Multifaceted Forensic Profiling of Mammalian Claw and Hair samples to Combat Wildlife crimes

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ABSTRACT:

The use of various animal body parts, for example meat, fur, fat, oil, bile, eyes, blood, viscera, gall bladder, bone, brain and skull or the whole animal for medicinal purpose and adornment supports a global trade in wildlife products. Resulting an increase in wildlife crimes and hence creating negative effects for the preservation of biodiversity, climate change, public health and security. Out of the 6399 mammal species, 26% are threatened with extinction. Forensic technologies could be an important component for strategically investigating and prosecuting wildlife crimes. This review aims to:

- Summarize problem and how forensics can address the issues. The specific forensic technologies of value in investigating wildlife crimes are discussed at length.
- Provide an overview of widely used destructive and non-destructive forensic techniques on mammalian claws and hair samples to aid wildlife crime-solving process.
- Explain the role of forensic techniques in efficient species identification of mammals, and application of this information by the law enforcement agencies against wildlife crimes.
- Compare various forensic techniques for adoption of utmost technique for species identification and its differentiation among mammal species. Also, finer intra-comparison of the molecular forensic techniques, specific primer comparisons and its identifying power demonstrates the great advancements in the field of wildlife for the identification of exact species involved in any wildlife crime.

KEYWORDS: Forensics, Mammals, Non-destructive equipment, Species Identification, Wildlife.

INTRODUCTION:

Since time immemorial humans have evolved in coexistence with wildlife and nature [1]. Some flora and fauna possess characteristics that are worshipped by humans, especially in Asia. Yet the same characteristics make these species vulnerable to hunting and poaching, as the products derived make them a commercially beneficial proposition. Consequently, the products are always in high demand and widely consumed in south east Asian countries. They include the use of numerous parts of animals like meat, fur, fat, oil, bile, eyes, blood, viscera, gall bladder, bone,

brain, skull etc. Besides direct consumption these products are also used for decorations as trophies to showcase status or increase aesthetic value. These practices predominantly occur in 51 countries, mostly in Asia, Africa and Latin America [2]. Despite their believed medicinal and fabled values some of these animals can adversely impact human wellbeing by spreading diseases [3]. The spread of Covid – 19, which likely originated from consumption of wild animals, is a well-known example [4].

Hunting and poaching of wildlife species may increase their vulnerability to local or global extinction [5]. The hunting, exploitation, possession or international trade of wildlife or their products in violation to national and international laws is termed as wildlife crime [6]. The World Wildlife Crime Report states that wildlife crime has negative effects for the preservation of biodiversity, climate change, public health and security [7]. The increasing human population and its growing dependency on use of plants and animals has become a dynamic phenomenon, sometimes criminal, and are increasingly threatening the existence of many wildlife species [8]. If we only consider the mammals, their numbers have increased from 6399 to 6,596 known species [9] [10], out of which 26% are threatened with extinction [11]. Thus, it is critically important that wildlife crimes are tackled strategically, and forensic technologies could be a vital tool for investigating and prosecuting such crimes, while serving as a deterrent to these kinds of offences. This review summarizes the problem and explains how forensics can address these issues. The specific forensic technologies of greatest value in investigating the wildlife crimes are also discussed at length.

WILDLIFE FORENSICS:

The wildlife forensics aids the investigations of wildlife crimes. Unlike in human forensics where humans are both victim and offender, in wildlife forensics evidence is analyzed to form a link between the animal victim and the human offender [12]. It assists in both species' identification and determination of geographical origin of the captured species, which may in turn help determine the severity of the crime and potential location respectively. In one such case of wild boar poaching from a national park of Italy, the bloodstain on a knife was analyzed using a molecular technique to identify the species and penalize the offender [13]. Several such cases of poaching for example, Guanaco in Chile (Marín, 2009); Sardinian mouflon on the island of Sardinia [14] and capybara, Chaco chachalaca and Pampas deerin Brazil [15] were solved using the molecular techniques. Through these and many other cases, wildlife forensics has demonstrated its value as a tool for answering wildlife crimes.

In 1963, following many years of increasing illegal wildlife trade, 184 countries joined as signatories to Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) to keep a check on the global illegal trade [16]. The CITES species are categorized, with varying degrees of protection, into three appendices. In India, the same level of protection to the wildlife was envisaged to be provided through the enactment of 'The Wildlife Protection Act, 1972' that regulates national parks, sanctuaries and zoos along with protecting the wildlife by categorizing them into six schedules with varying degrees of protection [17]. The rapid

decline of wild flora and fauna in India had caused a grave concern in the nation. It was felt essential by the government of India to form laws for the protection of wildlife in the nation. This act has been amended several times in order to keep up with the changing scenario of wildlife crimes. The Wildlife Stock Declaration Rules were framed by the government of India in1972. In 1982, the act was further amended and Wildlife (protection) Licensing rules were framed. Added amendment was made to form rules for protection of wildlife and introduction of specified plants to the list, and condition for possession by license. Subsequently, National Zoo Policy was framed in 1998, and Wildlife Stock Rules and National Board on wildlife were framed in 2003, and were further amended in 2014. In 2006, National Tiger Conservation Policy and Rules were framed. The Zoo Rules were framed in 2009. Further amendments in National Tiger Conservation Authority and for Wildlife Wardens were made in 2012. Amendments regarding prohibiting sale of zoo animals were also made in 2012 [18] [19]. An amendment of the same was introduced by Indian Government as Wildlife Protection Amendment Act (2022) further increasing the protection for wildlife [20]. Despite several laws and policies being put in place in India and simultaneously in many other countries across the globe, illegal hunting and trading continues. Enforcement of these laws has not been effective in reducing the illegal trade or in bringing offenders to justice. Failing in enforcement may also be due to the lack of scientific investigation techniques, which could aid in detecting such crimes and in gathering sufficient evidence to prosecute offenders. Forensic science can play an important role in yielding better results.

FORENSIC TECHNIQUES & ITS APPLICATIONS IN WILDLIFE CRIMES:

Globally, an array of forensic methods is used to examine wildlife products, varying according to the type of accessibility, accuracy, and precision. Samples from confiscated wildlife products that forensic experts typically analyze include blood, meat, viscera, fur, nails, bones, teeth, tusk, genitals, feces, horns, etc. Here, we are reviewing two most commonly found mammalian samples at crime scenes, i.e., claws and hair samples.

CLAWS:

Felid claws are one of the most commonly traded wildlife products in illegal wildlife trading due to their small size and high demand. They are often worn as lucky charms or jewelry, and sometimes displayed as trophies. Various forensic tools have been used for species identification using claw samples, with varying degrees of success.

Morphological examination of 18 confiscated tiger and leopard claws performed by Sharma et al. [21] included burn test, X-ray screening and morphometric analysis followed by identification using DNA analysis. The burn test displayed the presence of keratin in the samples; X-ray screening exhibited no keratin density gradient or empty space present in the seized samples as seen in tiger or leopard claws, whereas morphometric analysis depicted four out of 18 confiscated claws were of leopard. Identification using DNA analysis of the four claws using

cyt-b and 16SrRNA genes matched that of *Bos taurus* gene sequence. Hence, the tests were able to distinguish between fake and real claw samples. (Refer table-I for details)

Claws	Numbers	Claw measurement (mean ± SE)		
		ac (in mm)	bc (in mm)	bc/ac
Tiger	23	22.56 ± 0.64	22.64 ± 0.50	1.01 ± 0.02
Leopard	49	16.55 ± 0.26	15.45 ± 0.26	0.94 ± 0.01
Seized	18	18.53 ± 0.35	21.68 ± 0.88	1.18 ± 0.05

Table -I:*Results of morphometric analysis: ac = the distance from the external coronary dermo-epidermal interface to the epidermis of the skin fold connecting the palmer flanges of the coronary horn, bc= the distance from the claw tip to the epidermis of the skin fold connecting the palmer flanges of the coronary horn* [21]

In his thesis, Italiya (2019) analyzed claw samples of lion, tiger and panther to compare microscopic and physical characteristics. (Refer table-II and III for details)

Characters	Tiger	Lion	Panther
Color	Dark blond	Light blond	Medium dark blond
Surface	Rough	Slightly smoother	Smoother and dark
Curve	Medium curve	Medium curve	Complete curve
Circle ring	Outer circle ring Absent	Outer circle ring present	Outer circle ring absent
Thickness	Broad	Medium thick	Bellow to lion claw thickness
Tip point	Broad and High Elliptical	Broad and Elliptical joint	Pointed and round
Hollow cavity	Present	Present	Present
Root portion	Open and uniform	Open and uniform	Open and uniform
Root cavity	Large open	Naturally medium open	Naturally small open

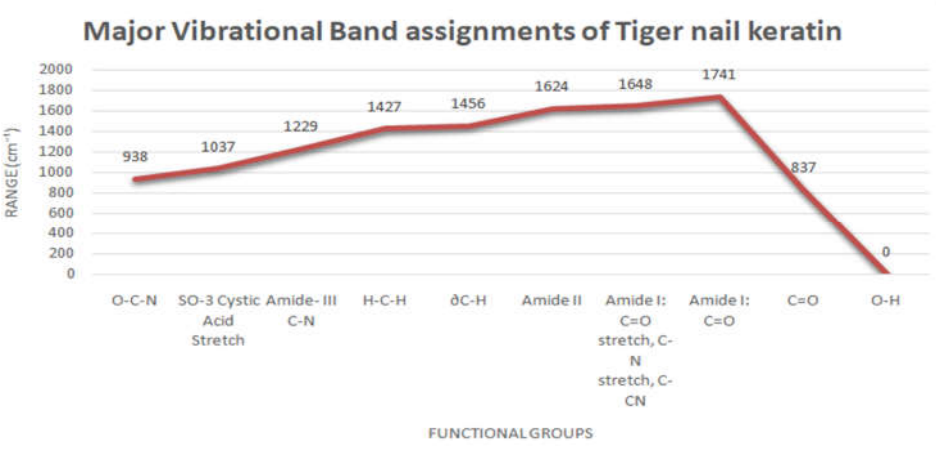
Table-II: *Physical characteristics of Tiger, Lion and Panther claw samples.* [22]

Characters	Tiger	Lion	Panther
Color	Dark Golden brown	Dark Golden brown	Dark Golden brown

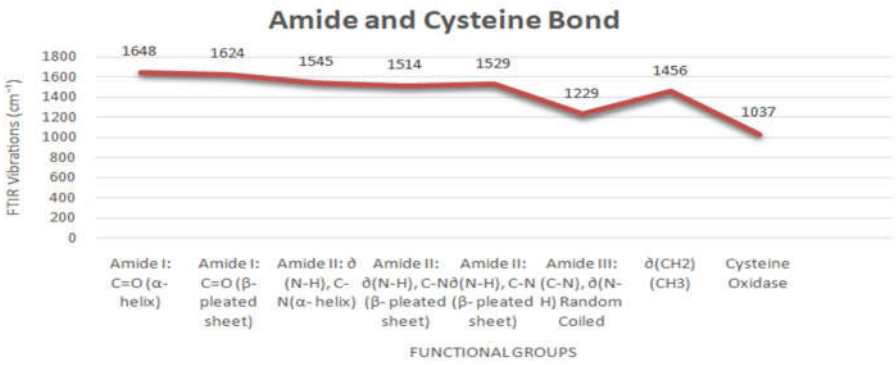
Pigmentation from Tip to root	Dense in Complete claw	Light and gradually increases	Very lower to higher
Hollow cavity	Near to tip	Complete	Complete
Scale	Diamond Petal	Diamond Petal	Diamond Petal

Table-III: *Microscopic characteristics of tiger, lion and panther claw samples.* [22]

Italiya et al. [23] studied 36 tiger claw (18 male and 18 female) samples using the Attenuated Total Reflectance-Fourier Transform Infra-Red (ATR-FTIR) technique. Scanning using ATR-FTIR and curved fit analysis of the samples in the spectral range 2000-500 cm^{-1} (spectral range for keratin) [24] exhibited regions comprising of bands like cystic acid vibrations, deformation and amide-I, II, III, CH2, CH3. The major vibrational bands found in tiger claw keratin were O-C-N; -SO-3 Cystic Acid Stretch; Amide – III C-N; H-C-H; δ C-H; Amide-II: 60% C-N stretch 40% N-H in plane band Minor Contributions C-C, N-C stretch, C=O; Amide-I: 80 % C=O stretch, C-N stretch, C-CN; Amide-I: C=O (α helix); C=O; O-H tyrosine with backbone containing peptide bonds and sulfur containing bonds. This helped providing the unique vibrational bands to identify the claw samples in future analysis. (Refer graph –I and II for details)



Graph-I: *Results of FTIR-ATR spectrum analysis of vibrational bands of functional groups present in keratin of tiger nail.* [23]



Graph-II: *FTIR-ATR vibrations in amide and cysteine bonds.* [23]

ATR-FTIR supplemented with principal component analysis (PCA), linear discriminant analysis (LDA) and partial least squares-discriminant analysis (PLS-DA) models were used by Sharma et al. (2019) [25] to differentiate 31 reference claw samples of 15 Indian Leopards and 16 Royal Bengal Tigers and 10 fake claw samples. PLS-DA and LDA models exhibited significantly high differentiation between the Indian Leopard, Royal Bengal Tiger and fake claw samples whereas; PCA could not make a distinction between any of the samples. Besides, the analysis to differentiate between the unknown samples by PLS-DA model gave the R-square value of 0.99, which is highly significant for analytical examinations making it more preferable to use as a supplement instead of PCA and LDA models.

Khedkar et al. [26] used COI bar-coding to identify 21 unknown claw samples confiscated in Nasik, India. For the examination, mitochondrial COI genes were amplified using a mixture of novel primer. This led to the identification of three samples as *P. pardus* and eight samples as *P. leo* on species level whereas the five samples could only be identified to family due to non-availability of reference animal sequences. COI gene bar-coding was able to accurately differentiate leopard, lion and other samples on family level out of 21 unknown claw samples.

In their research, Ashrifurrahman et al. identified samples from unlawful trafficking using genetic indicators such as the CO1 gene. The 20 claw samples used in this study included 13 samples from the Dharmasraya Sumatran Tiger Rehabilitation Centre and seven suspected samples from cases of illegal wildlife trade in West Sumatra, Indonesia. Each sample was separated, sequenced, and subjected to PCR analysis. With 99.60%–99.70% similarity with the *P. tigris* reference sequence and 99.90%–100% with *P.t. sumatrae*, the results verified that every sample was *P.t. sumatrae*. Phylogenetic analysis revealed a monophyletic group with an average intraspecies sequencing divergence of 0 to 0.4%, supporting the identification of species. [27]

HAIR :

Hair is one of the unique identifying characteristics of mammals. It is easily shed over many places posing as valuable evidence for forensics. Sometimes the hairs of domestic animals are also found at crime scenes so it becomes necessary to differentiate and identify if the species belongs to a protected group.

Lisa Knecht [28] authored a chapter in a book titled Wildlife Forensic describing the various features of hair morphology which can be easily visualized using Scanning Electron Microscope (SEM). Hair samples of many mammals were examined and compared with the standards on the basis of their length, color, shape, size, curliness, pigmentation, appearance of medulla and stiffness. The observations displayed color variation due to uneven distribution of melanin and pigment granules. Roots of the mammal hairs were classified according to their shapes namely, club shaped, wine glass shaped, spade shaped and non-distinct. The medulla types and scale patterns were observed varying with different species for example, eastern pipistrelle with medulla absent and coronal scale pattern; deer mouse hair with uniserial ladder medulla; Appalachian cottontail hair with multiserial ladder medulla and double chevron scale pattern; American bison hair with simple unbroken amorphous medulla; Canadian lynx hair with simple

unbroken amorphous medulla; American marten hair with unbroken cellular medulla; Black bear hair with unbroken vacuolated medulla; Least shrew hair with unbroken with cortical intrusions medulla and Elk hair with unbroken lattice medulla. The study aided in documenting the unique characteristics of the mammalian hair for future references.

Thitika Kitpipit and Phuvadol Thanakiatkrai [29] studied tiger guard hair (24 hair samples per individual of four tigers) to observe 23 tiger hair morphological characteristics under light microscope providing a detailed qualitative and quantitative analysis. The observation exhibited variations due to individual tiger, body region and hair section. There were minute intra-species variations namely hair index, scale seperation, hair length and scale pattern in the samples. The data produced included nine qualitative characteristics in percentage and 14 quantitative characteristics. (Refer table IV and V for details)

Hair color	Black	36.8	**Scalepattern**	Regular wave	49.7
	Brown	6.9		Single chevron	40.9
	White	38.9		Irregular wave	5.2
	Yellow	17.4		Streaked	2.8
Cortex color	Black	9.0		Mixed	1.4
	Brown	38.9	**Cross-sectioncolor**	Black	42.0
	White	33.0		Brown	0.3
	Yellow	19.1		Red	1.4
Medulla type	Simple	64.6		White	18.4
	Uniserial ladder	4.2		Yellow	23.3
	Absent	24.0		Mixed	14.6
	Mixed	7.2	**Cross-sectionshape**	Circular	49.0
Scalemargin	Crenate	30.9		Concavo-Convex	27.8
	Rippled	29.9		Oval	23.3
	Smooth	34.4		Absent	5.2
	Mixed	4.9	**Cross-section medulla size**	Medium	81.6
Scaleseparation	Close	35.8		Small	13.2
	Distant	6.6			
	Near	56.9			
	Mixed	0.7			

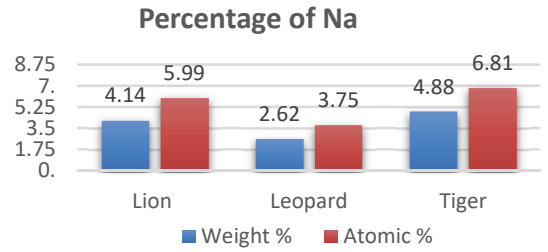
Table-IV:*Qualitative data of ninety-six hair samples (percentage mean of proximal, middleanddistalparts of tigerhairscalculated)* [29]

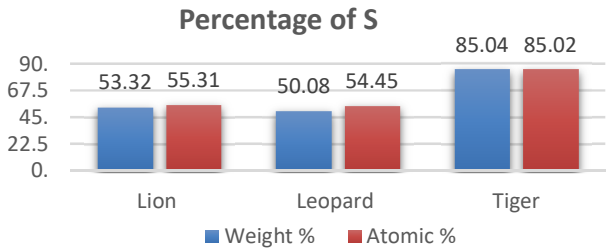
Features	Proximal	Middle	Distal
Length (mm)		14.4±7.9	

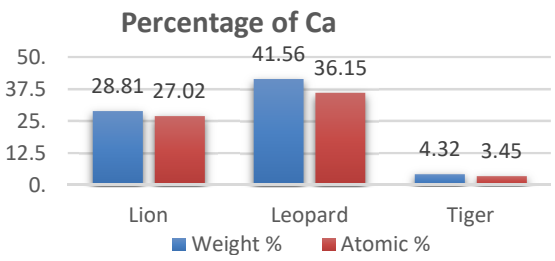
Proximalwidth (µm)		50±19.1	
Maximum width (µm)		73.1±21.1	
Medulla width (µm)	16.2±11.8	26.3±13.9	27±10.3
Scalewidth (µm)	36.4±9.4	46±12.6	34.4±13.6
Scaleheight (µm)	8.6±2.1	7.3±1.7	5.1±1.7
Cuticlewidth (µm)	1.4±0.7	1.8±1.1	1.9±1.2
Minimum diameter (µm)	53.8±20.3	67.4±19.8	55.5±21.1
Maximum diameter(µm)	61.2±23.0	77.6±23.0	62.5±23.4
Medullaryfraction	34.9±8.9	42.8±8.7	40.7±8.0
Hair width index		68.3±13.3	
Medulla index		35.2±11.3	
Hair index	87.9±8.7	86.9±9.8	89.1±11.4
Cuticle Index	2.4±1.6	2.4±1.6	3.4±2.0

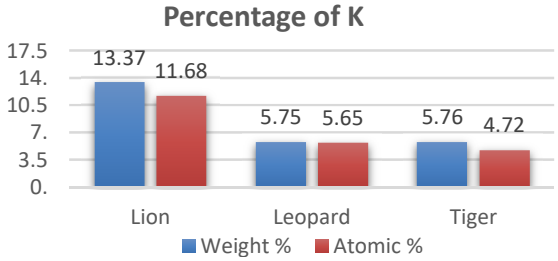
Table-V:*Quantitative data of ninety-sixtiger hair samples (mean± standard deviation calculated)* [29]

Dahiya M S and Yadav S K [30] used SEM coupled with Energy Dispersive Spectrum (EDS) to observe the morphological characteristics for species identification by small fraction of hair and their elemental analysis. They observed the cuticular scale measurements and layer difference pattern of 90 hair samples of lion, tiger and leopard (30 samples each) using scanning electron microscope. The scale layer pattern difference in overlapping of each scale showed a difference of 6.72431 ± 0.4, 7.869655 ± 0.38 and 9.592897 ± 0.5 µm for lion, tiger and leopard respectively. The elemental analysis by EDS coupled with SEM revealed the percentage of elements present in the samples aiding in species identification process along with potential to provide the geological origin of the sample. (Refer Graph III, IV, V and VI for details)









Graph-III, IV, V & VI: *The weight percentage and atomic percentage of (graph-III) sodium (Na), (graph-IV) sulphur (S), (graph-V) calcium (Ca) and (graph-VI) potassium (K) respectively in lion, leopard and tiger* [30]

A study was conducted by Karmacharya et al. (2018) [31] to examine 13 skin samples and two blood smeared knives using molecular forensics approach to identify the species and their sex. The samples were examined using a PCR assay that used tiger specific mtDNA Cytochrome-b primers and amplified 162 bp target PCR. The PCR products were compared with the reference baseline prepared by analyzing tiger fecal matter from different national parks of Nepal. Sexes of the identified samples were determined by amplifying the Amelogenin gene of sex chromosomes. Examination of samples revealed that all 15 samples were of tiger having ten males showing two PCR bands (194 bp and 214 bp) and five females showing one PCR band (214 bp). The analysis aided the experts to confirm the species along with differentiation of sex of the identified species.

Singh et al. [32] studied hair samples of few selected wild felids using morphological method and forensically informative nucleotide sequencing (FINS). For the identification and differentiation, they selected the following species: leopard cat (*Prionailurus bengalensis*), jungle cat (*Felis chaus*) fishing cat (*Prionailurus viverrinusis*), wild cat (*Felis silvestris*) and caracal (*Caracal cracal*). Tissue and hair samples of all the felids were collected except wild cat (only tissue sample collected). Microscopic examination of the hair samples showed irregular, smooth, near wave pattern for cuticle and percentage medulla index between 60-80 same as other felids. These observed characteristics alone were not informative enough to differentiate between the members of felid family. For FINS they observed maximum variation in Cyt b in comparison to12S rRNA and 16S rRNA genes. (Refer table VI for details)

Genes	Variable Sites	Parsimony Informative Sites	Singleton Variable Sites	Percentage nucleotide composition (T/C/A/G)
12SrRNA	21	11	10	23.0/22.7/36.2/18.0
16SrRNA	28	19	9	23.5/23.0/32.8/20.7
Cyt-b	65	44	21	27.95/29.25/28.9/13.9

Table-VI: *Number of variable sites, parsimony informative sites, singleton variable sites and percentage nucleotide composition observed in the genes of leopard cat, fishing cat, jungle cat, wild cat and caracal.* [32]

Basic Local Alignment Search Tool (BLAST) result for the mentioned sequences showed 99-100% similarity with their respective species. Hair morphological characteristics along with FINS accurately identified and differentiated the members of felid family on species level.

In research conducted by Talia et al. [33], fifteen hairs from the lumbar area of an animal that had been confiscated were analysed using a variety of criteria, including size, shape, thickness, colours, and cuticular and medullary patterns. Medullary patterns were analysed using optical

microscope pictures, while cuticular patterns were analysed using Scanning Electron microscope (SEM). Measurements were made to determine the medulla diameter, total hair diameter, cuticle thickness, and number of scales in 100µm after images were examined using an optical microscope. PCR was utilized to extract and amplify DNA from tissue samples for genetic identification. Scanning electron microscopy (SEM) and optical microscope images were used to investigate the hair sample. The medullary pattern was trabecular, and the cuticular pattern was wavy, with 11 cuticles per 100 µm. The HCO, LCO, and 16Sar/16Sbr areas were amplified, according to PCR verification. According to the Panthera onca or Jaguar mitochondrial DNA reference sequence, DNA analysis revealed 93% compatibility with COI gene regions and 100% compatibility with amplified 16S ribosomal gene regions.

A study by Shinta et. al. [34] used FTIR (KBr pellet used) spectroscopy to discriminate between Indian grey mongoose hair, domestic cattle hair, human hair, and synthetic fibre. The results showed that synthetic fibre could be visually distinguished from hair, suggesting that seized brushes may contain hair or other materials. However, a slight spectral difference was observed in the hair of Indian grey mongooses compared to domestic animals. The primary biochemical component of animal fibres is keratin. The study found no visual difference in the spectra (except Indian grey mongoose hair) as all hair is made of keratin proteins. The study identifies amide A as the primary amide in keratin, with peaks at 2965 cm−1 and 2930 cm−1 representing asymmetric and symmetric n(CH3). Chemometric analysis was used to distinguish Indian grey mongoose hair from domestic cattle hair, human hair, and synthetic fibre. The study used PLS-DA analysis to differentiate between Indian grey mongoose hair, domestic cattle hair, human hair, and synthetic fibre based on their IR radiation absorption intensity. The model, consisting of three latent variables, showed species-wise clustering and differentiation, with an R-square value of 0.9 and an RMSE value of 0.13. The model effectively highlighted differences in FTIR spectra. It reveals that keratin protein in hair varies across species, impacting IR absorption properties. PLS-DA analysis identified variations in amide I and II regions of hair, allowing for discrimination. FTIR spectroscopy combined with chemometric analysis can identify Indian grey mongoose hair, domestic cattle, synthetic fibre, and human hair.

DISCUSSION:

This study could act as the compilation of major forensic techniques used between 2004- 2024 to aid in investigating wildlife crimes. The study has effectively high-lighted the importance of forensics in wildlife protection and to trace the transition of forensic tools from destructive to non-destructive techniques along with the list of these tools and observations based on their analysis. The various destructive and non-destructive techniques employed in identification of mammals or mammalian products across the globe have been catalogued. Traditionally the forensic scientists depended mostly on the destructive methods as they had the maximum accuracy. Some of these techniques are reviewed in this study, namely Genetic and Molecular techniques, COI bar coding, FINS, Burn test, PCR (cyt b gene, D-loop, NDH dehydrogenase,

Amelogenin gene). Other studies not included in the paper also include use of ARMs PCR, nested PCR and SSPs for forensic identification of species.

These techniques are capable of accurately identifying the species with the disadvantage of being expensive, time consuming and destroying the part of sample used in the analysis. With the advancement of science over the years, the forensic techniques have also advanced and have gradually transitioned from using destructive techniques to non-destructive techniques. The non-destructive techniques reviewed in this study are X-ray screening, morphometric analysis, ATR-FTIR, ATR- FTIR Coupled with PLS-DA, Microscopy, SEM, EDS- SEM. Other techniques that are widely used now a days include XRF, hand-held XRF, ICP-MS, ICP-AES, ICP-MS, ICP-AES for species identification of other wildlife products by analyzing the elements present in the samples. They also help in determining their geo-location and hence, locating the wildlife poaching hotspot.

Non-destructive techniques provide a cheaper, faster and accurate way for forensic analysis of wildlife samples. The use of non-destructive techniques rather than destructive ones will also help the police to maintain the chain of custody.

CONCLUSION:

The study shows how the various forensic techniques are vital to the determination of species of animals and how this information can be conclusively and decisively used by the law enforcement agencies in prevention, investigation and detection of crimes against wild lives. The development in forensic science and emergence of newer methods and technologies coupled with advanced researches and databases have increased the power of wildlife identification up to species level in a very convenient manner. The best choice of methods adopted for identification and its differentiating ability is also made known through inter-comparison of various forensic techniques. The finer intra-comparison of the molecular forensic techniques and its identifying power demonstrates the great advancements in the field of wildlife forensics which when properly implemented would prove to be great enablers in identification of exact species involved in any wildlife crime.

Whereas, the use of the non-destructive techniques to make these identifications can prove to be a better technique as compared to the destructive techniques. The forensic experts can make use of non-destructive as primary techniques for identification. The use of destructive techniques should be an option in case of insufficient accuracy while testing some samples.

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