and tear strength were determined as per the IUP 6 and 8 methods respectively[13,14]. Lastometer test was carried out as per the method IUP 12[15].

3 Results and Discussion

3.1 Elemental and Structural Characterization of Stingray Skin.

Commercial tanners producing stingray leathers in India face major constraints as the leather becomes harder especially in the middle part after tanning. This is due to the calcification of the stingray during the mature stage. The calcium content of the raw stingray skin determined by AAS method is given in Table 1. The calcification in the middle layer of the skin is comparatively more to other parts of the skin. The calcium content as Ca in the middle portion of the skin has been found to be 6.61% w/w and in the side portion it is 5.16% w/w. Due to calcification the middle part of the stingray becomes harder after tanning. To minimize the calcium content and to impart fibre opening and splitting a suitable modification has been made in the pickling process in the modified chrome tanning experiment. The pickling process is repeated two times using hydrochloric acid followed by aging during pickling as it enhances the fibre opening and splitting. During first pickling, when it is left for overnight in the bath the pH increases to around 5.0. This increase is due to the dissolution of calcium salts present in the skin. To complete the dissolution process, the pickling process is repeated for two times with intermediate ageing in the modified process.

Table 1 Calcium content of stingray skin

Description of the sample	Calcium content as Ca (% w/w)
Middle part of the skin	6.6
Side part of the skin	5.2

The EDX spectrum obtained by the EDX analysis of stingray denticle is given in the Figure 1. The higher a peak in a spectrum, the more concentrated the element is in the specimen. From the EDX spectrum it is confirmed that elements Calcium, Phosphorus, Nitrogen are concentrated more in the denticle surface where the electron beam is bombarded. The scanning electron micrographs of stingray denticle at a magnification of 300000 X is given in Figures 2. The calcification of stingray denticle is very clear in this micrograph taken at higher magnification.

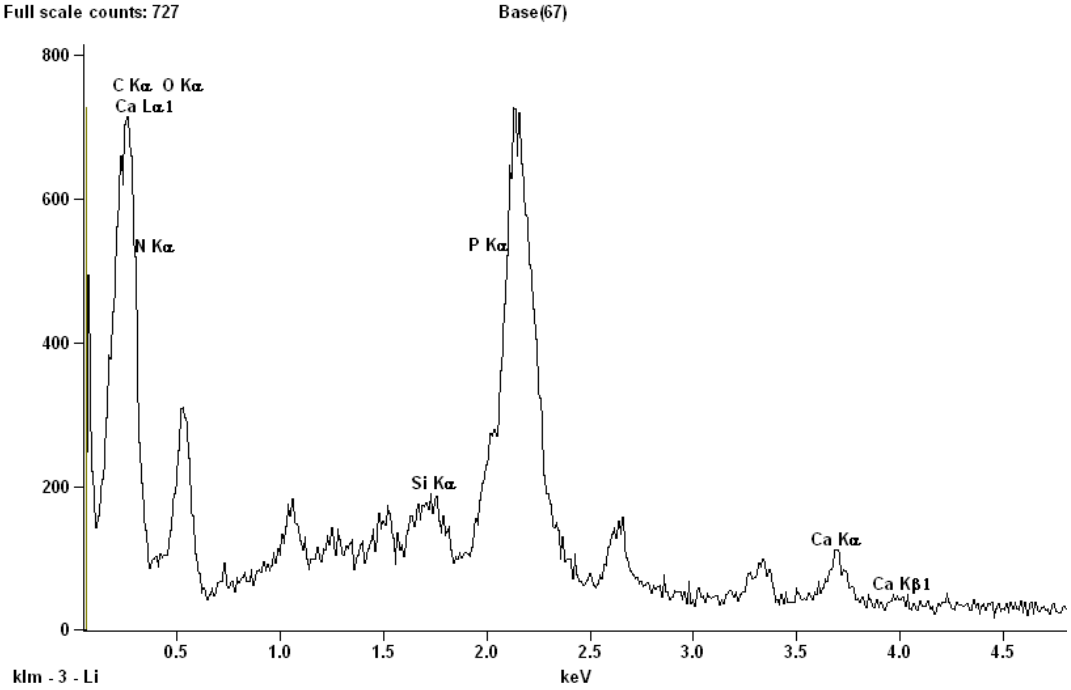

Figure 1 EDX spectrum of stingray denticle

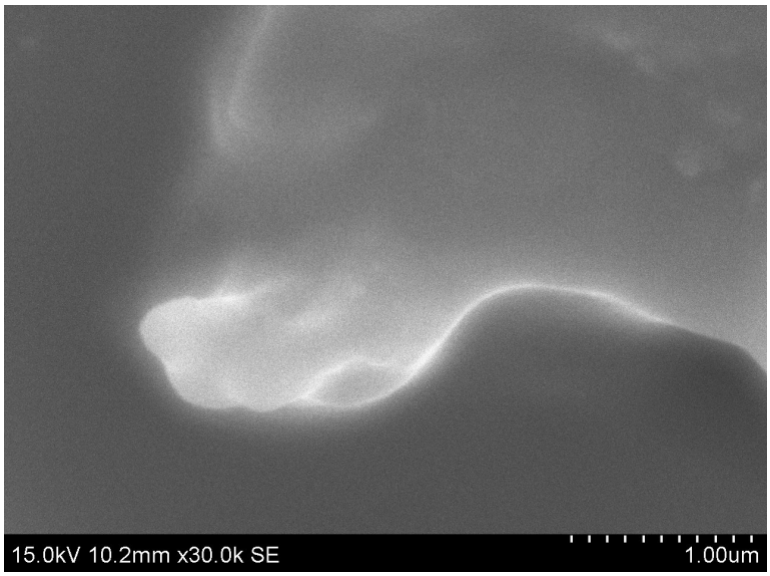

Figure 2 Scanning electron microphotograph of stingray denticle at 300000x magnification

3.2 Histological Studies on Stingray Skins

The optical photographs taken for the soaked, limed, and xyalanase treated stingray skins are set out in Figures 3-5. From the photographs it is confirmed that the stingray skins have more compact parallel fibre bundles arranged in a lamellar manner. Opening up of fibre bundles is very clear in the xyalanase treated skin.

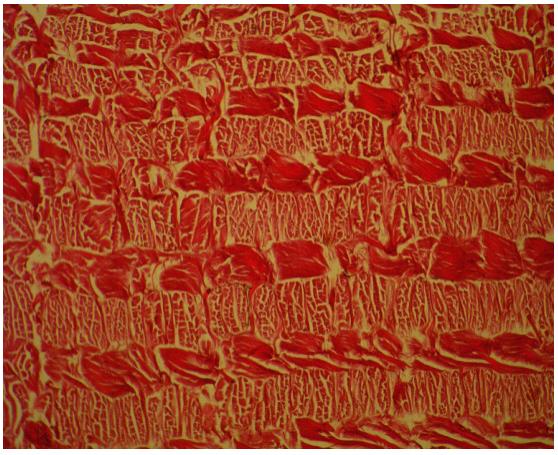

Figure 3 Soaked skin

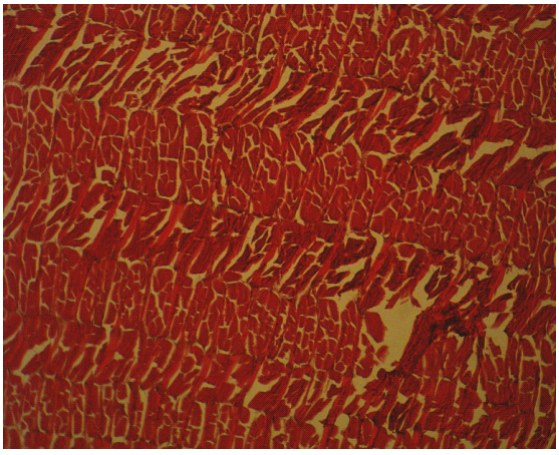

Figure 4 Limed

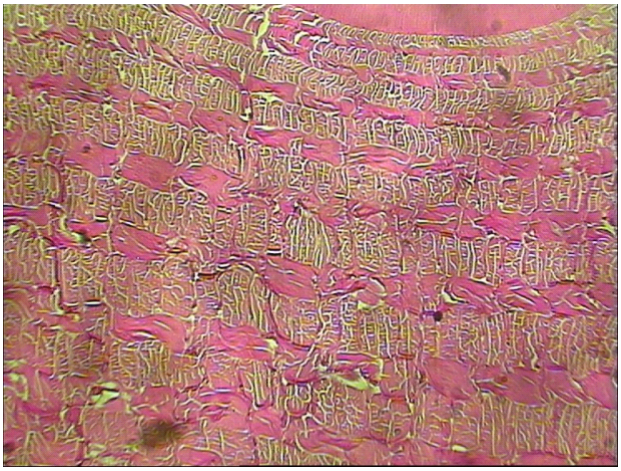

Figure 5 Xyalanse treated skin

3.3 Effect of Tanning system on the Fibre Structure of Stingray Leather

Scanning electron microphotographs of stingray leathers showing their cross section at a magnification of 500x are given in the Figures 6 & 7. From the micrographs it is confirmed that the leathers produced under process 1 have loose packing of fiber bundles. The appropriate modification made in the pickling process and the optimized amount of fatliquor given in the tanning experiment brings softness in the final leather through improved fibre separation achieved in tanning operation. The fibre bundles of xyalanase treated leathers remain compressed resulting in rigid leather which is very clear from the micrographs.

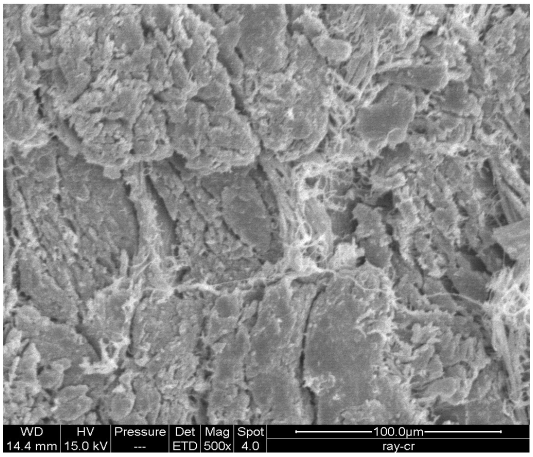

Figure 6 SEM for Leather from Process 1

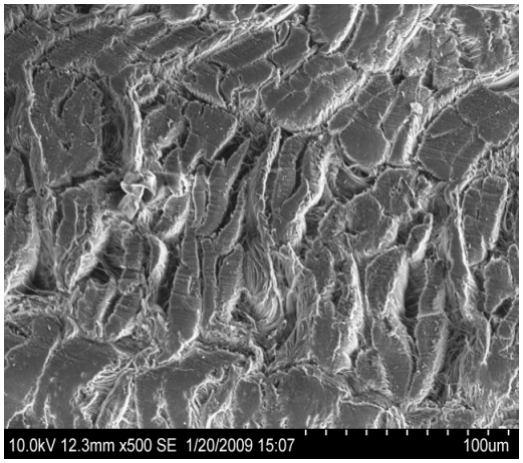

Figure 7 SEM for Leather from Process 2

3.4 Physical Testing Data

The strength properties of stingray crust leathers are given in Table 2. In general stingray leathers possess high strength properties. The strength properties of leathers obtained from process 1 are higher compared to process 2 (xyalanase treated) stingray leather. In stingray skins the parallel collagen fibre bundles are associated with calcified denticles, imparts high strength properties to the crust leather.

Table 2 Strength properties of stingray leathers

Strength Properties	Process 1	Process 2(xyalanase)

Tensile Strength Kg/cm^2	275	264
Elongation at break %	38	32
Tear Strength Kg/cm	92	80
Grain crack load, Kg	32	30
Distension at grain crack in mm	5.7	5.2

3.5 Applications of Stingray Leather

Figure 8 shows the geometry of hand reflex zones present in the palm of the hand. By stimulating the hand reflex zones, functions of the target organs can be controlled by more intensive blood circulation flowing to the target organs, resulting in enhancing the body energy and immunity. The various products which are used to stimulate the hand and foot reflex zones are given in figure 9. To stimulate the foot reflex a zone, stingray leather with denticles is used as insole/insock material for the production of footwear.

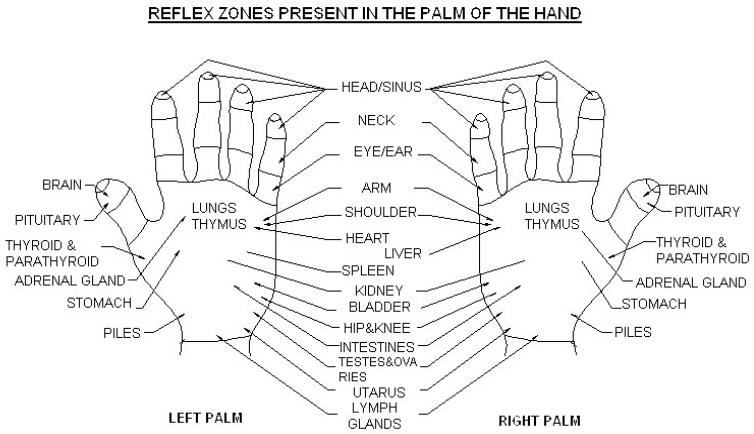

Figure 8

Figure 9 Various products from Stingray Leather

4 Conclusions

Through this article we report a novel route for the effective utilization of stingray leather and also offers a simple and trouble free method to practice the traditional reflexology technique. Fibre splitting during pickling and fatliquoring during tanning process imparts good fibre separation to the crust leather resulting in soft leather. Xyalanase could be effectively used to remove the pigments adhered to the stingray skin as well as to open up the fibre structure.

Acknowledgement

The author R. Karthikeyan wishes to thank Council of Scientific and Industrial Research (CSIR), Govt. of India for receiving the financial assistance in the form of Senior Research Fellowship.

References

[1] P.K. Talwar; A.G. Jhingran. Inland fishes of India and adjacent countries. (eds.) A.A. Balkema, Rotterdam. 1991, 1, 43-44 Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi

[2] B.L. Chaudhuri. Freshwater sting-rays of the Ganges. *J. Proc. Asiatic Soc.* 1912, 7: 625–629.

[3] M.N. Dean; W.A. Chiou; A.P. Summeres. Morphology and ultrastructure of prismatic cartilage calcification. *Microsc. Microanal.* 2005, 11: 1196-1197.

[4] P.R. Last; B.M. Manjaji-Matsumoto; P.J. Kailola; Himantura hortlei sp.nov., a new species of whipray (Myliobatiformes: Dasyatidae) from Irian Jaya, Indonesia. *Zootaxa* 2006, 1239:19-34.

[5] J. Dandidier. Acupressure: For Health, Vitallity and First Aid. Element, Rockport (1997).

[6] S.L.Tsay; M.L. Chen; Acupressure and quality of sleep in patients with end-stage renal disease – A randomized controlled trial. International Journal of Nursing Studies, 2003, 39: 245-251.

[7] M.L. Chen; L.C. Lin; J.G. Lin. The effectiveness of acupressure in improving the quality of sleep of institutionalized residents. *Journal of Gerontology,* 1999, 54A (8), M389-M394.

[8] J. Williamson. A Guide to Precision Reflxology. Salisbury, Wilts:Quay Books (1999).

[9] L.Grealish; A. Lomasncy; B. White-Man. The effects of foot massage and reflexology on decreasing anxiety, pain and nausea in patients wih cancer. Cancer Nursing, 2000, 23:237-43.

[10] HONG; Jae-Hyung. Acupressure Shoe International Publication Number PCT WO 2004/043185 (2000).

[11] S.Turucz Acu-pressure massage insoles US Patent No: 4,841,647 (1989).

[12] Official Methods of Analysis of the AOAC International (Association of Official Analytical Chemists) 18th Edition, 2005, United States.

[13] IUP: 6 Measurement of tensile strength and percentage elongation. *J Soc Leather Technol Chem*, 2000, 84: 317.

[14] IUP: 8 Measurement of tear load –Double edge tear. *J Soc Leather Technol Chem*., 2000, 84: 327.

[15] Official methods of analysis, *Society of Leather Technol. Chem;* Herts, (1965).