iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 13 **Issue:** IV **Month of publication:** April 2025

DOI: https://doi.org/10.22214/ijraset.2025.68372

www.ijraset.com

Call: 08813907089 | E-mail ID: ijraset@gmail.com

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538
Volume 13 Issue IV Apr 2025- Available at www.ijraset.com

Dandruff as a Forensic Biomarker: A Novel Approach in Trace Evidence Analysis

Sandhya Pandya
Parul University, India

Abstract: ***The forensic application of mitochondrial DNA (mtDNA) recovered from dandruff sampled using different methods, such as nail clippings, combs, hairbrushes, pillowcases, and clothes, is investigated here. Though sometimes neglected in forensic investigations, dandruff holds nucleated cells that contain useful genetic information. This paper seeks to develop a successful and effective means of recovering mtDNA from dandruff, particularly in instances where traditional sources of DNA are not available or are deteriorated.Dandruff samples were also obtained from volunteers by employing a variety of non-invasive approaches: nail clippings accompanied by attached dandruff, hairbrushes, combs, pillowcase, and apparel. mtDNA was isolated through a commercial kit for DNA isolation, and detection was done with the help of gel electrophoresis. For confirmation of accuracy and reliability of the extracted DNA, quantitative analysis was carried out to determine DNA yield, while Polymerase Chain Reaction (PCR) amplification and sequencing was carried out to check mtDNA quality and purity. The results confirmed successful mtDNA extraction, where high-quality sequences were applicable for forensic use.The study confirms that dandruff is a suitable and easily accessible source of DNA for forensic analysis. Its ability to be easily collected from personal items without contact makes it particularly useful in circumstances where traditional DNA samples are unavailable. The method can be applied to aid crime scene investigations, suspect identification, cold case solution, mass disaster victim identification, and DNA degradation research.Also to be considered are ethical concerns surrounding consent and privacy, as dandruff naturally flakes off and can be sampled without the knowledge of the individual. The current study presents dandruff as a significant and useful forensic source of DNA and calls for future research that would allow the implementation of this method in forensic analysis.***

Keywords: DNA extraction, PCR, electrophoresis, forensic genetics, mtDNA, dandruff, and nail clippings

I. INTRODUCTION

A. Overview

The mitochondria is present in cell in abundant amount, as we know that the function of mitochondria is to provide energy to cell for biological activities, however there are more than that. In aspect of Forensic Science, Genetics the mitochondria has significant role here. If we trace back very far to the beginning of mitochondria researches have established that the mitochondria was a bacterial cell engulfed by the eukaryotic cell but wasn't digested and the symbiotic relationship began there. The human mitochondrial DNA (mtDNA) is a 16 569 bp double-stranded circular molecule and includes 37 genes encoding two rRNAs, 22 tRNAs and 13 polypeptides.Human mitochondrial DNA has many uses i.e. missing person identity, war or disaster victim identification, maternal inheritance cases, government databases etc. In comparison to nuclear DNA(nDNA) mitochondrial DNA(mtDNA) is present in abundant amount however that’s not only benefit but mtDNA can sustain in harsh environment conditions also for sample which are exposed for too long can also be useful for mtDNA analysis unlike nDNA which degraded gradually as passage of time.

Traditionally blood, saliva, semen these biological fluids are used as primary sources for DNA analysis in Forensic investigations however dandruff can also be used as a source for DNA analysis,, is a flaky skin that sheds from scalp. However dandruff hasn’t been thoroughly studied in forensic science due to its readily availability at any particular crime scene. Nonetheless the use of dandruff as a primary source of DNA has potential advantages which includes that dandruff is less invasive and easily accessible than that of traditional biological fluids/evidences. Dandruff also contains Higher quantity of DNA than any other biological evidences, helps in obtaining complete DNA Profile of an individual from small quantity of evidence. There aren't many organic extraction procedures or kit-based techniques specialized for dandruff analysis, even though it has the highest quality genetic materials. Nevertheless, we still receive the best results from this kit-based practical.

B. Mitochondria

Due to their primary involvement in energy generation through oxidative phosphorylation, the mitochondrion is regarded as an indispensable organelle frequently referred to as the powerhouse of the cell. More significantly, mitochondria are now understood to contribute heavily to several functions of a cellular nature such as metabolic signalling, calcium homeostasis, and apoptosis. Recent discoveries now give an explanation of how dynamics and function characterize the relationships that exist both towards health and illness.

1) Mitochondrial features

- Copy Number: Many hundreds to thousands of mitochondria per cell each with many thousands of copies of mtDNA allowing it to more readily be extracted from old, degraded samples that may not easily yield nuclear DNA with only two copies per cell.
- Maternal Inheritance: mtDNA is transmitted from the mother only, and it is hence feasible to trace maternal lineage. It is of the greatest use in identifications involving missing persons or mass catastrophes where relationship can be demonstrated by maternal family relationship
- Lack of Recombination: mtDNA does not undergo recombination in reproduction like nuclear DNA. This stability allows for a consistent lineage but it can restrain at some extent the identification of an individual as relatives share the same mtDNA sequence.
- High Mutation Rate: mtDNA has a quite high mutation rate in comparisn to nuclear DNA. This characteristic can be utilized in determine between close relatives, such as monozygotic twins, by identifying individual mutations or SNPs.

2) Forensic Significance of mitochondria

- Identification of Degraded Samples: mtDNA is of tremendous value in forensic investigations with very degraded biological materials like bones, teeth, and hair. Through mtDNA, scientists have the power of obtaining it regardless of missing nuclear DNA.
- Mass Disaster Victim Identification: Since mtDNA offers genetic data from compromised samples, its examination is widely utilized in disaster victim identification (DVI).
- Exoneration Cases: Defence lawyers increasingly use mtDNA testing to reverse convictions based on weak or degraded evidence, thereby facilitating the exoneration of innocent individuals.
- Studies in History and Archaeology: Apart from criminal cases, mtDNA has been utilized to analyse ancient human remains and historical artefacts, thereby shedding light on ancestry and population genetics.

C. Mitochondrial DNA or mtDNA

Mitochondrial DNA (mtDNA) is immensely useful in forensic science, particularly in degraded biological samples where nuclear DNA is not viable. Its intrinsic features make it enormously useful for the identification of human individuals in various forensic applications.

Key Features of Mitochondrial DNA in Forensics

1) High Copy Number: There is copious amount of mitochondria in every cell of human body whether it is blood cells, skin cells, calciferous tissues etc and it is compared to two copies of nuclear DNA. This copiousness makes successful analysis from deteriorated sources like bones, teeth, and hair possible.

2) Matrilineal Inheritance: mtDNA is only passed down from the mother to the offspring, so maternal family members will share identical mtDNA sequences. This characteristic enables forensic analysts to make comparison using reference samples obtained from maternal relatives, especially for application in missing persons cases and mass disaster recoveries.

3) Resistance to Degradation: mtDNA may be retrieved from environmental degradation-exposed samples, thus providing a solid source of genetic data when nuclear DNA has degraded.

4) How Mitochondrial DNA is different from nuclear DNA?

5) Mitochondrial DNA and nuclear DNA are major organelles pf eukaryotic cells, both having unique structures, functions and characteristics. These are mentioned below :

a) Location and Structure

Mitochondrial DNA is found inside the mitochondria, which are organelles that are present in the cell which is responsible to generate energy. It is a circular molecule and contains about 16,569 base pairs, coding for 37 genes, of which 13 are proteins necessary for mitochondrial function, 22 tRNAs, and 2 rRNAs.

Nuclear DNA however, is located in the cell centre or nucleus and is arranged as linear chromosomes. The human nuclear genome contains approximately 3 billion base pairs that code for 20,000 to 25,000 genes responsible for a vast array of cellular processes.

b) Inheritance Patterns

mtDNA is passed on solely from the mother (maternal inheritance), since sperm mitochondria are normally destroyed and disintegrated following fertilization. This leads to a haploid genome that does not recombine. Therefore it is utilized in cases of maternal inheritance in forensic science.

Whether, nDNA is inherited from both parents (biparental inheritance) and is diploid, consisting of two copies of each chromosome. It recombines during meiosis, which is responsible for genetic variation among offspring.

c) Genetic Characteristics

- Mutation Rates: mtDNA is more mutable than nDNA. This increased rate is contributed to, in part, by its closeness to reactive oxygen species produced during mitochondrial ATP production.
- Gene Expression: Transcription of mtDNA is polycistronic (several genes per mRNA), whereas transcription of nDNA is monocistronic (one gene per mRNA) and conforms to the universal codon usage rule.
- Histone Packaging: mtDNA does not have anything to do with histone proteins, which play a vital role in packaging of nDNA as chromatin bodies inside the nucleus. This contrasts with the mode of regulation and expression of both types of DNA.

d) Functional Implications

Mitochondrial DNA chiefly contains genes essential for oxidative phosphorylation and metabolism of energy. Nuclear DNA contains a wider spectrum of proteins used in numerous cell processes such as development, cell cycle control, and adaptation to environmental changes.

Presence of nuclear-embedded mitochondrial sequences (NUMTs) reflects past exchanges of mtDNA into the nucleus, an aspect of evolutionary specialization and communication between these two genomic systems.

Such differences illustrate how mitochondrial and nuclear DNA complementarily contribute to cell function and transmission. The insights from such distinctions are vital in areas like genetics, evolutionary biology, and medicine.

D. Dandruff

Development of Dandruff The primary cause of dandruff is due to an overgrowth of a yeast-like fungus known as Malassezia, which exists naturally on the scalp. The fungus is sustained by the oil secreted by the skin. If it is in its favor to overgrow—such as with more secretion of oil or irritation of the skin—it may establish an inflammatory cycle and over-shedding of skin cells. In individuals with dandruff, skin cells that would normally take about 28 days to develop and then fall off might be replaced every 2 to 7 days, resulting in visible flakes. But individuals having sensitive skin and recurring dry skin also experience such issues as dandruff and itching of scalp.

Dandruff is a scalp infection that results in small flakes of dead skin to shed, and itching and reddening. Dandruff is usually non-contagious and harmless but embarrassing to most individuals. Formation of Dandruff The yeast fungus Malassezia, which forms naturally on the scalp, has been considered as the primary cause of dandruff. The fungus feeds on the oils secreted by the skin. Also, most people have dry scalps, which are due to dead skin cells and help in forming dandruff.

Benefits of Utilizing Dandruff in Forensic Science

1) Non-Invasive Sampling: Dandruff is readily collected from people or personal belongings, a less invasive method than using blood or saliva samples.

2) Stability and Resistance to Degradation: Dandruff is more stable and less prone to degradation than most biological samples, as it is vital to preserve DNA integrity over time.

3) Higher DNA Density: It was found through experiments that dandruff has a higher human DNA concentration, hence a higher possibility of a full DNA profile being obtained. With 30 to 40 ng of DNA enough, one could obtain DNA from as low as 1.0 to 1.5 mg of dandruff.

E. Sources of DNA evidence

DNA evidence is a major component of forensic science, with verifiable means of identifying criminals involved in criminal cases. There are various biological fluids that can be sources of DNA evidence and have varied types and quantities of DNA recoverable to be analyzed.

Sources of DNA Evidence

1) Whole Blood: It is one of the most commonly used sources and contains high concentrations of nucleated cells, thus ideal for DNA profiling. The blood can be preserved with anticoagulants and kept under refrigeration for later analysis.
2) Saliva: This bodily fluid can be taken on several surfaces or even directly from the individuals themselves. Saliva contains sufficient DNA, whose concentration is about 5,000 ng/mL.
3) Semen: Particularly beneficial in the instance of sexual assault, semen is a densely packed DNA source that is normally found to contain up to 250,000 ng/mL.
4) Urine: Less commonly used due to decreased DNA content (1-20 ng/mL), urine may serve as a source where other sources cannot be accessed.
5) Other Biological Sources: They include swabs from the vagina, postcoital samples, and also bite injuries where they have saliva. All these samples can provide invaluable DNA evidence in forensic investigations. This can be used to identify offenders by performing DNA profiling but not always to find offenders but to identify victims of mass disaster, paternity cases, government records etc.

F. Traditional sources of DNA vs Dandruff as non invasive source of DNA

In contrast to more conventional sources like blood and saliva, dandruff has become a novel form of DNA evidence in forensic research, with both benefits and drawbacks. Conventional DNA Evidence Sources Typical Sources: Blood is a good source for DNA extraction since it is incredibly dependable and rich in nucleated cells. Although saliva contains nucleated cells, it may not recover as well as blood. Although they provide high-quality DNA, tissues and organs are frequently more invasive to get. Hair: Helpful if there is a follicle, although the quality of the DNA varies greatly.

Benefits of Conventional Sources: established techniques for analysis and extraction. It is possible to acquire high-quality DNA profiles, which are essential for identification in criminal instances.

Benefits of Using Dandruff as a DNA Evidence Source

1) Accessibility: Dandruff is easy to gather from people without their active participation because it is non-invasive.
2) Stability: DNA purity can be preserved for a longer period of time because dandruff is less prone to deterioration than other biological materials. Dandruff may have a larger concentration of human DNA than other biological materials, according to studies, which increases the possibility of getting a full profile.

G. DNA Extraction methods from keratinized cells/tissues

For DNA extraction from keratinized tissues and cells, there are conventional techniques as well as kit-based approaches available conventional techniques take good amount of time and efforts but kit-based approaches are way more convenient and practical and works fine for time limitations

DNA recovery from keratinized cells, including hair shafts, nails, dandruff and stratum corneum of the skin, needs specific techniques to degrade the rigid keratin matrix and liberate nucleic acids. The main techniques and aspects for DNA isolation from these robust cellular structures are listed below:

Chemical Digestion with Reducing Agents

A variation of the phenol-chloroform technique employing dithiothreitol (DTT) and proteinase K works well to dissolve keratinized material such as hair shafts. Important steps are:

Digestion Buffer: Mixes DTT (powerful reducing agent to cleave disulphide bonds in keratin), SDS (anionic detergent), and proteinase K (to break down proteins).

Incubation: Incubate at 56–60°C for a long period of time (up to 4 hours) to completely dissolve the hair or keratinized tissue.

Organic Extraction: Phenol-chloroform-isoamyl alcohol extracts proteins and cellular waste, and then ethanol precipitation to purify DNA.
This process provides PCR-ready DNA appropriate for mitochondrial or nuclear targets, although purity of the DNA can be variable compared to blood samples.
Kit-based methods for DNA extraction from dandruff include the utilization of commercially purchased DNA extraction kits that are specifically formulated for biological samples like skin flakes, hair, and buccal cells. These kits simplify the process by offering pre-formulated buffers, enzymes, and columns or beads for DNA purification.

H. DNA Quality and Quantity check

To preserve the integrity and viability of DNA samples for analysis, quantitation and quality control (QC) are critical processes. The following is a step-by-step description of methods utilized for determining DNA quality and quantity.
DNA Quality Control (QC)
Purpose of DNA QC:
Integrity:
Ensures that the DNA remains whole and not fragmented, which is important for applications downstream, such as sequencing.

Purity:
Verifies against contamination by proteins, RNA, or other organic solvents that may influence results.

Methods of DNA QC:
Gel Electrophoresis:
Unrestricted or pulse-field gel electrophoresis (PFGE) can be employed to determine the size and integrity of genomic DNA.
Ordinary gel electrophoresis gives an overall sense of integrity but might not properly represent the size of high molecular weight DNA.

Spectrophotometry:
DNA purity can be determined through absorbance measurements at 260 nm and 280 nm. The ratio260/A280can be used to estimate purity. A260/A280should be between 1.7 and 1.9 for pure DNA.This technique can signal contamination; e.g., a ratio well above 1.8 can indicate RNA contamination.

I. UV-VIS Spectrophotometer

Principles of UV-Visible (UV-Vis) spectrophotometry
Ultraviolet and Visible absorption spectrophotometry is the method founded on attenuation of electromagnetic radiation measurement by an absorber [9]. Such radiation, has a spectral range roughly between 190–800 nm, which also vary in terms of energy ranges, and mode of excitation from other allied regions (Table 1). This attenuation is caused by the reflection, scattering, absorption or interferences. Nonetheless, precise measurements of the attenuation can be accomplished recording.
Instrumentation Components In order to measure how much light a sample absorbs, a UV-Vis spectrophotometer is made up of many main components that cooperate. The following are:

1) Light Source : Lighting Source To cover or illuminate both the visible and ultraviolet spectrum, the spectrophotometer needs a sufficiently stable high-intensity light source. Lamps that emit a continuous spectrum in both UV and visible ranges (190–400 nm) and tungsten-halogen lamps (400–800 nm) are common sources.
2) Entrance Slit : Slit at the Door The entrance slit controls the incident light beam's width and direction to provide uniform illumination for the monochromator. By doing this, stray light is reduced and resolution is improved.
3) A monochromator A broad spectrum is emitted by the light source, which is separated into separate wavelengths by a monochromator.An entry slit, among them, is an entry slit.A dispersion device (e.g., prism or diffraction grating) let only the desired wavelength of light pass through an exit slit.The dispersion device separates different wavelengths and then passes the desired wavelength to the sample.
4) The Cuvette or Sample Holder : A cuvette is generally employed to hold the sample, which can facilitate even light transmission. The cuvette materials differ based on their application: glass or plastic cuvettes (visible range), quartz cuvettes (UV range). While cuvette lengths differ based on application, the default is 1 cm.

5) Detectors

The role of a UV-Vis detector is to transform an electric signal from a light signal. Ideally, it should respond over a broad wavelength range, respond sensitively but with little noise, with a linear range of response, respond quickly, enable miniaturization and a low sample consumption. In attempting an ideal detector, numerous detectors, subsequently described, have been made in recent years.

A data acquisition system processes the electrical signal from the detector and converts it into a transmittance or absorbance measurement. Uses UV-Vis spectrophotometry is used extensively in both industrial and scientific activities, including: Identification and measurement of chemical compounds is known as chemical analysis. Assays for protein, DNA, and enzyme activity in biochemistry and the biological sciences Pharmaceutical analysis, including quality control and drug formulation, Environmental science: tracking air and water pollutants, and Material science:Characterization of thin films, coatings, and nanoparticles.

For research on molecule absorption in the ultraviolet and visible spectrums, UV-Vis spectrophotometry is a dependable analytical technique. The method applies the Beer-Lambert Law and principles of light-matter interaction to give precise and dependable measurements for numerous research and commercial uses. It is a fundamental technique in current-day analytical laboratories because it is easy, sensitive, and versatile.

J. Polymerase Chain Reaction

PCR amplification finds significance in the recovery and analysis of mitochondrial DNA (mtDNA) from keratinized tissues such as dandruff and hair shafts. It is challenging to analyze keratinized cells for DNA due to the presence of trace quantities of DNA and inhibitors. However, owing to the high number of copies and stability of mtDNA, it is a significant target under such circumstances.

1) PCR Amplification in mtDNA Isolation from Keratinized Tissues:

- Microdissection and Analytical PCR: It entails individualizing certain portions of keratinized tissues followed by PCR amplification for the analysis of mtDNA. It is especially handy when working with trace amounts of DNA characteristic of such samples.
- Quantitative PCR (qPCR): qPCR is used to quantify and detect mtDNA damage and copy number variations in keratinized cells. qPCR is sensitive enough to detect without the necessity of mitochondrial isolation.
- Long-Range PCR: Long-Range PCR is used to evaluate mtDNA integrity by amplifying larger DNA fragments, which gives information on possible damage in the mitochondrial genome.
- Direct PCR Amplification: Direct PCR amplification from difficult samples, e.g., touch DNA, is made possible by certain protocols without pre-extraction of DNA. It can be used for keratinized tissues as well to make the analysis more efficient.

2) Challenges and Considerations:

Keratinized tissues present certain challenges because of the presence of inhibitors such as melanin, which can influence the efficiency of PCR. It is essential to optimize DNA extraction techniques and use the right DNA polymerases to maximize amplification success rates.

In conclusion PCR amplification is part and parcel of mtDNA extraction and analysis in keratinized tissues. Using specialized techniques and overcoming inherent challenges can enhance the validity of results in forensic and research contexts.

K. Gel Electrophoresis

Electrophoresis in Gel Overview A popular laboratory method for separating, visualizing, and analyzing proteins and nucleic acids (DNA and RNA) according to their size and charge is gel electrophoresis. Gel electrophoresis is essential for verifying the existence of retrieved mitochondrial DNA (mtDNA) and evaluating its quality and integrity in the context of mtDNA study from dandruff isolated from nail clippings.

The Gel Electrophoresis Principle : The foundation of gel electrophoresis is the idea that when an electric field is applied, negatively charged DNA molecules flow across a gel matrix. The following factors determine how quickly DNA pieces move, DNA molecule size, Larger DNA molecules travel slowly, while smaller pieces travel farther and faster.

Gel concentration: Higher agarose concentrations provide higher resolution for smaller DNA fragments, whilst lower concentrations are ideal for bigger fragments. Electric field strength: Migration speed is influenced by the voltage across the gel, but too high a voltage might distort or overheat the gel.

Different kinds of gel electrophoresis exist. These are as following

1) Agarose gel electrophoresis is a popular molecular biology technique for separating nucleic acids, including mtDNA.
2) PAGE, or polyacrylamide gel electrophoresis, It is typically used to assess tiny proteins or DNA because of its higher resolution. The most effective method for assessing mtDNA is agarose gel electrophoresis.

Materials and Reagents

- Agarose powder is utilized to prepare the matrix gel or casting gel in which the sample is placed and that is utilized to separate DNA.
- An electrophoresis buffer, for this we have Tris-Acetate-EDTA (TAE) or Tris-Borate-EDTA (TBE) buffer is utilized to provide pH and ionic strength.
- Intercalating dye Ethidium Bromide (EtBr) or SYBR Safe dye can be employed to visualize DNA under UV light. Handle EtBr dye carefully due to its extremely carcinogenic nature always wear gloves and lab glasses, mask and apron.
- The DNA ladder (molecular weight marker) is a reference for the estimation of fragment size. The ladder is not required if the goal is to only visualize the DNA loading dye and density reagent : to enhance loading by providing color contrast and enhanced sample density, since loading dye Bromophenolblue may be utilized other dyes may be xylene cyanol, orange G, and cresol red and to increase sample so that it won't get confused in buffer glycerol may be utilized in volume of 10%/volume of sample.
- The power supply and electrophoresis chamber supply the electric field to allow for DNA migration.
- A Gel Documentation System or UV transilluminator can be employed to locate and photograph stained DNA.

II. MATERIALS AND REQUIREMENTS

A. *Materials*

The following materials and equipment were utilized in this research:

1) *Reagents and Kits*

- Commercial DNA Extraction Kit
- Lysis buffer (included in the kit)
- Ethanol (used in washing steps in extraction)
- Elution buffer (used for final recovery of DNA)
- Agarose powder (used in gel electrophoresis)
- Ethidium bromide or SYBR Safe stain (used for visualization of DNA)
- 1X TAE (Tris-Acetate-EDTA) buffer (used in running the gel)

2) *Equipment*

- Microcentrifuge
- Vortex mixer
- Water bath/incubator (utilized in lysis step)
- Gel electrophoresis system
- UV transilluminator or Gel Documentation System
- Micropipettes and sterile filter tips
- Sterile microcentrifuge tubes
- PCR unit
- Thermocycler

III. LITERATURE REVIEW

1) Sara, dua. MicroBites, 3 April 2024. "Dandruff as Explained by Forensic Science." According to Sara Dua's article "Dandruff as Explained by Forensic Science," dandruff can be used as a biological sample for DNA analysis in forensic science.

Given its ease of collection and nucleated cell composition, dandruff can be used in forensic DNA analysis in place of more traditional biological samples like blood and saliva. The study aims to understand how Malassezia bacteria and yeasts cause dandruff and how their stability in many environments makes them useful as forensic agents. Its application in forensic analysis has to become commonplace.

2) Marek, Uvizl, and colleagues, "Comparative Genome Microsynteny Illuminates the Fast Evolution of Nuclear Mitochondrial Segments (NUMTs) in Mammals." Evolution and Molecular Biology, vol. 41, no. 1, 2024. In their study "Comparative Genome Microsynteny Illuminates the Fast Evolution of Nuclear Mitochondrial Segments (NUMTs) in Mammals," Uvizl et al. examine nuclear genomes from 45 different mammalian species to examine mitochondrial DNA segments called NUMTs.Rapid turnover of NUMTs, preferential integration into intergenic, transposon-rich areas, and their potential as phylogenetic markers in the evolution of the mammalian genome are shown by their findings.

3) Silva, D. A. D., and E. F. Carvalho, "Forensic Use of Human Mitochondrial DNA: A Review." Volume 96, Issue 4, **2024**, Anais da Academia Brasileira de Ciências.Due to its large copy number and maternal inheritance, mitochondrial DNA (mtDNA) is useful in forensic research for examining deteriorated materials.The precision and dependability of mtDNA analysis have increased thanks to developments like Next-Generation Sequencing. Heteroplasmy and nuclear mitochondrial DNA segments (NUMTs) are obstacles that call for uniform standards for reliable forensic use.

4) 4.Rajput, I., and Mandal, P. "Isolation and Extraction of DNA from Dandruff: A Novel Approach for Forensic Science." Volume 10, Issue 5, 2023, pages 481–487 of the International Research Journal of Engineering and Technology. In their study "Isolation and Extraction of DNA from Dandruff: A Novel Approach for Forensic Science," Rajput and Mandal investigate the use of dandruff as a non-invasive source of DNA for forensic examination. In contrast to other biological samples, dandruff has a higher quantity of human DNA and is less likely to degrade, thus they devised a straightforward and affordable technique to extract high-quality DNA from it. The precision and effectiveness of forensic investigations may be improved by this method.

5) Claudia Tanja Mierke, "Structure and Function of the Mitochondrion." Springer, **2020**, pp. 141–161 in Cellular Mechanics and Biophysics. As Claudia Tanja Mierke explains in "Structure and Function of the Mitochondrion," mitochondrial structure has an impact on cellular dynamics. Crucial metabolic processes can be maintained by the organelle because of its double-membrane structure, which consists of an inner membrane that is firmly folded to form cristae and a peripheral membrane. The cycle of mitochondria involves both fusion and fission to maintain cellular equilibrium. They have the capacity to alter their distribution and shape, which impacts cytoskeletal structure and, in turn, cell invasion and migration. Diseases like cancer and neurological conditions change the dynamics of the mitochondria.

6) "Comparative Study on Methods of DNA Genotyping Between Single Piece of Dandruff and Buccal Cells." Vol. 6, no. 1, 2017, pp. e354–e356; Forensic Science International: Genetics Supplement Series.Dandruff is evaluated by the authors of "Comparative Study on Methods of DNA Genotyping Between Single Piece of Dandruff and Buccal Cells," as a substitute DNA source for forensic examination. Volunteers' dandruff was collected, and the DNA profiles from buccal cells and single dandruff flakes were compared.Volunteers' dandruff was collected, and the DNA profiles from buccal cells and single dandruff flakes were compared. According to the study, full STR profiles were obtained from 56% of dandruff samples that were oscillated during extraction as opposed to 11% that were not.This implies that dandruff can yield DNA profiles similar to those from buccal cells if processed properly, providing a good substitute in forensic investigations.

7) Amy Katherine Reeve and Eve Michelle Simcox, "An Introduction to Mitochondria, Their Structure and Functions." Amy Katherine Reeve et al., editors, Mitochondrial Dysfunction in Neurodegenerative Disorders, Springer, 2016, pp. 3–30. In "An Introduction to Mitochondria, Their Structure and Functions," Eve Michelle Simcox and Amy Katherine Reeve highlight the significance of mitochondria for cell survival and integrity by describing their critical function in cellular energy balance. Due to their dependence on oxidative phosphorylation, they emphasize how mitochondrial failure can have a significant impact on cells, especially neurons. In the chapter, mitochondrial dysfunction is also linked to a number of neurological and neurodegenerative conditions.

8) Walther, Parson, and colleagues. "Mitochondrial DNA Heteroplasmy in the Emerging Field of Massively Parallel Sequencing." pp. 131–139 in Forensic Science International: Genetics, vol. 18, **2015**. The authors of the paper "Mitochondrial DNA Heteroplasmy in the Emerging Field of Massively Parallel Sequencing," talk about how massively parallel sequencing (MPS) technologies have improved the analysis of mitochondrial DNA (mtDNA). They point out that although mtDNA typing has long been a useful technique in forensic genetics, newer studies suggest that forensic casework can benefit from examining the complete mitochondrial genome. MPS techniques have improved the precision and resolution of mtDNA analysis by making full mtGenome sequencing of forensic specimens both practical and affordable.

9) According to Julia Szendroedi and colleagues, "The Role of Mitochondria in Insulin Resistance and Type 2 Diabetes Mellitus." 92–103 in Nature Reviews Endocrinology, vol. 8, no. 2, 2011. According to Julia Szendroedi and colleagues' analysis in "The Role of Mitochondria in Insulin Resistance and Type 2 Diabetes Mellitus," mitochondrial dysfunction plays a part in metabolic disorders. They note that people with Type 2 diabetes mellitus (T2DM) frequently have lower levels of mitochondria in their liver cells and skeletal muscle, as well as a decreased ability to oxidatively phosphorylate. The study makes a distinction between hereditary insulin resistance, which is linked to a decrease in mitochondrial content, and acquired insulin resistance, which is linked to a limited ability of the mitochondria to adapt. The authors also point out that certain (pre)diabetic patients may have improved insulin sensitivity as a result of lifestyle and medication changes that improve mitochondrial activity.

10) An article by Laura L. Clay Montier and colleagues titled "Interactions between Nuclear and Mitochondrial DNA in Mammalian Cells: Functional Implications." Vol. 10, no. 5, pp. 414–419, Mitochondrion, 2010. According to Laura L. Clay Montier and associates' paper "Interactions between Nuclear and Mitochondrial DNA in Mammalian Cells: Functional Implications," nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) in mammalian cells are interdependent. They point out that most mitochondrial proteins are encoded by nuclear genes, made in the cytosol, and transported into mitochondria even though mitochondria have their own genome.To preserve cellular energy production and general function, this calls for exact coordination between the two genomes. Numerous illnesses, like as metabolic and degenerative problems, might result from disruptions in this connection.

11) Yohko Yamada et al. "Comparison of Different Methods for Extraction of Mitochondrial DNA from Human Pathogenic Yeasts." pp. 122–125 in Japanese Journal of Infectious Diseases, vol. 55, no. 4, **2002**. examined several techniques for removing mitochondrial DNA (mtDNA) from harmful yeasts, with an emphasis on chemical treatments, mechanical disruption, and enzymatic lysis. According to their research, specific extraction methods produce more pure and substantial amounts of mtDNA, which are necessary for precise genetic study of these yeasts.

12) Hanna Andréasson and colleagues, "Real-Time DNA Quantification of Nuclear and Mitochondrial DNA in Forensic Analysis." pp. 402–404, 407–411 in Biotechniques, vol. 33, no. 2, 2002, developed a real-time TaqMan assay to quantify nuclear and mitochondrial DNA in forensic samples, enhancing sensitivity and accuracy in analyzing degraded or minimal DNA quantities.

13) In **1998**, Herber and colleagues published "DNA Typing of Human Dandruff." Journal of Forensic Sciences, vol. 43, no. 3, pp. 648–652. In "DNA Typing of Human Dandruff," B. Herber and associates explore the possibility of using dandruff as a DNA source for forensic purposes. Nucleated cells were discovered to be present in dandruff particles, which are mainly made up of corneocytes, but partially broken down by epidermal differentiation processes. The study used a variety of short tandem repeat (STR) polymorphisms to evaluate samples from two criminal cases and 35 individuals. Surprisingly, 90% of the samples passed DNA genotyping, suggesting that dandruff can be a reliable source of DNA for forensic analysis.

14) Pages. 268–272 in "Forensic Applications of Mitochondrial DNA." Trends in Biotechnology, vol. 16, no. 7, 1998, the polymorphic character and maternal inheritance of mitochondrial DNA (mtDNA) make it useful in forensic investigations. They emphasize how well it works to find missing people, victims of war, those involved in large-scale disasters, and people implicated in criminal cases. DNA-sequence information is still required to confirm a match, even though a number of screening techniques have been developed to lessen the requirement to sequence samples that do not match.

15) "Forensic Use of Human Mitochondrial DNA: A Review." Analysis of the Brazilian Academy of Sciences, vol. 96, no. 4, 2024. Forensic science relies heavily on mitochondrial DNA (mtDNA) analysis, particularly in cases when nuclear DNA is scarce or damaged. For identifying human remains and examining contaminated materials, its high copy number per cell and maternal inheritance make it indispensable. The identification of the Romanov family and the victims of the tyranny in Guatemala are notable examples of applications.By facilitating thorough sequencing and the identification of heteroplasmy, advances in sequencing technologies like Next-Generation Sequencing (NGS) have improved mtDNA analysis. But problems like heteroplasmy interpretation and nuclear mitochondrial DNA segments (NUMTs) still exist, hence strong criteria are needed to guarantee trustworthy forensic applications.

IV. METHODOLOGY

Kit based techniques are also used to isolate DNA from dandruff.

Methodology: Mitochondrial DNA Isolation and Visualization from Dandruff on gel electrophoresis and amplification using PCR and sequencing.

A. Sample Collection and Preparation

Dandruff samples were collected by nail clippin, comb, pillow case etc of the volunteers, volunteers were asked to scratch the nails, comb their scalp gently with sterile comb and also sterility measures where taken by askin them tto sterilise their hands were sterilized by 70% ethanol or commercially available sanitizers, they were asked to scrach gently their scalp and dandruff is trapped in nail clippings were clipped, and same for comb and nails as well as combs and dandruff flakes collected from pillow case were stored in sterile paper bag, after sample collection dandruff is scraped from the nail clippings and stored in sterile 2.0 mL microcentrifuge tubes and stored at -17°C until processing. The samples ranged from approximately 10 mg per sample and visually inspected for dust and scalp debris contamination prior to DNA extraction. Pre-washing was omitted in processing to ensure maximum recovery of DNA.

B. DNA Extraction Protocol

DNA was extracted according to a tissue DNA extraction protocol modified for use with dandruff samples. The process proceeded as follows:

1) Digestion

All the samples were resuspended using 180 µL Resuspension Bufferand 20 µL Proteinase K (20 mg/mL).
Suspension was vortexed and incubated in a water bath at 55°C for 4 hours for complete digestion of cell material.
Samples were just vortexed to homogenize after digestion.

2) Lysis

200 µL of Lysis Solution was added to the samples.
The samples were vortexed for 15 seconds and heated at 70°C for 10 minutes to lyse the cell membrane and release DNA.

3) Binding to Spin Colum

200 µL of 96-100% ethanol was added to the lysate to facilitate DNA binding.
The whole lysate was transferred to a Miniprep Spin Column in a 2.0 mL collection tube.
The column was centrifuged at 10,000 rpm for 1 minute and discarded flow-through.

4) Washing Steps

The column was washed twice to remove impurities:

- Prewash: 500 µL diluted Prewash Solution was added and then centrifuged at 10,000 rpm for 1 minute.
- Wash: Add 500 µL diluted Wash Solution and centrifuge at 13,000 rpm for 3 minutes to dry the column.
- Centrifuge the empty column for further 1 minute to remove any leftover ethanol.

5) Elution of DNA

Elution of the DNA, add 100 µL of Elution Buffer to the column directly.
The column was incubated in room temperature (15-25°C) for 5 minute and spun at 10,000 rpm for 1 minute.
A second elution with an additional 100 µL of Elution Buffer was performed to obtain maximum DNA yield.
The eluate was then transferred into a new 2.0 mL microcentrifuge tube and stored at -17°C until the analysis, storage condition is must as a small error in handling will denture our samples.

C. PCR amplification

There is limited research on mtDNA extraction and analysis from dandruff samples. It does not give a clear PCR-based protocol for mtDNA extraction from dandruff samples. For thorough PCR protocols for keratinized tissues, you can refer to hair shaft and nail sample studies, which have similar biochemical characteristics to dandruff.
Although there are few specific studies on the extraction of mitochondrial DNA (mtDNA) from dandruff, those involving keratinized tissues such as hair shafts and nails are of considerable interest to effective PCR-based techniques. Below are some relevant studies:

D. Preparation of Agarose Gel for Electrophoresis

One percent agarose gel was prepared in order to observe the purified DNA:

1) Preparation of Agarose Solution

0.75 g of agarose powder was measured and dissolved in 75 mL of 1X TAE buffer, electrophoresis kit. The reagents are included in the kit.

The solution was heated in 10-second intervals until dissolved completely.

The solution cooled to around 50°C or until we can touch with bare hands, and ethidium bromide (EtBr) as staining reagent was added to a final concentration of 10.0 µg/mL.

The gel was filled in a gel casting tray, the tray was sterilized by 70% ethanol, the gel became solid after a while.

2) Electrophoresis Buffer

New 1X TAE buffer was prepared to be used for gel run.

E. Gel Electrophoresis for DNA Visualization

1) Sample Preparation

Loading Dye: Bromophenol blue was mixed with each DNA sample in a proportion of 1:5 for proper visualization in electrophoresis.

Adding Glycerol: Since the samples would drain out of the wells, 20% glycerol was added to all the samples.

2) Loading and Running the Gel

The agarose gel was loaded and aligned inside the electrophoresis chamber, and 1X TAE buffer filled to the level at which the gel was submerged.

Sample Loading:

Individual samples (5-10 µL) were loaded separately into separate wells with care.

The gel was loaded for 120V for 45-30 minutes until the half-way migration of the dye inside the gel.

F. Visualization and analysis of DNA

The gel was then placed upon a UV transilluminator to view bands of DNA upon electrophoresis.

Presence or lack of DNA bands was recorded via gel documentation software.

G. Troubleshooting and Interpretation

No bands were suspected due to no bands, low concentration of DNA, degradation, problem of loading, or problem of electrophoresis.

Faint or smeared bands indicated suboptimal extraction, inadequate staining, or degraded DNA as the probable causes.

V. RESULTS, EXPECTED OUTCOMES AND HYPOTHESIS

A. Results

Overview of this study's main goals were to ascertain whether extractable mitochondrial DNA (mtDNA) is present in dandruff made from nail clippings and to assess how well a kit-based DNA extraction technique works. To verify that genetic material was present, the collected DNA was examined using gel electrophoresis. With only one sample displaying a discernible DNA band and no DNA ladder for size comparison, the data, however, show notable variability in DNA recovery.

B. Hypothesis

The research relies on the hypothesis that mitochondrial DNA (mtDNA) can be effectively isolated from dandruff collected from nail clippings through a kit-based method, and the isolated DNA can be identified through gel electrophoresis. As dandruff is composed of shed epidermal cells that could contain both mitochondrial and nuclear DNA, it would be anticipated that there would be an adequate amount of DNA to be analysed by forensic means.

Expected Outcomes

1) Effective DNA Extraction from Dandruff

It is expected that the DNA extraction protocol with a commercial kit will be able to provide detectable DNA from dandruff collected from nail clippings.

Gel electrophoresis would verify the success of DNA extraction by showing visible bands, demonstrating effective extraction.

2) Variability in DNA Yield

As dandruff is an atypical forensic source of DNA, the yield of DNA recovered can differ greatly from sample to sample.

Varying rates of shedding, dandruff mix, and efficiency of collecting dandruff may result in different concentrations of DNA.

DNA yield may be less than that from blood, saliva, or buccal swabs but ideally remain quantifiable.

3) Difficulty in Verifying mtDNA Presence

Although the DNA extracted should include mitochondrial DNA, gel electrophoresis itself is not sufficient to verify that the DNA extracted is strictly mtDNA or a combination of mitochondrial and nuclear DNA.

Without PCR amplification with mtDNA-specific primers, the research cannot absolutely confirm that mtDNA is present in the sample.

This limitation implies that even if DNA extraction is carried out successfully, its forensic applicability for mitochondrial DNA analysis is questionable.

4) Forensic Potential of Dandruff as a DNA Source

If DNA could be reliably isolated from dandruff, this argues in favor of dandruff as an effective, non-invasive source of DNA for forensic analysis.

Although PCR and sequencing are not carried out here, the results can be used as a springboard for more advanced methodologies in the future for mtDNA profiling from dandruff.

In forensic use, dandruff found at crime scenes or on personal items may yield useful genetic material for identification.

5) Limitations that might Impact by the Results

No DNase I treatment: The DNA extracted can have both nuclear and mitochondrial DNA, hence it is not easy to decide on the amount of mtDNA without additional testing.

No PCR or sequencing: Without sequencing and amplification, there is no way to be certain that mtDNA specificity has been established, which constrains the study in making conclusions for sure regarding mitochondrial DNA presence.

Reliance on gel electrophoresis: As gel electrophoresis only verifies the existence of total DNA but does not quantify mtDNA independently, its forensic value is uncertain.

6) Wider Implications of the Study

Despite its limitations, this study adds to the increasing interest in alternative, non-invasive forensic DNA sources. If dandruff routinely yields detectable DNA, it may stimulate additional forensic investigation into its applicability for crime scene analysis, missing persons investigations, and degraded sample analysis.

The study may also motivate future research employing:

PCR-based validation to establish mtDNA presence.

Quantitative analysis (Nanodrop/Qubit) for accurate DNA yield quantification.

DNase I treatment to isolate mtDNA from nuclear DNA.

Comparative analysis of dandruff with other DNA sources

VI. CHALLENGES AND LIMITATIONS OF RESEARCH

All scientific research carries inherent limitations and challenges that influence its scope, accuracy, and applicability. Although this study effectively examines the extraction and occurrence of DNA from dandruff collected from nail clippings, various factors restrict its forensic value and general effectiveness. These limitations are occasioned by methodological limitations, absence of sophisticated validation methods, and the biological nature of dandruff as a DNA source.

A. Difficulties in Extracting DNA from Dandruff

1) Differences in DNA Quality and Yield

Dandruff is a shed skin fragment that does not always contain intact cells with nuclei or mitochondria, in contrast to more conventional forensic DNA sources like blood, saliva, or buccal swabs. Due to variations in people's rates of skin shedding, personal cleanliness practices, and exposure to the environment, the DNA yield may range considerably between samples. The amount of dead keratinized cells in different dandruff flakes may vary, which could affect the outcome of DNA extraction. DNA recovery success is not guaranteed for every sample because the extraction procedure is predicated on the idea that dandruff contains intact cells.

2) Contamination Risks

As dandruff is an outside shed material, it has a higher risk of environmental contamination with sweat, dust, bacteria, or other outside DNA sources.

In sample gathering and processing, laboratory equipment, pipettes, or even the researcher's own DNA may cause cross-contamination.

Lacking rigorous contamination control, the recovered DNA may include outside contaminants, impacting the validity of results.

3) Inability to Standardize Sample Collection

Unlike body fluids, dandruff cannot be harvested entirely under controlled conditions.

The quantity of dandruff picked up under nail clippings differs between individuals, resulting in variability in sample size.

Standardized collection methods (e.g., by using sterile scalp scrapers or sticky tapes) would have enhanced the reproducibility of sample collection, but they were not employed in the study.

B. Limitations in Methodology

1) Lack of PCR-Based Validation

The most striking limitation of the study is that it lacks Polymerase Chain Reaction (PCR) analysis.

MtDNA-specific primer PCR amplification would have determined if the isolated DNA includes mitochondrial DNA.

The study cannot tell nuclear DNA and mitochondrial DNA apart without PCR and hence cannot check if the isolated DNA is forensically useful for mtDNA analysis.

PCR would also have enhanced the detection sensitivity, enabling the study to work with even small quantities of mtDNA.

2) Reliance on Gel Electrophoresis for DNA Verification

Gel electrophoresis was utilized to verify the existence of isolated DNA, but it has serious limitations:

It gives only qualitative verification (presence or absence of DNA), but not precise quantification.

It fails to distinguish between nuclear DNA and mitochondrial DNA.

It is unable to verify the purity of isolated DNA (i.e., if it includes contaminants).

A better method would have been qPCR (quantitative PCR) or sequencing, but these were not accessible for this research.

3) No Application of DNase I Enzyme to Remove Nuclear DNA

DNase I treatment is a proved technique to selectively break down nuclear DNA, with only mitochondrial DNA remaining.

This important step was not carried out due to insufficient time and enzyme availability.

Therefore, the isolated DNA can be a combination of nuclear and mitochondrial DNA, lowering the specificity of results of this study.

4) No Quantitative DNA Measurement

No fluorometric quantification methods like the following were used in this study:

Qubit Fluorometer (for precise DNA concentration)

Nanodrop Spectrophotometer (for purity analysis, e.g., determining the A260/A280 ratio)

Only gel electrophoresis was employed, which is less accurate in measuring the specific DNA concentration and purity.

C. Forensic and Practical Limitations

1) Limited Applicability Without

mtDNA Sequencing mtDNA forensic identification is based on sequencing the hypervariable regions (HV1, HV2) of mitochondrial DNA.

Because this research does not involve sequencing, it cannot ascertain whether the DNA obtained is appropriate for forensic identification.

In forensic science, mtDNA is applied to degraded samples or unidentified remains, but without sequencing, this research cannot establish if dandruff yields usable forensic markers.

2) Lack of Control Samples

The research might have to comprised:

Positive controls (samples of known mtDNA) to confirm extraction efficiency.

Negative controls (blank samples) to confirm that there was no contamination.

No proper control samples due to limitations on resources, lessening the validity of the results.

3) Ethical and Practical Issues with Sample Collection

As opposed to blood or buccal swabs, which may be gathered with informed consent, dandruff naturally falls off and might unknowingly be placed on items or surfaces.

In forensic contexts, the gathering of dandruff as evidence of DNA comes with ethical and privacy issues if gathered without one's knowledge.

D. Suggestions to Mitigate Limitations in Future Research

Future research should include the following to mitigate these limitations:

1) PCR Amplification & Sequencing

Amplify using mtDNA-specific primers and verify the presence of mitochondrial DNA.

Sequence hypervariable regions (HV1, HV2) to examine mtDNA variability for forensic profiling.

2) Enhanced DNA Quantification Procedures

Utilize Qubit or Nanodrop to measure exact DNA concentration and purity.

Do qPCR in order to discriminate between nuclear and mitochondrial DNA.

3) DNase I Treatment to Remove Nuclear DNA

Add treatment with DNase I enzyme to remove nuclear DNA so that the isolated DNA is purely mitochondrial.

4) Improved Sample Collection & Standardization

Design a controlled collection protocol, e.g., employing adhesive scalp strips rather than nail clippings.

Provide sterile conditions to reduce the risk of contamination.

5) . Control Samples

Use positive and negative controls to ensure extraction efficiency validation and contamination exclusion.

Due to methodological limitations and the absence of mtDNA-specific confirmation, this study's forensic usefulness cannot yet be established, despite its successful demonstration that DNA can be retrieved from dandruff isolated from nail clippings. The possibility of identifying the mitochondrial origin of the recovered DNA is limited by the absence of PCR, sequencing, DNase I treatment, and quantitative DNA analysis. Future research must overcome these limitations by integrating increasingly complex molecular techniques to improve the precision, specificity, and forensic value of mtDNA extracted from dandruff.

VII. FORENSIC IMPLICATIONS AND APPLICATIONS OF THIS RESEARCH

A. Scientific and Forensic Implications

1) Extending the Scope of Forensic DNA Sources

This research presents proof that dandruff collected from nail clippings can be a viable source of DNA.

Forensic DNA analysis has long been dependent on blood, saliva, semen, hair roots, and buccal swabs. Nevertheless, in situations where these bodily fluids cannot be accessed, dandruff may provide a backup, non-invasive source of genetic material.
The occurrence of mtDNA in dandruff indicates that even tiny shed biological pieces could be of forensic value, especially in missing person cases, crime scene evidence, or degraded human remains.

2) Gathering Non-Invasive Samples for DNA Analysis

The fact that dandruff may be collected non-invasively is another important benefit of using it as a DNA source. Dandruff is naturally shed and can be passively collected from personal items such as clothing, pillows, and combs, unlike blood or buccal swabs, which need the person's active participation. In circumstances where direct DNA collection is not feasible (such as cold cases or dealing with an unwilling suspect), this has important applications for forensic investigation.

3) Potential in Degraded DNA Investigations

In decomposed or highly degraded remains in forensic cases, nuclear DNA tends to degrade with exposure to the environment, whereas mtDNA is more stable because of its greater copy number per cell.
If dandruff is present in ancient remains, mass disaster scenes, or buried evidence, it may be analysed for mtDNA as a means of long-term identification.
This research adds to post-mortem DNA recovery research, offering insights into how dandruff can be used as a long-lasting source of genetic informatlong-te9

B. Applications for Criminal and Legal Investigations

1) Use in Criminal Investigations

Forensic experts use the biological evidence that criminals inadvertently leave at crime sites to create DNA profiles. Although the most valuable evidence is blood, semen, or fingerprints, dandruff from a suspect's skin or hair can also be detected on weapons, clothing, or furniture. This study suggests that dandruff-based DNA evidence may be used to establish a suspect's presence at a crime scene.

2) Use in Cold Cases and Unsolved Crimes

Most unsolved crimes do not have the conventional DNA evidence, and the investigators usually find it hard to collect usable genetic material from crime scenes.
If dandruff is an effective source of DNA, then forensic investigators could look into cold cases by re-screening stored evidence for shed skin cells.
The research's results might urge law enforcement agencies to widen their forensic DNA practices to the examination of dandruff and other unorthodox biological samples.

3) Proof in Cases of Domestic Abuse and Sexual Assault

In cases of sexual assault or domestic abuse, victims and suspects frequently make intimate physical contact, which may result in the sharing of biological evidence. Although DNA analysis of perspiration, skin, and bodily fluids is common, dandruff can potentially serve as proof linking a suspect to a victim. The argument that dandruff is trace evidence that can be used in forensic examinations of physical altercations is strengthened by this study.

C. Medical and Genetic Research Implications

1) Role in Genetic and Ancestry Studies

Because mtDNA is inherited maternally and very conserved, it is commonly applied in ancestry testing, population genetics, and evolutionary biology.
mtDNA, if it could be reliably isolated from dandruff, may prove to be a new source for genetic lineage analysis.
These results of this study may lead researchers to investigate the use of dandruff-based DNA extraction for genealogical analysis.

2) Future in Personalized Medicine and Genetic Screening

As personalized medicine advances, scientists are seeking non-invasive means of obtaining DNA for genetic screening and disease risk testing.

If dandruff yields good-quality DNA for sequencing, it may be applied to future diagnostic uses for genetic diseases, mitochondrial disorders, and inherited conditions.
This research provides the foundation for investigating dandruff as a possible sample source for genetic diagnostics.

D. . Ethical, Privacy, and Security Implications

1) Ethical Issues in DNA Collection

As dandruff is shed naturally and can be harvested without the knowledge of the person, it poses issues of consent and privacy.
Police or private organizations may be able to harvest dandruff-based DNA without letting the person know, creating ethical issues in forensic analysis and personal data protection.
This research emphasizes the necessity of ethical standards on the procurement and utilization of shed DNA in forensic and research purposes.

2) Databases for DNA and Biometric Security

National and international forensic databases, such as CODIS (Combined DNA Index System), are increasingly using DNA profiling. Police might add dandruff-derived mtDNA profiles to forensic databases to help identify unidentified people if dandruff DNA analysis were to become more accurate. Background checks, criminal investigations, and biometric security systems may all benefit from this.

3) Threat of DNA Theft and Abuse

As technology improves, the capability to recover traceable genetic information from minute biological residues becomes a threat of DNA theft, identity theft, and genetic discrimination.
This research highlights the potential threats of off-grid genetic profiling and highlights the need to develop legal safeguards against the abuse of shed DNA.

E. Future Research and Applications of Technology

1) Developing Forensic DNA Extraction Techniques

This research proves the necessity for better DNA extraction and purification techniques specifically designed for dandruff and other shed biological materials.
Future research may include:
Designing high-yield extraction procedures for mtDNA from dandruff.
Investigating the effects of varying environmental conditions on the preservation of dandruff DNA.
Developing standardized forensic protocols for dandruff-based DNA analysis.

2) Coupling with Next-Generation Sequencing (NGS)

Thanks to the Next-Generation Sequencing (NGS) technologies, scientists are able to study minute samples of mtDNA with great accuracy.
If extraction of mtDNA using dandruff becomes efficient, it would be coupled with NGS to analyze mtDNA in depth for genetic studies, mutation screening, and forensic identification.
This work adds to the basis for making progress in the field of forensic genomics and DNA sequencing.

3) Possible Application in Environmental DNA (eDNA) Research

Environmental DNA (eDNA) is applied in wildlife research and ecology to identify genetic traces left by organisms in the environment.
Since human dandruff has recoverable DNA, it may be utilized in forensic eDNA research to trace human movement, determine individuals in environmental samples, and investigate microbiome dynamics on personal items.
This may find applications in biodefense, surveillance, and forensic anthropology.
This research has broad implications in forensic analysis, genetics, biomedical research, and ethical considerations of DNA acquisition. By showing that dandruff present in nail clippings yields extractable DNA, the research opens the door to non-invasive DNA analytical techniques and indicates the forensic uses of shed biological matter.

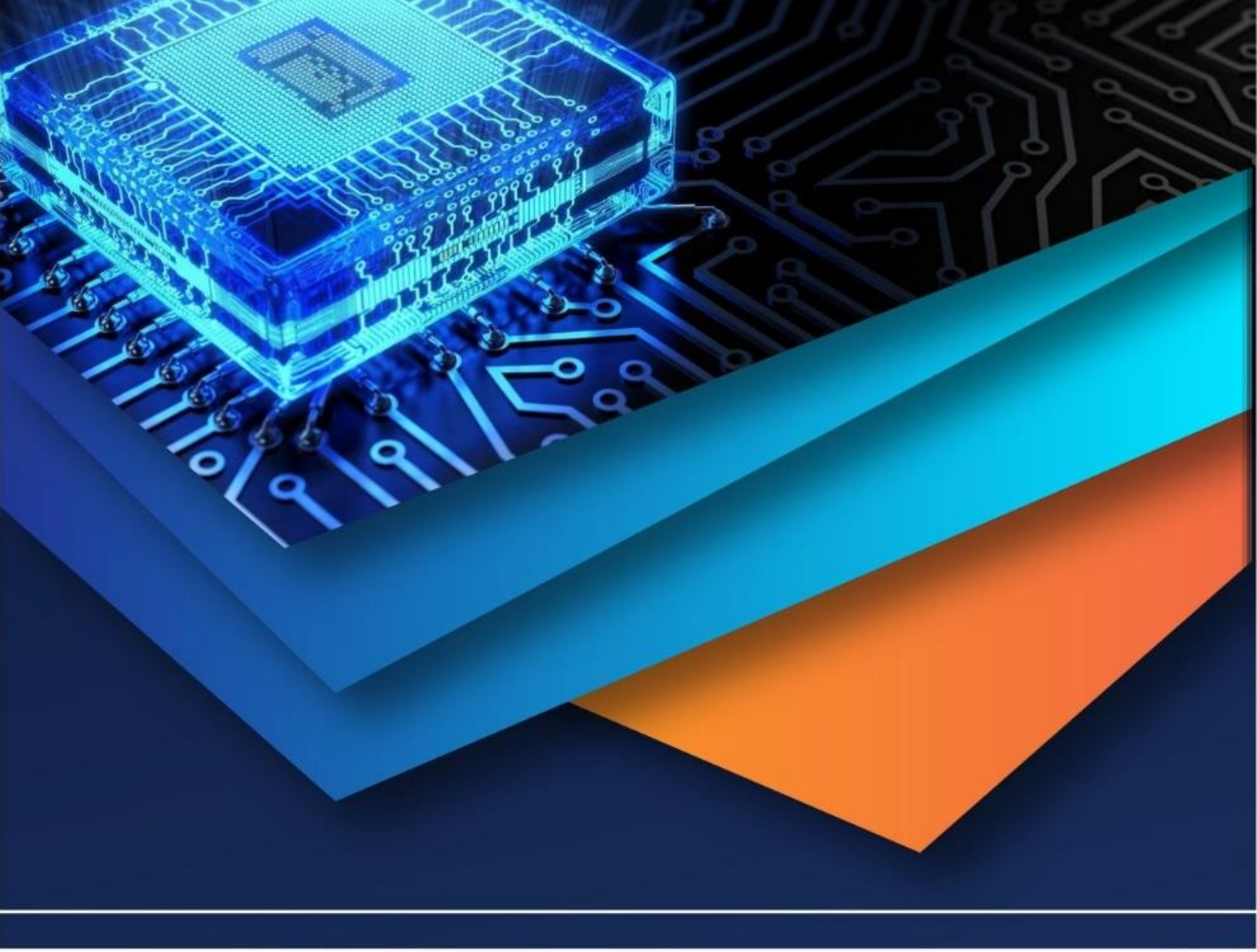
However, the research also puts some critical questions forward regarding privacy, ethical issues, and technological progress in DNA forensics. Although more research should be conducted to enhance the accuracy of dandruff-based mtDNA analysis, this research lays a solid groundwork for future research on other DNA sources.

By broadening the scope of forensic DNA analysis to include non-traditional sample types, this study may pave the way for new identification methods, clear up cold cases, and transform forensic evidence collection and analysis.

REFERENCES

[1] Medicolegal Aspects and Examination Techniques in Exhumation Cases with Alleged Human Rights Violation https://juniperpublishers.com/jfsci/JFSCI.MS.ID.555755.php
[2] Forensic use of human mitochondrial DNA: A review https://www.scielo.br/j/aabc/a/5DgryBp8CYHqG9JKDG5RYdB/
[3] Mitochondrial DNA on Wikipedia https://en.wikipedia.org/wiki/Mitochondrial_DNA
[4] Mitochondrial DNA in human identification : a review https://pmc.ncbi.nlm.nih.gov/articles/PMC6697116/
[5] Mitochondrial DNA in forensic use https://pmc.ncbi.nlm.nih.gov/articles/PMC8457767/
[6] The mitochondrial DNA analysis in Forensic identificationhttps://papers.ssrn.com/sol3/papers.cfm?abstract_id=3563058
[7] Mitochondrial DNA in forensic use, merging topics in life sciences https://portlandpress.com/emergtoplifesci/article/5/3/415/229502/Mitochondrial-DNA-in-forensic-use
[8] National institute of justice Non-STRDNAMarkers:SNPs, Y-STRs, LCN and mtDNAhttps://nij.ojp.gov/nij-hosted-online-training-courses/non-str-dna-markers-snps-y-strs-lcn-and-mtdna/mitochondrial-dna-non-nuclear-dna-markers/mitochondrial-dna/applications
[9] Forensics and mitochondrial DNA: applications, debates, and foundations https://pubmed.ncbi.nlm.nih.gov/14527299/
[10] Forensic applications of mitochondrial DNA https://pubmed.ncbi.nlm.nih.gov/9586238/
[11] Mitochondrial DNA and methods for forensic identification https://juniperpublishers.com/jfsci/JFSCI.MS.ID.555755.php
[12] Forensic use of human mitochondrial DNA : A review https://www.scielo.br/j/aabc/a/5DgryBp8CYHqG9JKDG5RYdB/
[13] What are the differences between nuclear DNA and mitochondrial DNA https://www.aatbio.com/resources/faq-frequently-asked-questions/what-are-the-differences-between-mitochondrial-dna-and-nuclear-dna
[14] Nuclear-embedded mitochondrial DNA sequences in 66,083 human genomes https://www.nature.com/articles/s41586-022-05288-7
[15] Mitochondrial and nuclear genes conflicting portrait of human origins https://pubmed.ncbi.nlm.nih.gov/9029794/
[16] Mitochondrial DNA : Inherent complexities relevant to genetic analysis https://pubmed.ncbi.nlm.nih.gov/38790246/
[17] Mitochondrial DNA-like sequences in the human nuclear genome. Characterization and implications in the evolution of mitochondrial DNA – PubMed https://pubmed.ncbi.nlm.nih.gov/3003363/
[18] mtDNA and Mitochondrial Diseases | Learn Science at Scitablehttp://www.nature.com/scitable/topicpage/mtdna-and-mitochondrial-diseases-903
[19] Nuclear and mitochondrial DNA repair: similar pathways? – PubMedhttps://pubmed.ncbi.nlm.nih.gov/16050976/
[20] Mitochondrial DNA: Inherent Complexities Relevant to Genetic Analyses – PMChttps://pmc.ncbi.nlm.nih.gov/articles/PMC11121663/
[21] Large Variation in the Ratio of Mitochondrial to Nuclear Mutation Rate across Animals: Implications for Genetic Diversity and the Use of Mitochondrial DNA as a Molecular Marker – PubMed https://pubmed.ncbi.nlm.nih.gov/28981721/
[22] Mitochondrial DNA and nuclear DNA from normal rat liver have a common sequence – PMC https://pmc.ncbi.nlm.nih.gov/articles/PMC390140/
[23] Nuclear genetic control of mitochondrial DNA segregation – PubMed https://pubmed.ncbi.nlm.nih.gov/12539044/
[24] The Three Genetics (Nuclear DNA, Mitochondrial DNA, and Gut Microbiome) of Longevity in Humans Considered as Metaorganisms – PMC https://pmc.ncbi.nlm.nih.gov/articles/PMC4017728/
[25] Nuclear DNA influences variation in mitochondrial DNA | Broad Institute https://www.broadinstitute.org/news/nuclear-dna-influences-variation-mitochondrial-dna
[26] Nuclear vs. mitochondrial DNA - Understanding Science https://undsci.berkeley.edu/teach-resources/nuclear-vs-mitochondrial-dna/
[27] Differences in nuclear DNA fragmentation and mitochondrial integrity of semen and prepared human spermatozoa | Human Reproduction | Oxford Academic https://academic.oup.com/humrep/article-abstract/15/7/1552/2915364
[28] Comparison of oxidative base damage in mitochondrial and nuclear DNA – PubMed https://pubmed.ncbi.nlm.nih.gov/9586801/
[29] A comparison of mitochondrial and nuclear DNA status in testicular sperm from fertile men and those with obstructive azoospermia – PubMedhttps://pubmed.ncbi.nlm.nih.gov/12042280/
[30] Interference of nuclear mitochondrial DNA segments in mitochondrial DNA testing resembles biparental transmission of mitochondrial DNA in humans – PubMed https://pubmed.ncbi.nlm.nih.gov/33846581/
[31] Structure and Function of the Mitochondrion | SpringerLinkhttps://link.springer.com/chapter/10.1007/978-3-030-58532-7_5
[32] An Introduction to Mitochondria, Their Structure and Functions | SpringerLink https://link.springer.com/chapter/10.1007/978-3-319-28637-2_1
[33] The role of mitochondria in insulin resistance and type 2 diabetes mellitus – PubMed https://pubmed.ncbi.nlm.nih.gov/21912398/
[34] The role of mitochondria in insulin resistance and type 2 diabetes mellitus – PubMed https://pubmed.ncbi.nlm.nih.gov/21912398/
[35] SciELO Brazil - Forensic use of human mitochondrial DNA: A review Forensic use of human mitochondrial DNA: A review https://www.scielo.br/j/aabc/a/5DgryBp8CYHqG9JKDG5RYdB/?utm_source=chatgpt.com
[36] Mitochondrial DNA heteroplasmy in the emerging field of massively parallel sequencing – ScienceDirect https://www.sciencedirect.com/science/article/pii/S1872497315300089
[37] Comparative Genome Microsynteny Illuminates the Fast Evolution of Nuclear Mitochondrial Segments (NUMTs) in Mammals | Molecular Biology and Evolution | Oxford Academichttps://academic.oup.com/mbe/article/41/1/msad278/7485626
[38] Interactions between mitochondrial and nuclear DNA in mammalian cells are non-random – ScienceDirecthttps://www.sciencedirect.com/science/article/abs/pii/S1567724916301325

[39] Advancements in Forensic DNA Analysis: Challenges and Future Directions in Molecular Biology , Biomedical Sciences, Science Publishing Group https://sciencepublishinggroup.com/article/10.11648/j.bs.20241003.11?utm_source=chatgpt.com
[40] Isolation and Extraction of DNA from Dandruff: A Novel Approach for Forensic Science https://www.academia.edu/104326125/Isolation_and_Extraction_of_DNA_from_Dandruff_A_Novel_Approach_for_Forensic_Science
[41] Comparative study on methods of DNA genotyping between single piece of dandruff and EZ-tape – ScienceDirect https://asmedigitalcollection.asme.org/forensicsciences/article/43/3/648/1184196/DNA-Typing-of-Human-Dandruff
[42] DNA Typing of Human Dandruff | ASTM J. Forensic Sci. | ASME Digital Collection https://asmedigitalcollection.asme.org/forensicsciences/article/43/3/648/1184196/DNA-Typing-of-Human-Dandruff
[43] Dandruff as explained by forensic sciencehttps://micro-bites.org/2024/04/03/dandruff-as-explained-by-forensic-science/
[44] Comparative study on methods of DNA genotyping between single piece of dandruff and EZ-tape – ScienceDirect https://www.sciencedirect.com/science/article/abs/pii/S1875176817302251
[45] Comparison of different methods for extraction of mitochondrial DNA from human pathogenic yeasts – PubMed https://pubmed.ncbi.nlm.nih.gov/12403909/
[46] Dandruff as a potential source of DNA in forensic case workhttps://www.academia.edu/9794056/Dandruff_as_a_potential_source_of_DNA_in_forensic_casework
[47] Dandruff as a potential source of DNA in forensic casework https://pubmed.ncbi.nlm.nih.gov/9670516/
[48] The invisible witness : air and dust as a DNA evidence of human occupancy in indoor premises https://www.nature.com/articles/s41598-023-46151-7
[49] Dandruff as a potential source of DNA in forensic casework https://www.academia.edu/9794056/Dandruff_as_a_potential_source_of_DNA_in_forensic_casework
[50] Dandruff : why it is, why it happens and how to managehttps://www.webmd.com/skin-problems-and-treatments/understanding-dandruff-basics
[51] Dandruff : the most commercially exploited skin disease https://pmc.ncbi.nlm.nih.gov/articles/PMC2887514/
[52] What is dandruff? Symptoms, causes, diagnosis, treatment and prevention https://www.everydayhealth.com/dandruff/guide/
[53] Dandruff : Causes, symptoms, diagnosis and effective treatmentshttps://www.sakraworldhospital.com/symptom-detail/dandruff
[54] Dandruff : Symptoms and causes – Mayo clinichttps://www.mayoclinic.org/diseases-conditions/dandruff/symptoms-causes/syc-20353850
[55] Dandruff : Causes, treatment and prevention https://www.healthline.com/health/dandruff-itchy-scalp
[56] What is dandruff?https://www.news-medical.net/health/What-is-Dandruff.aspx
[57] Dandruff : Causes and treatment https://www.medicalnewstoday.com/articles/152844
[58] A simple method of genomic DNA extraction from human samples PCR – RFLP Analysis – PMChttps://pmc.ncbi.nlm.nih.gov/articles/PMC3792701/
[59] DNA Extraction from keratin and chitinhttps://pubmed.ncbi.nlm.nih.gov/30875044/
[60] DNA samples QC and NGShttps://www.qiagen.com/us/knowledge-and-support/knowledge-hub/bench-guide/ngs/dna-sequencing/dna-sample-qc-for-ngs
[61] DNA QC and Quantitation – Qubit cost in Indiahttps://dnalabsindia.com/test/dna-qc-and-quantitation-qubit
[62] Why quality control (QC) is crucial in accurate NGS testinghttps://3billion.io/blog/why-quality-control-qc-is-performed-for-ngs-testing
[63] Quantifying DNA? Here are five methods to consider https://blog.addgene.org/five-methods-for-quantifying-dna
[64] How do I determine concentration, yield and purity of a DNA Sample? https://www.promega.in/resources/pubhub/enotes/how-do-i-determine-the-concentration-yield-and-purity-of-a-dna-sample/
[65] Quantification of DNA https://www.qiagen.com/us/knowledge-and-support/knowledge-hub/bench-guide/dna/analysing-dna/quantification-of-dna
[66] Quality and quantity of Deoxyribonucleic Acid (DNA) from preserved tissues and purified unidentifiable human corpse – PMC https://pmc.ncbi.nlm.nih.gov/articles/PMC3969639/
[67] Detection in UV-VIS Spectrophotometry : detectors, detection system, and detection strategies – Science direct https://www.sciencedirect.com/science/article/abs/pii/S0263224118311904
[68] UV-Vis spectroscopy https://www.analytik-jena.com/products/chemical-analysis/molecular-spectroscopy/uv-vis/
[69] UV-VIS Spectrophotometry : Principle, parts, uses and limitations https://microbenotes.com/uv-vis-spectroscopy/
[70] UV-Vis spectroscopy : Principle, strength, and limitations and applications https://www.technologynetworks.com/analysis/articles/uv-vis-spectroscopy-principle-strengths-and-limitations-and-applications-349865
[71] What is Uv-Vis/ UV-Visible spectroscopy? Principles overview https://www.agilent.com/en/support/molecular-spectroscopy/uv-vis-uv-vis-nir-spectroscopy/uv-vis-spectroscopy-spectrophotometer-basics
[72] UV VIS | UV VIS Spectrophotometer | https://www.thermofisher.com/in/en/home/industrial/spectroscopy-elemental-isotope-analysis/molecular-spectroscopy/uv-vis-spectrophotometry.html
[73] UV-Vis | NIR Spectrophotometers https://jascoinc.com/products/spectroscopy/uv-visible-nir-spectrophotometers/
[74] Electrophoresis of DNA in agarose gels, polyacrylamide gels in free solution – PMR https://pmc.ncbi.nlm.nih.gov/articles/PMC2757927/
[75] Agarose gel electrophoresis of the separation of DNA fragments – PMC https://pmc.ncbi.nlm.nih.gov/articles/PMC4846332/
[76] Gel Electrophoresis – Tan – 2007 – Biochemistry and molecular biology https://iubmb.onlinelibrary.wiley.com/doi/10.1002/bmb.83
[77] Agarose gel electrophoresis to assess PCR product yield : Comparison with Spectrophotometry, Fluorometry and qPCR https://www.tandfonline.com/doi/full/10.2144/btn-2021-0094
[78] Band – collision Gel Electrophoresis https://www.nature.com/articles/s41467-019-11438-9
[79] Nucleic Acid Gel Electrophoresis overview and history https://www.thermofisher.com/in/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/na-electrophoresis-education/na-separation-overview.html
[80] DNA Profiling in forensic science – a review https://pmc.ncbi.nlm.nih.gov/articles/PMC8635824/
[81] Electrophoresis as a tool in forensic science https://www.news-medical.net/life-sciences/Electrophoresis-as-a-Tool-in-Forensics.aspx
[82] Applications of electrophoresis techniques in forensic science https://legaldesire.com/application-of-electrophoresis-techniques-in-forensic-science/
[83] Agarose gel electrophoresis based RAPD – PCR an optimization in conditions to rapidly detect similarity of alert pathogens for the purpose of epidemiological studies https://www.mdpi.com/2310-2861/8/12/760



10.22214/IJRASET

45.98

IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY