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Research Article

NON DESTRUCTIVE TECHNIQUES FOR INDIVIDUALIZING PORCUPINE QUILL THROUGH TRACE EVIDENCE ANALYSIS

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ABSTRACT

As we all know there is an increasing rate of poaching of the various items obtained from the various wildlife animals. One of its kind is the Indian crested porcupine quills. To the best of our knowledge, no research work is reported till date for the identification of the quills using any non-destructive techniques which can prove to be useful in the field of wildlife forensic. The present study, introduces for the first time the application of microscopic examination, FTIR ATR andED-XRF as a promising valuable examination tool for identifying porcupine quills in wildlife crime. The microscopic study showed clearly defined scales patterns which are unique to Indian crested porcupine quills having the mean value of 17.40 μm and showing standard deviation of 9.66 while the FTIR ATR study showed that a peak of disulphide linkage and amide back bone (amide – I, II, III) which can be effectively utilized for their identification. As well as the XRF analysis showed that the quills contains about 84% of sulphur in it and also provide data for the differentiation of black-white-black and white quill. As all the techniques implied in this study are non-destructive, the samples can be saved for further research analysis which also maintains its evidential value in the court procedure. The results obtained after applying the techniques draw the conclusion that Indian crested porcupine quills can be distinguished while preserving the sample which will have far reaching implications in wildlife forensics especially in poaching cases.

INTRODUCTION

Hair is an inherent characteristic existing only in mammals and such mammals are placed under the class called Trichozoa i.e. hairy mammals or Pilifera i.e. carrying hair (Bonnet 1892; Noback 1951). The hair cover of mammals are subjected to the direct effect of various environmental factors and also undergo evolution due to which they have acquired many adaptive features in the form of spicules, semi-spicules, bristles, spines, semi-spines etc., which are due to the presence of several generation of hair follicles in ontogeny (Sokolov 1977; Sokolov and Chernova 1998; Chernova and Kuznetsov 2001). The old world porcupines are unique kind of mammalian group possessing specialized quill-like epidermal structure. In porcupines, hair feature is altered to become spines. These spines of Hystricidae provide them adaptive advantages and protective covering against predator attack (Hoey et al. 2004). Hystrix have shorter tail and rattle quills which vary in lengths. These quills located at the end of the tail are slender and of greater diameter for about the terminal fifth.

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The expanded portion is hollow and thin walled, thus the together vibration of quills produce a hiss like rattle. The Indian Crested Porcupine (Hystrixindica), or Indian Porcupine, is a member of the Old World porcupines. It is a large rodent, found throughout southern Asia and the Middle East. In Hystrixindica possess four types of quills i.e. at head, shoulder, neck, and limbs. The long quills are raised into a crest like structure and cover head, back and nape. The side and back half of the body are covered with stout, cylindrical quills up to 35 cm long and marked with alternating light and dark bands. The longest quills grow from its shoulders and is about a 23-30 cm large. Its tail is covered with short, hollow quills that can rattle when threatened. It has broad feet and long claws for digging. When attacked, the Indian Crested Porcupine raises its quills and rattles quills on its tail.Porcupine quill is an outgrowth of epidermis skin which is considered as made up of keratin (Harding HWJ and Rogers GE 1972). Quill is a hard, broad, thick, hair like structure and consists of three parts - cuticle, cortex and medulla. Phenotypically Indian Porcupine quill is of two types i) Black –White –Black ii) total White (Cho WKet al. 2012). Porcupines are famed for their quills, which are actually large, stiff hairs that help defend the animals against natural predators. If the predator persists past these threats, the

porcupine launches a backwards assault, hoping to stab its attacker with its quills. It does this so effectively that most brushes between predators and the Indian porcupine end either in death or in severe injury (Chakravarthy AK et al. 2006).

Porcupine quills possess various useful applications as they are also used during the process of paddy threshing, mix some quills with the seeds to protect it from the pest and insects during storage, also used as bio-pesticide, for making handicrafts. The poaching is done due to the importance of the quills in the foreign market as a decorative article. The meat is used for edible purpose. The large scale farming is done in the foreign countries and hence transportation from the different areas to foreign countries due to good price in market encourages poaching. Porcupine quills possess antibiotic properties due to some fatty acids coating on quills (Roze U 1990, Brooks EGE2010). Not only in India but also in different countries, quills are used at medicinal level and also used as traditional and artificial ornaments like bowls, fins of cap etc (Ettinger P 2010, Burgess R and Rimington C 1929, Roze U 1990, Lyford CA 1979). Poaching is encouraged due to the high price paid by restaurants for the porcupine meat and also due to the supply of quills to luxury urban market. In general, black quills worked best for loom work,fine quills from the neck were ideal for embroidery and thin quills found near the belly were used for delicate lines. As porcupines have moved into urban and rural areas of the country where very few, if any, predators are still found, predation levels have diminished completely and the only known threat to porcupines are humans. The link of porcupine trade is the middleman or dealer, the retailer, the products, the increase in the availability of quill products and the Internet. For example, the Wakedain Oakdale, California USA i.e "African Porcupine Quill 6-9" is sold for $1.95 and is great for roach pins and hair sticks. The Atlantic Coral Enterprise in St. Augustine, Florida i.e "African porcupine quills / From 4" to 14" is sold in lots of 50, 100 and 1000 / skip by the middleman, who are direct importers!" (Nick C 2006). Two cases of poaching were reported in the newspaper from Gujarat, one from Anand district near a Borsad Social forestry area and the other one fromGir-Somanath District near a Kodinar Social forestry area. Bekker JG, King AT (Bekker JG, King AT 1931) in their work reported the sulphur content of quills using destructive method and they found 1.35% per quill (0.79%) in dry ash. Not much work is reported till date in relation to the identification of the Indian Crested Porcupine (Hystrixindica) via any non-destructive technique. Animals have different hair pattern which can be detected and measured by the application of various non-destructive techniques. Advantages of analysing quill samples also include their easy and non-invasive collection, the small sample size requirement for analysis and their easy storage at room temperature for long time. This facilitates the setting up of wildlife animal identification library using different advanced tools which helps to develop effective governance and rule of law and improve the quality of criminal justice responses to wildlife crime.

The present study deals with the study of the Indian Crested Porcupine (Hystrixindica) using non-destructive techniques to individualize it as well to form an identification library using advanced tools which will be help in tracing the porched articles and can improve the quality of criminal justice responses to wildlife crime. The study showed the application of compound microscopic analysis, Fourier transform infrared spectroscopy – Attenuated total reflection (FTIR-ATR) analysis and Energy Dispersive X-ray Fluorescence (ED-XRF) analysis for the non-destructive analysis of the Indian Crested Porcupine (Hystrixindica) for identification of species. The present study for the first time showed the application of non-destructive analysis in wildlife crime investigation especially in relation to forensic context. The compound microscopic analysis was done for the measurements of scale present in the quill. The FT-IR (ATR) is a vibrational spectroscopic technique used for the non-destructive identification of molecular species which can be classified according to their chemical structures (Tu AT 1982, Baddiel CB1968, Miller JV,andBartick EG 2001). The analysis showed the fingerprint region contains bands such as Amide-I, II, III, CH, deformation and cysteic disulphide vibrations. Energy Dispersive X-ray Fluorescence (ED-XRF) was done as it has shown applicability for Forensic scientists by matching samples associated with suspects namely dirt or sand on clothing or shoes samples obtained from crime scenes as it has revealed applications in elucidate an elemental fingerprint, without analysing the evidence destructively.

MATERIALS AND METHOD

Sample collection

For the present study, priory permission was taken for the collection and examination of the porcupine quill from the Principal Chief conservation of forest, Gujarat. All the samples were then collected from the Sakkarbaug Zoo, Junagadh, Gujarat, India. The total of 60 Indian Crested Porcupine quill, 22black-white-black and 48 white quill, were collected and analysed under microscopic examination, FTIR-ATR and ED-XRF.Only those samples which was priory confirmed by the Zoo to be of Quill was considered as the standard Quill samples in the preset study. The quill samples were first subjected to a clear wash of acetone and then air dried. After that the samples were examined using non-destructive examination methods such as microscopic examination, Fourier transform infrared spectroscopy - Attenuated total reflection (FTIR-ATR) analysis and examination under Energy Dispersive X-ray Fluorescence (ED-XRF).

Microscopic Examination

This technique is used for the examination of porcupine quill scale pattern. The quill surface was examined under compound microscope (Leitz CFM-2, 40X–100X, Leica Microsystems, Wetzlar, Germany) in order to determine outer layer structure of the quill. Before examining the scales pattern under microscope, the samples were first washed and then placed over the cellulose sheet of 1 inch X 3 inch. Cotton swap dipped in acetone was then rubbed over the surface of the quill. As the acetone reaches to the bottom portion which is already in contact with the cellulose sheet, it makes scales impression over the surface of sheet. This sheet was then analysed under microscope having a camera attachment. The photographs taken were then analysed by using Leica Quine Software.

FT-IR (ATR) Spectroscopy

In the Fourier transform or interferometric type of spectrometer a two – beam, variable-path-length interferometer is used in lieu of a monocromator. Fourier transform spectrometrydescribes the use of such a device since

its output is not regular spectral data but the Fourier transform of data. Such instruments are becoming increasingly important, especially in IR spectrometry. The key advantage of this types of spectrometer over a conventional dispersive instrument is that in many energy – limited situations it offers superior signal-to-noise ratio. The advantage of the method is due to the fact that in an interferometric unit energy throughput is inherently greater (no entrance slit is required) and also all wavelength present reach the detector during the entire time required to observe a spectrum.

Infrared spectroscopy is a widely used technique that for many years has been an important tool for investigating chemical processes and structures. The combination of infrared spectroscopy with the theories of reflection has made advances in surface analysis possible. Specific IR reflectance techniques may be divided into the areas of specular reflectance, diffuse reflectance, and internal reflectance. The latter is often termed as attenuated total reflectance (ATR) and will be the focus of this work.

Fourier Transform Infrared - Attenuated Total Reflectance (ATR) Spectroscopy is an innovative technique for monitoring the transport process of low molecular weight species. It enables the monitoring of individual species in-situ, while providing additional chemical information on any changes that may be occurring during the transport process.

In the present, the FTIR-ATR spectroscopy was used to evaluate the composition of quill. ATR is chosen as it is primarily a surface technique having the depth of analysis approximately 2 micrometres (Merrill and Bartick 2000). The ATR data can be considered conclusive with regard to structure identification and can prove to be useful as a non-destructive technique for the identification of Indian Crested Porcupine.

The infrared spectrometer used was an Alpha-IR ESP (Enhanced Synchronization Protocol) equipped with apotassium bromide (KBr) beam splitter and a deuterated triglycinesulfate (DTGS) detector. The ATR accessory was of Bruker IR Technologies (Danbury, Connecticut) with resolution capacity of 8 cm^{-1} and equipped with zinc selenide (ZnSe) crystal. Analysis of all the 60 samples and background scans were taken in Transmittance mode with a spectral range of 2000-500 cm^{-1}.

For the first time the application of FT-IR ATR technique is applied in wildlife animal porcupine quill examination. Quill is an epidermal out growth and made up of sclera-protein. It has a basic composition of keratin and higher amount of cystine amino acid. The strongest bond present in the composition of quill are sulfur bond linkage known as disulfide linkage (BekkerJG and KingAT 1931, Harding HWJ 1972).

Energy Dispersive X-ray Fluorescence (ED-XRF)

Energy Dispersive X-ray Fluorescence (ED-XRF) basically is an analytical technique used for the elemental analysis of a sample. It deals with interaction between source of X-ray excitation and a sample. It follows the principle that each element has a unique atomic structure permitting a unique set of peaks on its electromagnetic emission spectrum.The number and energy of the X-rays emitted from a sample can be measured using an energy-dispersive spectrometer. As the energies of the X-rays are characteristic of the difference in energy between the two shells and of the atomic structure of the emitting element, **ED-XRF** allows the measurement of elemental composition of the specimen.

XRF is an analytical method to determine the chemical composition of all kinds of material. Materials can be of any form, solid, liquid, powder etc. The method is fast, accurate and non-destructive, and usually requires only minimum sample preparation. Applications are very broad including the analysis of metals, polymer, oil and food industries along with the fields like forensic, chemistry, geology, and mineralogy. The measurement time depends on the number of elements to be determined and the required accuracy, and varies between seconds and 30 min. The analysis time after the measurement is only a few seconds.

Figure 1 Indian Crested Porcupine quill

RESULTS AND DISCUSSION

Microscopic examination

Indian crusted porcupine quill scales were examined under 40X magnification. Figure 2 shows the images of the scales under microscope. The measurements of scale was done with the help of Leica Quine computer based software.

Figure 2 Indian porcupine quill Microscopic scale pattern (40x).

Table 1 shows the results of measurements of scale of quill. Total up to 14^{th} scale was counted in each scale impression in all the quill samples. The mean value of all the samples, as given in Table 1 is 17.40 µm and the standard deviation (S.D) is 9.66 with a standard error of 2.58. The maximum and minimum scale distances are found to be 38.85 and 1.05 µm.

FT-IR (ATR) Spectroscopy

The FT-IR ATR spectra of the Indian crested porcupine quill is shown in Figure 3, similar spectra was obtained in all the samples. The spectral (or "fingerprint") region between 2000-500 cm^{-1} was curve fitted as described into the molecular bases

for the discrimination observed by Chemo-metrics. The region contains bands such as Amide-I, II, III, CH, deformation and cysteic disulphide vibrations. Overall 6 bands were curve fitted and the results were presented. Curve-fit of the relative intensity are of the cysteic acid band (1007 cm^{-1}) as mention in Table 2.

Table 1 Measurements of scale under microscopic of Indian Crested Porcupine quill

Object	Class (µm)	Distance (µm)	Statistical (µm)
1	1-22.05	22.05	
2	22.05-45.15	23.10	Total: 243.60
3	45.15-71.40	26.25	
4	71.40-85.05	13.65	
5	85.05-100.80	15.75	Mean: 17.40
6	100.80-139.65	38.85	
7	139.65-140.70	1.05	
8	140.70-157.50	16.80	Standard
9	157.50-170.10	12.60	Deviation: 9.66
10	170.10-183.75	13.65	
11	183.75-201.60	17.85	Standard Error: 2.58
12	201.60-208.95	7.35	
13	208.95-214.20	5.25	Max: 38.85
14	214.20-243.60	29.40	Min: 1.05

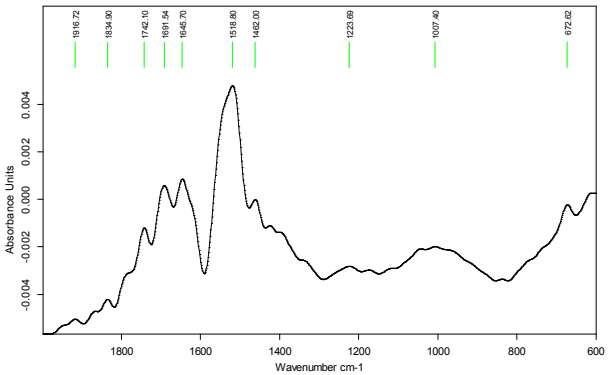

Figure 3 FT-IR ATR spectra of Indian crested porcupine quill

Table 2 Identified functional group and intensity

Sr.No.	Functional group	Peak intensity (cm^{-1})
1	$-SO^{-}_{3}$ Cystic Acid Stretch (O Bond Stretching).	1007.40
2	Amide – III C-N (Vibrations).	1223.69
3	H-C-H (Stretching).	1462.00
4	Amide-II: 60% C-N stretch 40% N-H in plane band Minor Contributions C-C, N-C stretch, C=O in plane band.	1518.80
5	Amide-I: 80 % C=O stretch, C-N stretch, C-CN.	1645.70
6	Amide-I: C=O (α helix).	1691.54

The frequencies of the amides are sensitive to peptide conformation and hydrogen bonding and can be used to characterize the secondary structure of the peptide backbone. Analysis of the relevant intensity area of Amide-I (α Helix 1645cm^{-1} and β Sheet1691cm^{-1}), Amide-II (α Helix 1518 cm^{-1}) and Amide-III (1223 cm-1) indicates secondary structure of the FT-IR ATR spectra of the Indian crusted porcupine. It therefore appears that there is no variation in the secondary structure in the porcupine quill keratin on the bases of the gender variation.

ED-XRF Analysis

All the 60 quill samples were analysed using ED-XRF and the results of the level of elements are present in Tables 3-5. Table 3 gives the level of main elements present in Black-White quill samples and Table 4 represents the level of the corresponding elements present in White quill samples. The results are the average values of the samples analysed.

Table 3 Element detected (%) in Indian Crested Porcupine quill (Black-White)

Sr.No.	Element	Concentration, %
1	Sulphur(S)	84.7
2	Calcium(Ca)	5.35
3	Phosphorus(P)	4.15
4	Potassium(K)	1.9

Table 4 Elements detected (%) in Indian Crested Porcupine quill (White)

Sr.No.	Element	Concentration, %
1	Sulphur(S)	83
2	Calcium(Ca)	4.86
3	Phosphorus(P)	4.85
4	Potassium(K)	0.65

Table 5 Metals detected under XRF in Black- White and White quill samples

Sr.No.	Metal	Black-White [B-W.Q] (conc. %)	White Quill [W.Q] (conc. %)
1	Aluminum(Al)	1	1.2
2	Lead(Pd)	0.7	-
3	Copper(Cu)	0.488	0.569
4	Zinc(Zn)	0.33	0.973
5	Iron(Fe)	0.28	0.419
6	Nickel(Ni)	0.26	0.347
7	Gold(Au)	0.21	-
8	Rhenium(Re)	0.17	-
9	Chromium(Cr)	0.11	0.13
10	Bromine(Br)	0.078	-
11	Silicon(Si)	-	1.7
12	Rubidium(Ru)	-	1.33

The results of XRF analysis of Indian crested porcupine quill showed the presence of sulphur, calcium, phosphate and potassium as the main elements present in quill samples. There is a high concentration of sulphur in both the Black White and White quill samples while other elements namely calcium, phosphate and potassium are relatively low. Also the concentration of all four elements are same in both black-white-black and white quill samples. This shows that the identification is very easy on the basis of these levels of the elements present in the quill. This data refers to Indian crested porcupine species only.

The Indian Crested Porcupine examined under XRF are mention in **Table 5** showed the following metals namely lead, gold, rhenium and non-metal bromine in black-white quill whereas they were absent in white quill. White quill contains silicon and rubidium but they are absent in black-white quill. The above results can be used as a data base for comparison both the types of quill and can prove to be useful for their identification purpose.

CONCLUSION

To the best of our knowledge, no research work is reported so far using non-destructive techniques for the identification of quill in the field of wildlife forensic. This study introduces for the first time FTIR ATR and ED-XRF as a promising valuable examination tool for identifying porcupine quills in wildlife crime. The FTIR ATR study showed that a peak of disulphide linkage and amide back bone (amide – I, II, III) are present in

the Indian crested porcupine quills which can be effectively utilized for their identification. The microscopic study showed clearly defined scales patterns which are unique to Indian crested porcupine quills having the mean value of 17.40 µm and showing standard deviation of 9.66 as well as the XRF analysis showed very promising results thatIndian crested porcupine quills contains about 84% of sulphur in it and also provide data for the differentiation of black-white-black and white quill. As all the techniques implied in this study are non-destructive, the samples can be saved for further research analysis which also maintains its evidential value in the court procedure. The results obtained after applying the techniques namely microscopic, FT-IR ATR and XRF draw the conclusion that Indian crested porcupine quills can be distinguished while preserving the sample which will have far reaching implications in wildlife forensics especially in poaching cases.

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