ISBN: 978-93-48620-76-7

Multidimensional Research Perspectives in Life Science

Editors:
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Dr. Tarkeshwar Kumar
Dr. Biplab Kumar Das
Dr. Alok Ranjan Sahu

Bhumi Publishing, India

First Edition: May 2025

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May 2025

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Published by:

Bhumi Publishing

BHUMI PUBLISHING

Nigave Khalasa, Tal – Karveer, Dist – Kolhapur, Maharashtra, INDIA 416 207

E-mail: bhumipublishing@gmail.com

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PREFACE

The field of Life Science stands at the forefront of scientific innovation, continuously evolving to address the complex challenges of human health, biodiversity, environmental sustainability, and biotechnology. This book, Multidimensional Research Perspectives in Life Science, is a sincere attempt to bring together a spectrum of contemporary research that reflects the dynamic and interdisciplinary nature of life science.

The aim of this volume is to provide readers—students, researchers, and academicians alike—with a panoramic view of current developments across various subfields of life science. From molecular biology to ecology, biotechnology to physiology, and microbiology to bioinformatics, the chapters included herein highlight the richness of research diversity and methodological approaches being employed today. Each contribution represents an effort to explore new frontiers, deepen our understanding of living systems, and propose innovative solutions for the betterment of society.

This compilation not only showcases original studies and critical reviews but also underscores the importance of integrated research. The intersection of biological science with technology, environmental studies, and pharmaceutical applications points to the necessity of collaboration across domains. As life science continues to expand its boundaries, it is imperative to foster a multidimensional perspective that appreciates both the complexity and the interconnectedness of biological systems.

We are confident that this book will serve as a valuable academic resource, stimulate new ideas, and inspire future research endeavors. It is our belief that through the shared knowledge and insights presented in these pages, readers will gain a deeper appreciation for the transformative potential of life science in addressing global challenges.

We extend our heartfelt thanks to all the contributing authors for their scholarly input and to the editorial and review team for their tireless efforts in shaping this volume. It is our hope that Multidimensional Research Perspectives in Life Science will enrich the academic discourse and contribute meaningfully to ongoing scientific progress.

- Editors

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TILAPIA LAKE VIRUS: UNRAVELING THE PATHOGEN'S HIDDEN IMPACT

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Abstract:

As the world's population continues to grow, aquaculture is becoming increasingly important in meeting the global demand for high-quality protein. Tilapia, a resilient and adaptable fish species, is widely cultivated for its nutritional value. However, the emergence of Tilapia Lake Virus (TiLV) is a serious threat to the world's tilapia trade, with substantial financial consequences. Addressing the challenge of TiLV is crucial for ensuring the long-term sustainability of tilapia production and maintaining food security for the growing population. This article emphasizes the significance of aquaculture in addressing food security challenges and the urgent need to mitigate the impact of TiLV on the tilapia fisheries sector.

Keywords: Aquaculture, High-Quality Protein, Food Security, Tilapia Lake Virus, Long-Term Sustainability

Introduction:

By 2050, it is expected that there will be more than 9 billion people on the planet, resulting in an astounding 70% surge in the worldwide demand for food, feed, and fiber. This population growth is closely intertwined with significant shifts in lifestyle and consumption patterns, primarily driven by the rapid urbanization taking place. As a consequence of these trends, it is expected that the consumption of grains and pulses will decrease, while there will be a substantial increase in the consumption of vegetables, fruits, meat, dairy, and fish. Aquaculture, a well-established industry, stands out as a pivotal source of high-quality protein for humanity (Nash, 2010; Gui *et al.*, 2018). As our global population continues to expand, the challenges of resource scarcity and environmental degradation are becoming increasingly evident. The needs of our society are changing, and land resources alone can no longer meet them. Consequently, our oceans are emerging as a new frontier for human sustenance and development, with fish playing a central role as a source of high-quality protein and a fundamental dietary staple (Baiden and Bissiri, 2007). Over the past few decades, aquaculture has emerged as the fastest-growing sector within agriculture. Since 2013, its production has even surpassed that of wild fisheries (FAO, 2020). Fisheries and aquaculture play a critical role in enhancing food security

and livelihoods on a global scale. Fish remains a vital nutritional source for nearly 3 billion individuals, providing essential minerals, while supplying over 50% of animal protein to 400 million people in the world's most economically disadvantaged regions. In developing countries, the sustenance of over 500 million people is either directly or indirectly connected to fisheries and aquaculture remarkably, the aquaculture sector has gained remarkable momentum, boasting an impressive annual growth rate of 7%, and fish products constitute more than 37% of internationally traded food production by volume. Aquaculture stands out as the fastest-growing sector that provides highly protein-rich foods. Globally, the average fish consumption per capita is approximately 20.2 kg (FAO, 2022).

Tilapia, often colloquially referred to as "aquatic chicken," is a popular choice among major cultivable species. Its annual global production ranges from 0.7 to 0.9 million tonnes per year (FAO, 2022). Asia serves as the main centre for the production of tilapia aquaculture, with Africa and the Americas contributing additional production. Notably, China leads the world in tilapia production, followed by Indonesia and Egypt. Nevertheless, it's essential to underscore that disease represents a significant concern in tilapia production. Various pathogens, including bacteria, parasites, and viruses, are prevalent worldwide, posing a substantial challenge for the sustainable growth of the sector. Tilapia is renowned for its resilience in suboptimal water quality and its ability to withstand many diseases that affect other farmed fish. However, the emergence of Tilapia Lake Virus (TiLV) has posed a significant threat to the global tilapia industry, marking the first major disease epidemic in tilapia aquaculture. In 2011, a new virus called TiLV was discovered in Israel with scientific reports of the disease surfacing in 2013. Subsequent research revealed that the virus linked to syncytial hepatitis of tilapia (SHT) bears a close genetic resemblance to TiLV (Eyngor *et al.,* 2014). Categorized as an OIE-listed disease, TiLV is highly contagious and endangers tilapia farming. Since its discovery in 2014, TiLV has drawn widespread attention within the aquaculture industry due to its association with substantial fish mortality and the severe consequences for the tilapia aquaculture sector. It has now been reported in 16 countries and the number continues to rise, thanks to improved diagnostic techniques and expanded surveillance efforts around the world. It poses a major threat to the tilapia aquaculture industry, with reported mortality rates reaching as high as 90%. The economic losses suffered by fish farmers due to TiLV are substantial and directly linked to the occurrence of this disease.

Aetiological Agent

Tilapia lake virus is a novel enveloped, negative-strand single-stranded RNA virus. They can contain capsid virion with multiple aggregates that are dense in electrons (del Pozo *et al.,* 2017). It composed of 10 segments, each of which encodes a protein (Eyngor *et al.,* 2014). The diameter is between 55 to 100 nm (Ferguson *et al.,* 2014). The ten segments each contain open

reading frames (ORFs), which could encode 14 different proteins (Acharya *et al.,* 2019). According to the Baltimore classification system, the tilapia lake virus belongs to group V, the monotypic genus tilapinevirus, which is the sole genus in the Amnoonviridae family and can contain only one species of tilapinevirus. The new unassigned genus *Tilapinevirus*, which includes the new species *Tilapia tilainevirus,* has been proposed taxonomically to the International Committee on Taxonomy of Viruses.

Global Distribution of Tilapia Lake Virus

There have been reports of the tilapia lake virus in 16 countries. (Jansen *et al.,* 2018). Some Asian countries included are Thailand (Dong *et al.*, 2017a), Chinese Taipei (OIE, 2017b), Amal *et al.,* 2018), Malaysia (Abdullah *et al.,* 2018), Indonesia (Koesharyani *et al.,* 2018), Philippines (OIE, 2017a), and Bangladesh (Debnath *et al.,* 2020). The disease has also been reported from Colombia (Tsofack *et al.,* 2017), Mexico (OIE, 2018b), the USA (Ahasan *et al.,* 2020), Peru (OIE, 2018c), and African countries like Egypt (Fathi *et al.,* 2017), Tanzania and Uganda (Mugimba *et al.,* 2018). In India, the tilapia lake virus was first spread in two states, namely Kerala and Kolkata (Behera *et al.,* 2018).

Mode of Transmission

Tilapia lake virus is transmitted in various ways; some routes are horizontal and some are vertical transmission. Direct horizontal transmission has been shown in cohabitation studies to be an important mode of transmission. The presence of TiLV in the gonads of breeders and in fry at 2-, 5, and 10 days post-hatching suggests that TiLV may be transmitted vertically. (Yamkasem *et al.,* 2019).

Host factor

The tilapia lake virus was infected with wild and farm tilapia, which they have observed in numerous nations. Red tilapia (O. sp.), Nile tilapia, and hybrid tilapia (O. niloticus × O. aureus) have all been shown to be susceptible to TiLV (Dong *et al.* 2017, Eyngor *et al.* 2014, Ferguson *et al.* 2014).

Disease sign at farm, tank, or pond level

The tilapia species exhibits general symptoms at the farm, pond, and tank, with symptoms like anorexia, abdominal swelling, loss of appetite, scale protrusion, several anemias, skin abrasion and congestion, poor body condition, and bilateral exophthalmia (Jaemwimol *et al.,* 2018).

Gross sign

Gross symptoms appeared within a month after the fingerlings are placed in the grow-out facilities. and mortality typically begin The gross signs may include bleeding at the fins and opercula bases, skin erosion, scale protrusion and loss, abdominal distension due to ascites, skin darkening, gill pallor, and swelling of the abdomen in addition to ocular changes such as

exophthalmia ("pop-eye") and severe cases presenting with shrinkage of the eyeball (phthisis bulbi) and lens opacity. Additionally, fish exhibit abnormal behaviors including Lethargy, loss of appetite, surface swimming, and loss of balance are some abnormal behaviors that fish display (Eyngor *et al.,* 2014; Ferguson *et al.,* 2014; Dong *et al.,* 2017a; Tattiyapong, Sirikanchana and Surachetpong, 2018). TiLVD-related deaths can range widely from 10% to 90%. Internally, the TiLV exhibits some abnormal conditions, fish with symptoms like watery, pale, and necrotic liver. In certain cases, liver tissue looks green or dark; other symptoms show enlargement of the skin and accumulation of watery fluid from the gallbladder in the abdominal cavity and intestines.

Microscopic Pathological Sign

The targeted organs of the Tilapia Lake virus are the spleen, kidney, liver, and brain. In the histopathological analysis, the most typical findings in the livers of infected fish include the formation of syncytial cells and massive hepatocellular necrosis with pyknotic and karyolitic nuclei. (Behera *et al.,* 2018; Tsofack *et al.,* 2017). The fish with TiLV infection had capillary congestion, proliferative glial cells, edema, and multifocal hemorrhages in their brains. (Amal *et al.,* 2018; Tsofack *et al.,* 2017). Increased melanomacrophage centers (MMCs) were seen in the anterior kidney along with numerous necrotic foci, and melanin granule dispersion was seen in the spleen.

Different Diagnostic Methods:

1) Virus isolation and cell culture

TiLV sensitivity was seen in the multiple cell line.

1	E-11	Eyngor *et al.* (2014); Tsofack *et al.* (2017).
2	OmB	Tsofack *et al.* (2017).
3	TmB	

Other cell lines for the detection of TiLV

1	CHSE-214	Eyngor *et al.* (2014).
2	BF-2	
3	BB	
4	EPC	
5	KE-1	
6	RTG-2	
7	FHM	

2) Molecular methods

There are numerous molecular TiLV tests available.

1	RT-PCR	• Using a particular primer, it's the first reverse transcription polymerase chain reaction (RT-PCR).	Eyngor *et al.* (2014).
2	Nested RT-PCR	• Assay for nested RT-PCR with reportedly improved sensitivity. • Able to identify TiLV in diseased fish samples that are preserved as well as fresh. • Tenfold higher sensitivity than one RT-PCR.	Tsofack *et al.* (2017).
3	Semi- nested RT-PCR	• It is alternative semi-nested RT-PCR assay. • A maximum of 7.5 copies for detection • capable of identifying TiLV in clinically healthy fish	Dong *et al.* (2017a).
4	RT-qPCR	• A recently created reverse transcription quantitative PCR (RT-qPCR) technique based on SYBR green. • Two viral copies per microliter is the detection threshold.	Tattiyapong *et al.* (2018).

Prevention and Control

It is mandatory to use drugs prior to a disease outbreak in order to prevent it. The commonly used prophylactics are

1. Vaccination

For controlling fish diseases, vaccines can be used as an effective prophylactic measure. A vaccine is an antigenic material that induces adaptive immunity against a specific pathogen. Interest in creating tilapia vaccines has increased recently as tilapia culture has grown exponentially and large-scale production has become common. TiLV vaccine development is ongoing in China, Thailand, and Israel. Tilapia can produce anti-TiLV antibodies in seven to ten days, according to research on humors' defense against TiLV (Tattiapong *et al.*, 2020). After being exposed to the virus. The majority of the time, an increase in antibodies happened within two weeks after the fish were re-exposed to TiLV (through IP injection). These demonstrated that tilapia maintained humoral memory and generated anti-TiLV antibodies to shield fish from further exposure. The TiLV has recently been created using β-propiolactone (BPL) inactivation (Zeng *et al.*, 2021). Montanide IMS 1312 adjuvant, combined with the inactivated vaccine, has an apparent high efficacy survival rate of 85.7%.

2. Immunostimulants

Immunostimulants are substances that stimulate one or more immune pathways, increasing the body's ability to fight antigens. Unlike vaccines, which target particular pathogens,

immunostimulants act to improve the immune system's overall response. It is broad-spectrum activity and very chief compared to the vaccines. There are different immunostimulants on the market. That can contain β-glucans, which are most commonly used in aquaculture. These immunostimulants bolster the immune system, both adaptive and innate, by enhancing the humoral complements, phagocytes, and lysozyme activities. Additionally, they may be usefull for antibody responses (Dong *et al.,* 2015). β-glucans, effective against viral and bacterial diseases.

3. Probiotics

Live microorganisms known as probiotics, which are mainly bacteria and yeasts found in fishes, normal pond environments or intestinal microflora, are beneficial to health. Probiotics may be added to feed, given to boost immune responses, or added to ponds to enhancing the water quality (Cha *et al.,* 2013). Probiotics are used in aquaculture to combat pathogens, including bacteria and viruses. It has been demonstrated that probiotic bacteria can influence fish immunity (innate and adaptive) directly by increasing lysozyme levels, phagocytic activities, cytokine production, and complement responses (Salinas *et al.,* 2005). The probiotic *Bacillus subtilis* C-3102 has been demonstrated to enhance the adherence of beneficial bacteria to the mucosal surfaces of the stomach of hybrid tilapia (*O. aureus* × *O. niloticus*) and enhance the intestinal production of cytokines such as TGF-β, IL-1b,and TNF (He *et al.,* 2013). In a TiLV challenge bioassay, probiotic supplementation was shown to have a reduced mortality rate of 25% compared to tilapia that were not fed the probiotic diet, which had a higher mortality rate of 32%. (Waiyamitra *et al.,* 2020).

4. Virus free Tilapia

The majority of tilapia farmers utilize SPF animals that are TiLV-free. The biosecurity facilities where the SPF tilapia are raised allow for constant monitoring of their health status through the use of standardized diagnostic techniques.

Conclusion:

As the world's population continues to grow and food demands change, the growing demand for high-quality protein is anticipated to be largely met by aquaculture. Among the various fish species, tilapia stands out as a popular choice due to its resilience and adaptability. However, the tilapia industry worldwide faces a serious threat from the emergence of Tilapia Lake Virus (TiLV), which could result in large financial losses. Addressing the challenge of TiLV is essential for ensuring the long-term sustainability of the tilapia sector and maintaining food security for billions of people worldwide.

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ADVANCEMENTS IN DIGITAL HEALTH: THE ROLE OF WEARABLES, TELEMEDICINE, AND AI-DRIVEN ROBOTICS

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Abstract:

The integration of technology and healthcare, led by digital health, is transforming patient care, illness management, and general well-being. This disruptive shift includes a wide range of breakthroughs, such as wearables, telemedicine, and other emerging technology. Wearable gadgets, for example, allow for continuous physiological monitoring and remote patient management, delivering crucial real-time data to both patients and healthcare practitioners. Telemedicine improves access to care, particularly in underprivileged areas, by enabling remote consultations and chronic illness management. Furthermore, artificial intelligence and machine learning are improving diagnostics, drug discovery, and specific treatment plans. Virtual and augmented reality are transforming medical education and rehabilitation procedures. The Internet of Medical Things (IoMT), along with blockchain technology, is enhancing data exchange and security in the healthcare sector. The current research focusses on improving sensor accuracy, seamlessly integrating digital health tools into existing healthcare systems, and tackling major ethical concerns such as data privacy and bias due to algorithms. Looking in advance, prospective directions include developing predictive and preventative healthcare models, implementing patient-centered care techniques, and achieving seamless digital integration. Overcoming interoperability difficulties and ensuring fair access are critical to attaining digitized health's full assure for transforming the provision of healthcare.

Keywords: Artificial Intelligence and Machine Learning, Digital Health, Internet of Medical Things (IoMT), Patient-Centered Care, Wearable Devices

1. Introduction:

The healthcare landscape has undergone a dramatic transformation in recent years, largely driven by technological innovations that aim to improve patient outcomes, enhance efficiency, and reduce the burden on healthcare systems. Among the most promising developments are digital health solutions, which encompass a broad range of technologies, including wearable devices, telemedicine platforms, and AI-driven robotic systems. These advancements have redefined healthcare delivery by enabling remote patient monitoring, early disease detection, and minimally invasive treatments. Wearable technology has witnessed

substantial growth, with devices now capable of continuously tracking physiological parameters such as heart rate, oxygen saturation, glucose levels, and physical activity. These wearables, equipped with sophisticated biosensors and artificial intelligence (AI) algorithms, offer real-time health monitoring, aiding in disease management and preventive care. The integration of machine learning in wearables has further enhanced their predictive capabilities, enabling early identification of health anomalies and personalized interventions [1].

Telemedicine, another pillar of digital health, has seen an unprecedented rise, particularly in response to the COVID-19 pandemic. The ability to conduct virtual consultations and remotely manage chronic diseases has improved healthcare accessibility, particularly in underserved regions. The integration of AI in telemedicine has led to the development of intelligent diagnostic tools, automated triaging systems, and virtual health assistants that enhance clinical decision-making [2]. Telehealth has not only minimized unnecessary hospital visits but has also contributed to reducing healthcare costs and improving patient satisfaction AI-driven robotics is revolutionizing various aspects of healthcare, from precision surgeries to patient rehabilitation and elderly care. Surgical robotics, such as the Da Vinci Surgical System, has enhanced the accuracy of minimally invasive procedures, reducing post-operative complications and hospital stays. In rehabilitation, robotic exoskeletons assist patients with spinal cord injuries and stroke-induced disabilities in regaining mobility. AI-powered companion robots have also been introduced in mental health and elderly care, providing emotional support and improving the quality of life for patients with neurodegenerative disorders [3].

Despite these remarkable advancements, the adoption of digital health technologies is not without challenges. Data privacy and cybersecurity remain primary concerns, given the sensitive nature of health information. Ensuring interoperability between different digital health systems and regulatory compliance across regions is also a significant hurdle. Moreover, disparities in digital literacy and access to technology pose barriers to the equitable implementation of these innovations. Addressing these challenges requires collaborative efforts from researchers, policymakers, and industry stakeholders to create robust frameworks that support the ethical and secure use of digital health technologies [4].

2. Wearable Technology in Healthcare

2.1 Evolution of Wearables

Wearable medical devices have evolved significantly from basic pedometers to sophisticated biosensors capable of continuously monitoring multiple physiological parameters. The global market for wearables is expanding, driven by the increasing prevalence of chronic diseases and the growing demand for remote patient monitoring (RPM) solutions. Wearable technology is projected to play a critical role in digital healthcare, with an estimated market value surpassing $80 billion by 2025 [5]. Wearable devices encompass a wide range of

technologies, each designed for specific medical applications. Some of the most widely used categories include smartwatches and fitness trackers, continuous glucose monitors (CGMs), wearable electrocardiograms (ECGs), smart clothing and textiles, wearable blood pressure monitors, wearable sleep monitors, and neurotechnology wearables. Smartwatches and fitness trackers, such as the Apple Watch and Fitbit, monitor physiological parameters like heart rate, blood oxygen levels, and physical activity. They play a crucial role in preventive healthcare by encouraging users to maintain an active lifestyle and detect early signs of cardiovascular abnormalities. Continuous glucose monitors like the Dexcom G6 and FreeStyle Libre continuously track glucose levels, allowing diabetic patients to manage their blood sugar more effectively without frequent finger-prick tests. These devices help in reducing hyperglycemia and hypoglycemia risks, enhancing overall diabetes management [6].

2.2 Clinical Applications of Wearables

Wearable electrocardiograms, such as AliveCor Kardia, detect arrhythmias, including atrial fibrillation, in real time. These wearables enable early diagnosis and intervention, potentially preventing severe cardiac events such as strokes. Smart fabrics embedded with nano-biosensors can track vital signs such as temperature, hydration levels, and muscle activity. These are particularly beneficial for athletes, elderly individuals, and patients undergoing post-operative recovery [7]. Wearable blood pressure monitors, like the Omron HeartGuide, provide real-time blood pressure readings, allowing for better hypertension management and reducing the risk of cardiovascular complications [8]. Wearable sleep monitors, such as the Oura Ring and Withings Sleep Analyzer, assess sleep patterns, detect sleep apnea, and provide insights into sleep quality. These tools aid in diagnosing sleep disorders and recommending lifestyle modifications. EEG-based neurotechnology wearables, like those developed by Muse and Emotiv, monitor brain activity and assist in managing neurological conditions such as epilepsy, ADHD, and stress-related disorders. They are also used in cognitive training and meditation [9].

2.3 AI Integration in Wearables

The incorporation of AI and machine learning has significantly enhanced the predictive and diagnostic capabilities of wearables. AI-powered cardiac monitoring analyzes heart rate variability and ECG data to predict cardiovascular events before symptoms appear. Machine learning processes vast amounts of wearable data to provide personalized health insights, improving disease prevention strategies [10]. Wearables equipped with accelerometers and gyroscopes can detect falls and automatically notify emergency contacts or healthcare providers, ensuring timely medical intervention for elderly individuals and patients with movement disorders. Advanced sleep trackers use AI to identify patterns related to sleep quality, disorders, and potential interventions, improving sleep hygiene and overall well-being [11].

2.4 Limitations and Challenges

Despite their benefits, wearable health technologies face several challenges, including data accuracy and reliability issues, privacy and data security concerns, user compliance and adoption difficulties, and interoperability with healthcare systems. Many wearable sensors still struggle with inconsistencies in measurements due to movement artifacts and improper device placement. Given the sensitive nature of health data, strong cybersecurity measures and compliance with regulatory standards (e.g., GDPR, HIPAA) are crucial. Many users abandon wearables after initial use due to discomfort, lack of motivation, or difficulties in interpreting health data [12]. Standardization is required to integrate wearable-generated data seamlessly into electronic health records (EHRs) for clinical use. Future advancements in wearable health technology may include implantable biosensors for continuous monitoring, AI-driven digital health assistants, and blockchain-based security measures for enhanced data protection. Continued research and innovation will drive improvements in accuracy, accessibility, and clinical utility, further solidifying wearables as essential tools in modern healthcare [13].

3. Telemedicine

3.1 Growth and Adoption of Telemedicine

Telemedicine has witnessed rapid growth, particularly during the COVID-19 pandemic. It has facilitated remote consultations, reduced hospital visits and improving healthcare accessibility [10]. Governments and healthcare institutions worldwide have implemented telehealth policies to expand coverage and integration into mainstream healthcare systems [11].

3.2 AI-Powered Telemedicine Platforms

Artificial intelligence has further enhanced telemedicine capabilities through chatbot-based symptom checkers and automated triaging systems. AI models trained on large datasets assist in preliminary diagnoses, reducing physician workload and improving efficiency [12]. Additionally, telemedicine platforms leveraging natural language processing (NLP) streamline patient-provider communication, ensuring precise documentation and interpretation [13].

3.3 Remote Patient Monitoring

Remote patient monitoring (RPM) enables continuous tracking of patient vitals through IoT-connected devices, facilitating early intervention in chronic conditions [14]. RPM systems have demonstrated significant efficacy in managing hypertension, heart failure, and post-surgical recovery by transmitting real-time health data to clinicians [15]. Telemedicine is not without limitations. Digital disparities, limited internet accessibility in rural regions, and patient reluctance to adopt virtual healthcare pose significant barriers [16]. Additionally, AI-driven telehealth must address concerns regarding algorithmic bias, patient consent, and data security to ensure equitable healthcare delivery [17].

4. AI-Driven Robotics in Healthcare

4.1 Robotic-Assisted Surgeries

The advent of robotic-assisted surgeries has revolutionized modern surgical procedures by enhancing precision, reducing human error, and minimizing invasiveness. Surgical robots, such as the Da Vinci Surgical System, enable surgeons to perform complex procedures with improved dexterity and control [18]. These systems utilize AI-driven real-time imaging, providing high-definition, magnified, and 3D visualization of the surgical site, leading to better patient outcomes. AI integration allows for intraoperative decision-making support, optimizing surgical techniques, and reducing complications. Furthermore, robotic-assisted surgeries have been linked to shorter hospital stays, reduced post-operative pain, and faster recovery times compared to traditional surgical methods [19].

4.2 Robotics in Patient Care and Rehabilitation

Beyond the operating room, AI-powered robotics are making significant strides in patient care and rehabilitation. Exoskeleton robots, such as the ReWalk system, assist patients with mobility impairments, including those recovering from strokes or spinal cord injuries. These wearable robotic devices help in regaining muscle strength and movement coordination, facilitating rehabilitation and independence. AI-driven robotic caregivers and assistants are also transforming elderly care by providing companionship, reminding patients to take medications, and assisting with daily activities. Automated robotic drug dispensing systems have improved medication adherence and minimized errors in pharmaceutical settings. Moreover, robotic rehabilitation systems incorporating AI algorithms personalize therapy sessions based on patient progress, ensuring more effective and tailored treatments [20].

4.3 Ethical and Technological Barriers

Despite the remarkable advancements in robotic healthcare solutions, several challenges and ethical concerns persist. The high cost of robotic surgical systems limits accessibility, particularly in low-resource settings. Additionally, there are concerns about job displacement in the healthcare workforce due to automation. Ethical considerations regarding AI decision-making in surgery and patient care remain an ongoing debate. The need for human oversight in robotic-assisted procedures is crucial to ensuring patient safety and maintaining trust in AI-driven medical interventions. Addressing these barriers requires the development of cost-effective robotic solutions, rigorous ethical guidelines, and continuous advancements in AI algorithms to enhance transparency, reliability, and accessibility in robotic healthcare applications [21].

5. Addressing Digital Health Disparities

5.1 Socioeconomic Barriers to Adoption

Digital health disparities remain a pressing issue, particularly in underserved communities where access to technology and healthcare infrastructure is limited. Socioeconomic factors such as education, income level, and geographic location play a significant role in determining digital health adoption rates. Rural and low-income populations often face challenges in accessing telemedicine services due to a lack of high-speed internet connectivity and limited digital literacy. Furthermore, the affordability of digital health tools, including wearable devices and AI-driven diagnostics, poses an additional burden on economically disadvantaged populations. Studies indicate that individuals from lower-income backgrounds are less likely to own smartphones or wearable health monitors, limiting their ability to engage with digital health platforms [22].

5.2 Policy Recommendations and Future Directions

To bridge these disparities, governments and healthcare organizations must implement strategic policies aimed at improving digital health accessibility. Expanding broadband infrastructure in rural areas is crucial to ensure reliable telemedicine and remote patient monitoring services. Additionally, initiatives that provide subsidized or low-cost wearable health devices to low-income populations can significantly enhance digital health inclusion. Digital literacy programs tailored for elderly populations and socioeconomically disadvantaged communities can improve their engagement with telehealth platforms, empowering them to manage chronic diseases more effectively [23].

Collaboration between technology developers and healthcare policymakers is vital to creating user-friendly, accessible digital health solutions that cater to diverse patient populations. Additionally, healthcare systems must integrate culturally sensitive approaches that address language barriers and provide multilingual digital health support. Future research should focus on developing AI-driven algorithms that consider socioeconomic and demographic factors to reduce bias in digital healthcare delivery. Ultimately, fostering public-private partnerships and leveraging emerging technologies can contribute to a more equitable and inclusive digital health ecosystem [24].

Conclusion:

Digital health is profoundly transforming healthcare, shifting from a reactive, clinic-centric paradigm to a proactive, patient-centric one. This revolution is being driven by unparalleled chances to improve patient outcomes via continuous monitoring and personalised therapies. Wearables, for example, provide real-time data collecting, enabling for early detection of health problems and proactive management of chronic illnesses. Telemedicine removes geographical boundaries, increasing access to specialised treatment, particularly among

underprivileged communities. Remote consultations and monitoring eliminate the need for in-person visits, which improves convenience and lowers expenses. Beyond that, AI-powered diagnostics and personalised treatment plans are improving care delivery, while VR and AR are transforming medical teaching and rehabilitation. Ongoing research and innovation are important to this progression, with an emphasis on increasing data accuracy, protecting data privacy, and smoothly integrating these technologies into existing healthcare systems. The goal is to build a future in which healthcare is not only more personalised and suited to individual requirements, but also more accessible, removing obstacles to care, and more efficient, maximising resource utilisation. This move aims to empower patients, improve health outcomes, and build a more sustainable and equitable healthcare system.

Acknowledgement:

The authors express their sincere gratitude to Sumandeep Vidyapeeth Deemed to be University, Vadodara, Gujarat for providing the necessary facilities and support to complete this chapter.

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SILK: A NEW DIMENSION TOWARDS BIOMEDICAL ENGINNERING AND SERICULTURE TECHNOLOGIES

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Abstract:

In 3D bioprinting, bioinks are specialized biomaterials that create tissue-like constructs for tissue engineering, drug testing, and regenerative medicine applications. Because of their remarkable biocompatibility, adjustable mechanical characteristics, and low immunogenicity, silk proteins—in particular, silk fibroin derived from Bombyx mori—have emerged as promising candidates among the various natural polymers explored for bioink development. In addition to providing structural support, silk-based bioinks create an environment that is permissive to cell adhesion, proliferation and differentiation. In order to enhance printing capability and recreate the characteristics of native extracellular matrix, silk fibroin has been blended with a variety of biological additives, including gelatin, alginate, or growth factors, in recent developments in bioink formulations. Complex, functional tissue constructions with enhanced mechanical integrity and cellular viability can be developed via this hybrid technique. The chapter covers opportunities for expansion and innovation in the sericulture sector going forward, such as utilizing advances in technology and devoting resources for research and development.

Keywords: Silk, Bioprinting, Silk Fibroin Protein, Biomedical Science.

Introduction:

In recent years, three-dimensional (3D) printing is a promising strategy to the biomedical field and it is regarded as a future alternative to current clinical treatments. Bioinks are cell-encapsulating biomaterials that are used in 3D printing process and they must be friendly to both printing process and 3D cell culture. The term bioink was first used in the context of organ printing in 2003 and was introduced together with the term biopaper. Thomas Boland from Clemson University patented the first bio-printing technique, “inkjet printing,” for printing viable cells. The primary objective of bioink is to promote cell adhesion, proliferation and differentiation through the creation of a supportive environment that simulates the natural extracellular matrix (ECM). They are fundamental to tissue engineering and regenerative medicine, enabling the development of functional, customized tissues for drug testing, disease modeling and transplantation. As technology advances, bioinks are evolving to more closely resemble the complexity of actual human tissues. Not only that it can alleviate the artificial organ or tissue shortage crisis, but it can also design and produce complex and precise microstructures

according to reconstruction of tissue engineering requirements. bioink plays a significant role in rebuilding the similar function of native tissue following the principle of tissue engineering. In the field of tissue engineering, the three strategies that were used to replace or repair native tissue: using cells, cytokines, or cell substitutes only; using biocompatible biomaterials only to induce tissue regeneration, combination of using cells, cytokines, and biomaterials.

More importantly, a series of advanced 3D printing techniques have been approved to achieve structural and functional consistency with model design, which means that competitive manufacturing technology is ready for tissue repair and transplantation. Bioink as a core of the 3D printing is the key to success for 3D printing products. Specifically, bioinks loading cells, growth factors and cues for bio-applications are still in the early stage in 3D printing. Therefore, it is an urgent need to seek an appropriate material as bioink for 3D printing.

Materials used in Bioink:

Polymers	Advantage
1. Alginate	a) Biocompatible, low toxicity and low cost b) Ease of cross linking c) Stability of constructs
2. Agarose	a) Non-toxic b) Maintains shape fidelity across broad range of temperatures
3. Collagen	a) Easy degradation b) Minimal immunogenicity c) Easy to process and modify
4. Fibrin	a) Rapid gelation b) Easily purified from blood providing autologous source
5. Gelatin	a) Decreased immunogenicity b) Contains cell-adhesive Arg-Gly-Asp motifs
6. Hyaluronic Acid	a) Fast gelation b) Controllable mechanics, architecture, and degradation
7. Hydroxyapatite	a) Keep good shape fidelity and produce porous

Processing of bioink

1. First, doctors make CT or MRI scans of the desired organ.
2. Next, they load the images into a computer and build a corresponding 3- D blueprint of the structure using CAD software.
3. Combining this 3-D data with histological information collected from years of microscopic analysis of tissues, scientists build a slice-by-slice model of the patient's

organ. Each slice accurately reflects how the unique cells and the surrounding cellular matrix fit together in three-dimensional space.

4. After that, it's a matter of hitting File > Print, which sends the modeling data to the bio-printer.
5. The printer outputs the organ one layer at a time, using bio-ink and gel to create the complex multicellular tissue and hold it in place.
6. Finally, scientists remove the organ from the printer and place it in an incubator, where the cells in the bio-ink enjoy some warm, quiet downtime to start living and working together.
7. The final step of this process making printed organ cells behave like native cells has been challenging. Some scientists recommend that bio-printing be done with a patient's stem cells.
8. After being deposited in their required three-dimensional space, they would then differentiate into mature cells, with all of the instructions about how to "behave." Then, of course, there's the issue of getting blood to all of the cells in a printed organ.

Currently, bio-printing doesn't offer sufficient resolutions to create tiny, single-cell-thick capillaries. But scientists have printed larger blood vessels, and as the technology improves, the next step will be fully functional replacement organs, complete with the vascularization necessary to remain alive and healthy.

❖ **Benefits of Bioink**:

- ✓ Artificial organ personalized using patients own cells.
- ✓ Eliminate organ donation.
- ✓ No DNA rejection.
- ✓ No waiting period.

❖ **Disadvantages of Bioink materials:**

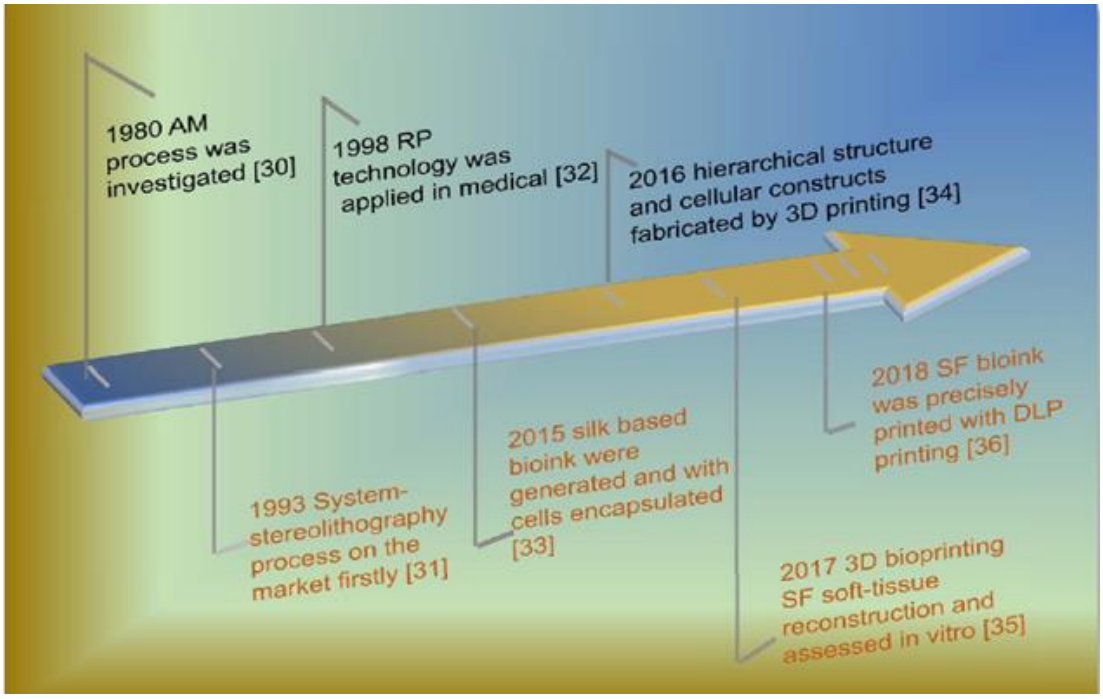
- ✓ Not degradable, poor cell adhesion.
- ✓ Slow gelation rate.
- ✓ Limited printability.
- ✓ Poor shape fidelity.
- ✓ Poor stability.

Scenerio of bioink with respect to sericulture:

One of the primary reasons being the ease of solubility in aqueous medium without the need of additional organic solvents. Silk can be processed into hydrogel (predominantly random coil conformation, water soluble) and in defined levels of crystalline conformations (predominantly β-sheet conformation, to make water-insoluble constructs), at higher concentrations (up to 30 wt. %). Amphilic nature of silk protein chains can be exploited to

generate precise volume of fibroin protein drops in the Pico to Nano liter orders or continuous filaments by optimizing the rheological properties of the ink (e.g., viscosity, surface tension) at wide range of pH and ionic strength. Even the biodegradation could be controlled either by modulating the secondary conformations (α-helix, β-sheet, β-turn content) or by controlling the length of fibroin chain during fibroin protein isolation process. Above all, Silk fibroin protein offers superior mechanical properties compared to other potential bioinks as a result of β-sheet conformation which can be precisely controlled to regulate cellular mechano transduction.

Timeline of the development of Silk fibroin based bioink in three-dimensional (3D) printing technology.



❖ **Advantages of Silk Fibroin Bioink:**

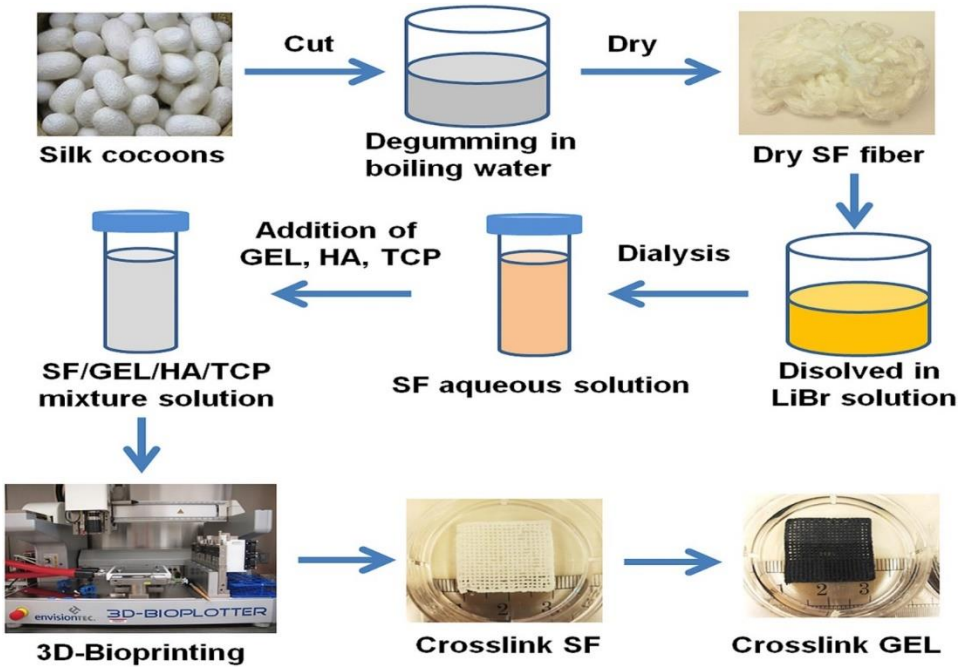
- ✓ Ease of structural modification
- ✓ Outstanding strength and toughness
- ✓ Flexible structural modification
- ✓ Diversity of methods for crosslink
- ✓ Controlled degradation

Processing of Silk Bioink:

Native *B. mori* silk is composed of silk fibroin protein coated with sericin protein and sericin is a group of soluble glycoproteins that are expressed in the middle silk gland of *B. mori* silkworms. By degumming, the sericin is removed; the Silk fibroin (SF) fibers could be dissolved and purified into an aqueous solution through dialyzing in de-ionized water. Based on aqueous solution system, the SF can be further processed into different types of materials in films, particles, fibers and sponge including hydrogel. However, there is a barrier hindering 3D printing

fabrication in SF bioink that is caused by low concentration and viscosity. Increasing its concentration and adding other high viscosity additives are perhaps useful strategies in improving its printing process ability and bio function ability.

To obtain high concentration SF solution, there are two approaches that are employed. One way is based on the SF purification protocol that is modified with some additional procedures. Specifically, SF solution is concentrated with a dialysis bag (Molecular Weight Cut Off (MWCO) ≈ 3000Da) in polyethylene glycol (PEG, Molecular Weight (MW) ≥ 20000Da) solution, or regenerated SF materials are re-dissolved in organic solvents (1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), Formic acid, etc.) to increase the concentration to meet the requirements of rheology of bioink. However, the bioactivity of silk proteins will be inevitably weakened by these complexing processes. Recently, adapting new dissolving systems for another effective way, the Ca^{2+}-formic acid binary solvent system and HFIP are studied as dissolving solvent directly for silk fibers to produce high concentration SF solution, which is easier for yielding over 20 wt.%. These unfriendly solvents will continue cutting the SF molecules chains in a further process that resulting in low SF molecular weight and viscosity. What is more, the hostile solvent residues have a detrimental effect on cell viability and encapsulating in 3D printing, which limited the applications of these solvents in 3D printing. As a second strategy, it is convenient and highly efficient to enhance the freestanding and viscosity of SF based bioink by blending other high viscosity biomaterials. Based on the principle of similar compatible, gelatin, chitosan, alginate and HA are mixed with SF solution to prepare SF based bioink. This strategy is more successful than other approaches in improving the SF solution with a high concentration and plastic ability for 3D printing.



Schematic of methods to optimizing the rheology of SF bioink

Prospects of Silk Fibroin Bioink for Biomedical Applications:

1. **Skin Tissue:** With the development of multi component bioink and printing technology, a series of biomedical applications have been reported based on the process of 3D printing. The skin is the largest complex organ in the human body and it is composed by three layers (the stratified squamous epithelium, the dermis and the hypodermis). Autografts and allografts are two strategies for skin repairing, which is still limited in donors and recipients to some extent. Specifically, the donor suffers from pain, second operation and scarring for the recipient. Recently, a gelatin-sulfonated silk composite scaffold was fabricated by a DIY pneumatic printing system, with the incorporation of growth factors which presented skin-like tissues and enhanced skin regeneration by printing technology. By the nano imprint lithography technique, SF film with skin tissue-like nano scale structures was fabricated to mimic the collagen morphology of natural dermal, which could alleviate scar formation. The silk based bioink combined with collagen are also employed to prepare artificial skin grafts and the network connective of neo-tissue increased a lot when compared with scaffolds that are derived from the freeze-drying method. Although SF as bioink to printing artificial skin-tissue is starting out, the available results regarding the histology and immune fluorescence characterization of the 3D printed grafts presented an applicable potential in skin tissue repair.
2. **Cartilage Tissue:** Cartilage damage and degeneration are common disease in the aged suffering from osteoarthritis, which has become an urgent need in clinical healthcare. Some challenges still existed in mimicking the fine structures of native cartilage tissue, especially in Nano-micro-ordered structures. Fortunately, when comparing to common approaches, the 3D printing fabrication manifested positive practicability. It appeared to be more promising for SF based bioinks with the recent study, though it was not wildly applied in tissue engineering. For example, by integrating SF with gelatin loading growth factors as bioink, it could be optimized in structural and function for cartilage repairing. Pure silk bioink with high concentration could be processed by direct-writing technology, which showed that 3D printing is a much more competitive method in resolution, cell viability and complex tissue formation.
3. **Bone Tissue:** Bone tissue engineering usually relies on bone structure, compositions, mechanics, and tissue formation which makes it crucial in obtaining a fundamental understanding of bone biology. Nevertheless, it has become the focus as to how to keep balance between bioactivity and mechanical properties for printing bone. As for mechanical performance, the bio-ceramics have been used frequently as an important element of bioink, including α-tri calcium phosphate (α-TCP) and hydroxyl apatite. The results showed great potential in bone tissue repair when combined with SF. For instance,

polylactic acid/hydroxyl apatite/silk ternary bioink, in fabricating bone clip, which demonstrated an equivalent mechanical property, good biocompatibility, and alignment when compared with other types of the bone clip. Another SF/hydroxyl apatite scaffold that was fabricated by direct-writing technology and their regular pore size was beneficial regarding cell growth in orientation. A low-temperature printing technology for the collagen/decellularized extracellular/SF scaffold preparation also showed higher cell proliferation and differentiation. When comparing to that of the collagen scaffold, the compressive modulus was highly improved due to the β-sheet formation of SF.

4. **Blood Vessel:** Vasculature within the tissues or organs is crucial in transporting oxygen and nutrients and in maintaining tissue functions. Though the quantity demanded is enormous, the thrombogenicity and low patency rate narrowed the clinic utility of artificial blood vessels, especially in repairing small diameter (in 4–6 mm) blood vessels. By convenience of 3D printing, it was greatly encouraged to manufacture blood vessel tissue engineering. SF and glycidyl methacrylate (Sil-MA) as blending bioink was used for building blood vessels in the hydrogel state that showed outstanding mechanical and rheological properties, which provide many possibilities for vessels, brain and ear with highly complex organ structures. Similarly, the SF incorporated melanin nano particles could be as a transparency modifier to adjust poly (ethylene glycol)-tetra acrylate to improve the printing resolution, and these features make it possible to fabricate blood vessels or vacant tubes. In advanced 3D printing technology for fabricating vessels, preference should be given to obtaining enough porosity and mechanical properties and non-thrombosis to combat thrombogenicity at early stage. Therefore, the characteristics of SF bioink should be optimized to satisfy target application and tissue engineering.

Role of Muga Silk Protein as Bioink:

Using proteins from Muga silk, scientists at IIT Guwahati have created a bioink with live cells that can be used for 3D printing of tissues, implants and even organs. Dr. Biman B Mandal's Biomaterials and Tissue Engineering Laboratory at IIT-Guwahati has been working with silk proteins to create artificial implants that mimic the biological architecture of real tissues and bone cartilage. His team has so far created prototypes of structural tissues such as bone,

cartilage and knee-meniscus as well as soft tissues such as liver, heart and skin. Liquid silk is used to create the bioink. This is obtained either by dissolving silk fibers into appropriate solvents or by direct extraction from silkworm glands. Mandal's laboratory has been working on creating artificial medical implants from silk proteins since 2012. They had earlier used silk proteins to create prototypes of artificial pancreas as well as an injectable gel to deliver insulin producing cells to treat diabetes. They has also developed implantable mats made of silk-proteins and bioactive glass fibers that can help treat arthritis. It also used silk–bone cement composite to create bone grafts. The bioink should be based on polymers that not only support live cells but also prevent them from getting damaged in the process of printing. The polymers should retain its properties throughout the printing process. For tissue engineering applications, we are always looking for polymers which would allow the cells to stick to it, and also give a suitable space for cells to grow and proliferate faster. Generally, people use different types of polymers such as collagen. Scientists use chemicals to bind cells to these polymers. Muga silk has 'attachment sites' naturally available. This silk is abundantly found in India but not available in any other country. Their process is much simpler than existing tissue engineering techniques. The team hopes that their research will pave the way for 3D printing fully-functional organ implants in the future. Mandal claimed that the 3D printed structures can also have applications in checking toxicity of drugs, and artificial organs can further help reduce the need to test drugs on animals.

❖ **Properties of Muga silk protein Bioink:**

1. Allow the cells to stick to it.
2. Give a suitable space for cells to grow and proliferate faster.
3. Process is much simpler than existing tissue engineering techniques.
4. Muga silk has 'attachment sites' naturally available.

Challenges in Silk Bioink Development:

- Optimizing the rheology of bioink is of paramount importance as high viscosity of bioink exhibits gel-like behavior to facilitate self-supporting filament extrusion.
- Bioinks should exhibit attributes of shear-thinning fluids (viscosity should reduce with increasing shear rate) so that they readily flow through small diameter nozzles of the printer. At the same time, they should exhibit instant shapere tention as soon as they are released from the nozzle.
- The cell-laden bioink should have sufficient mechanical stiffness, which is required to retain the structural integrity of filament post-printing, so that complex 3D multilayered patterns can be formed.
- Hydrated network to permit the exchange of gases, nutrients, and metabolite diffusion for effective cell viability.

- In extrusion-based printing technique, the diameter of this extruded filament is typically determined by several factors including the diameter of the nozzle, rheology of bioink, ink flow rate, pressure applied, line height (distance between the surface and the tip of the needle), and printing speed. Post-extrusion, two main material properties determine the stability of construct, viscosity, and surface wetting. While the viscosity of hydrogel depends upon gelling/cross-linking mechanism, molecular weight, concentration, temperature, and humidity, surface wetting is more of a function of interfacial energies between the substrate and bioink material.

Like many other biopolymers, silk fibroin protein does not inherently fulfill many of these requirements mainly due to two reasons. Silk fibroin inks display shear thinning behavior at low concentrations (i.e., <20 wt. %); the concentration range is not appropriate for printing. Beyond this 20 wt% concentration, it offers behavior of Newtonian fluid, where viscosity is not changed with increasing shear rate. On the other hand, during printing, macromolecular chains of fibroin protein undergo shear-induced (<100 s−1) conformational changes from random coil to β-sheet and crystallite formation, resulting from thermodynamic and kinetic processes. As a result, the extrusion process of silk fibroin ink is frequently interrupted due to clogging of the micro nozzle.

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QUALITY MODIFIERS FOR MEDICINAL PLANTS

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Abstract:

As the need for herbal medical products and materials continues to grow, it is essential to ensure that the quality of these things is maintained in order to properly satisfy this demand. The quality of these materials is affected by a wide range of factors, including those that are physical, chemical, and geographical in nature. Additionally, the quality of the herbals is contingent upon all of these factors. Aside from that, the issue of adulteration is also becoming a growing worry, which is causing the quality of herbal materials to become increasingly concerned. In order to evaluate the quality of the plant components that are utilized in herbal medicines, a number of different chemical and phytochemical assays, analytical procedures, and hyphenated analytical methods are utilized. It is possible to employ these procedures as a means of quality control, and they may also be utilized to evaluate the quality of herbal materials and herbal medicines.

Keywords: Quality, Herbal Plants, Affecting Factors

Introduction:

The quality and purity of herbal raw materials and finished products will need to be maintained in order to meet the growing demand for herbal medicines in both developing nations and established countries alike. This is an inescapable consequence of the growing demand for herbal remedies. Plants in their whole, plant parts, or extracts obtained from the plant are the three primary forms that herbal treatments can take. It is a very complicated process that takes into account a wide range of criteria while formulating herbal treatments.
It is because of this complicated composition that there is a requirement for standardization in respect to plants. In order to ensure that any herbal remedy will have a quality that can be duplicated, it is essential necessary to have a good control over the material that is used in the beginning. When it comes to maintaining control over the quality of the initial material, there are a number of considerations that need to be considered. These issues will be discussed in the parts that follow.

Factors affecting the quality of herbal plants: [1,2,3]

1. Validation and reproducibility of herbal Ingredients
2. Intra or inter species variation in herbal plants
3. Environmental adaptations in plants

4. Geographical location in herb growth
5. Plant Parts used
6. Time of harvesting for maximum herbal potency
7. Post Harvesting Factors
8. Contamination in Herbal Ingredients
9. Chemical Variation in Medicinal Plants

1. Validation and reproducibility of herbal ingredients

The macroscopical and microscopical features of herbal compounds must be carefully identified before they can be compared to legitimate material or real herbs that have been appropriately characterized. When referring to herbal components, it is important to use their binomial Latin names, which contain the specie and genus. Synonyms should only be used when they are appropriate. It is essential to have a solid understanding of the fact that the quality of various batches of the same herbal component might change owing to a variety of reasons, even if the authenticity of the item has been independently verified.

2. Intra or inter species variation in herbal plants

There are major variations between plants of different species, both within and between species, as well as between their primary and secondary metabolites. Because of this, there is a degree of variation in the various components, which makes standardization challenging. There is a connection between the nation of origin of the species and all of these changes, which are established by genetic data.

3. Environmental adaptations in plants

Numerous variables, including temperature, altitude, precipitation, and other circumstances influencing plant growth, impact the quality of herbal constituents within the same species, even when located in the same nation. This leads to significant changes in the herbal constituents found in some particular plant species. Numerous environmental factors regulate the variability of the myriad chemicals in a certain herbal medicine, topical cosmetic, or oral formulation. Thus, awareness of these aspects is crucial for achieving consistent plant quality. Plants are always subjected to many environmental conditions, many of which induce stress. The plant progressively acclimatizes to its evolving environment, resulting in alterations in the synthesis of these metabolites. Alterations occur in the genes and proteins, influencing the quantity of enzymes produced that participate in the synthesis of plant secondary metabolites. Consequently, the synthesis of these metabolites is modified. Various variables generally regulate the formation of certain secondary metabolites in plants. Secondary metabolites are often localized inside certain plant parts, tissues, and developmental phases. Environmental variables affect the synthesis of these chemicals. Throughout the years, plants have evolved and adapted to endure harsh climatic circumstances, including drought, heavy rainfall, extreme

temperatures, persistent humidity, and flooding. Throughout these evolutionary transformations, plants have altered their production patterns of secondary metabolites. Plants modify their production patterns to counteract environmental stress, which adversely impacts their fitness and survival. Numerous factors influence plant growth and development, as well as the secondary metabolites found therein, as discussed in the following sections.

3.1 Solar radiation

Solar radiation, particularly ultraviolet light, is necessary for the process of photosynthesis. It is damaging to the physiology of plants to be exposed to certain UV-B wavelengths (280–315 nm). Radiation that might be harmful to the plant should be avoided. The production of flavonoids, anthocyanins, hydroxycinnamic acids and their esters, anthocyanidins, and tannins is increased as a result of this transformation. In plants, flavonoids are responsible for a variety of functions.

Chemical structure relies on function. These compounds shield plants from UV radiation, herbivores, and illnesses. It absorbs chemicals effectively. Natural products scientists must do this because of their antifungal, antiviral, antibacterial, anti-inflammatory, antihepatotoxic, vasodilatory, lipolytic and antiallergic qualities.

Solar radiation exposure predicts a plant's metabolome and activity. Environmental issues boost production of additional molecules than polyphenolic compounds. The accumulation of glycol-alkaloids, such as α-chalconine and α-solanine, in potato tubers increases phytosterols in plant tissues. Former causes gastrointestinal or neurological problems. Plant sterols reduce cardiovascular disease, therefore they're good for you.

3.2 Soil nutrients

Plant nutrition regulates secondary metabolites. Plants favour major metabolites above minor ones. Nutrient-rich soil produces alkaloids and cyanogenetic glycosides. The plant cannot create nutrients in inadequate soil. Little soil phosphate promotes anthocyanidin and little iron increases phenolics. In nitrogen-deficient soil, plants produce more carbohydrate-rich metabolites, says the carbon nitrogen equilibrium theory. Phenyl alanine produces many alkaloids. Ammonia-lyase forms alkaloids from phenyl alanine. Proteins and phenols interact. Protein synthesis reduces phenols. As known, plant genetics and environment affect secondary metabolite production. Nutrition and pH are easily checked in Rodiolasachaliensis roots. Soil nitrogen is connected to medicinal plant cyano-genetic glycosides.

3.3 Stress

During the process of evolution, plants engage in interactions with their surroundings. They come into touch with a variety of abiotic components, including water, temperature, light, soil, and chemicals (fertilizers and minerals). For the purposes of their development, growth, and continued existence, plants require an adequate quantity of these parts. However, the plant

experiences stress as a result of either an increased or decreased amount of these abiotic components, which eventually results in variations in the quantities of metabolites that are produced or accumulated.

3.4 Water stress

Due to the fact that it acts as a channel for the transfer of nutrients and metabolites to all segments of the plant, water is an essential molecule in the physiological processes that occur within a plant. Water stress, also known as drought stress and salinity stress, is a condition that occurs when the availability of water is restricted or when the rate of transpiration reaches a higher level in plants.

3.5 Drought stress

Dry weather damages stomata, membranes, osmotic stress, and enzymes, affecting photosynthesis. Different plants and metabolites respond to drought. Drought reduces Chamomilla's volatile oil concentration. The drought stress promotes secondary metabolite production in *Artemisia annua, Hypericum perforatum, and Catharanthus roseus*. Increasing drought stress raises H. perforatum hyperforin. Drought increases Artemisinin in Artemisia and Hypericum Brasiliense, quercetin, betulinic acid, and rutin. In *G. longituba*, water stress reduces total flavonoids. Best for essential oils and secondary metabolites: 75% field water capacity for Ocimum basilicum and americanum. Dryness and salt stress raise *Achnatherum inebrians*' alkaloid levels. Here, Ergonovine beats Ergine. Phenolic compounds protect against winter moisture drought locally.

3.6 Salinity stress

It has been discovered that a plant's ability to produce secondary metabolites is depending on how salinized the soil is. One important trigger in this respect is the Na^+ content of the soil. *C. roseus* and *Rauwolfia serpentina* plants generate greater vincristine and reserpine in response to elevated salt stress. When *Ricinus communis* is subjected to salt stress, the amount of ricinine produced in the roots decreases and increases in the shoots. *Achillea fragrantissima* has a higher phenolic content. Caffeic, chlorogenic, and protocatechuic acid synthesis increases in *M. chamomilla*. The amount of essential oil in *Mentha piperita, Thymus maroccanus, Origanum vulgare, Majorana hortensis, M.* chamomilla, *Salvia officinalis, Mentha pulegium, and Mentha suaveolens* has started to decrease.

3.7 Chemical stress

A variety of chemical variables regulate metabolite formation in plants. Minerals, heavy metals, fertilizers, pollutants, gaseous toxins (CO_2 and ozone), insecticides, growth regulators and elicitors, $FeSO_4$, $ZnSO_4$, Miczink, $CuSO_4$, and other substances are included. Nitrogen and phosphorous, two crucial components for plant development and growth provided by fertilizers, have a major influence on the formation of Naphtho dianthrones (such as hypericin and

hyperforin) in St. John's Wort. Nitrogen and phosphorus also have a role in genetic modification. This has been uncovered through genomic and bioinformatics research. Phosphorus has also been proven to have a major effect on plant growth and secondary metabolite synthesis. It has been discovered to have a reduced link with the plant's essential oil concentration.

3.8 Temperature

Temperature stress affects plant enzymatic processes. Native antioxidant enzymes including superoxide dismutase, catalase, peroxidase, and others are particularly important in this setting. At 35 ℃, St. John's Wort shoot tissues had more hypericin, pseudo hypericin, and hyperforin. Temperature stress affects many vaporous compounds. Polyphenolic compounds are amazing molecules because they defend against heat. The peculiar behaviour of plant anthocyanins has been shown. In creating secondary metabolites, herbivore stress is important. The mechanical damage caused by herbivore insects stresses the plant, causing it to produce secondary metabolites. Ozone affects secondary metabolite production.

3.9 Ecological factors

In the age of "set-aside" land, farmers can cultivate herbs organically if their customary crops are unprofitable. This is a significant reason medical plant use is rising. Some wild species are threatened by the popularity of herbal remedies. For instance, Panax quinquefolium (American ginseng) now costs $1100 per kilogram due to high demand. Northern and Eastern American forests had it in abundance two centuries ago. Over-collection has caused plant extinction before. Roman ladies used carrot-family plant Silphion as a contraceptive. Silphion was hard to grow and was collected from the wild in big numbers, making it extinct in the 3rd century AD. Herbal medicine must be produced sustainably if it is to continue growing.

3.10 Seasonal variation

Other factors that can affect the concentration of secondary metabolites in plants include genetic regulation, gene expression, genotypes, biological and environmental factors, biochemical, physiological, ecological, and evolutionary processes, as well as seasonal variation and peculiarities within and between plants and species. The opium poppies (Papaver somniferum) contain around 80 alkaloids, including morphine, codeine, thebaine, narcotine, and papaverine, which are vital to the pharmaceutical industry. These alkaloids exhibit seasonal fluctuations at different phases of development. Alkaloid harvesting time and diurnal oscillations should be considered together to avoid alteration or degradation of the main chemical. If there is an irreversible rise or fall in the enzyme activity in the alkaloid synthesis pathway, the latex may lose the prior component following conversion.

4. Geographical location in herb growth [4]

This component simply combines the environmental factors stated above. Regional effects impact climate. Geographical and environmental factors greatly affect PSM production.

Some plants adapt better to certain climates. Positive environmental factors boost plant growth and secondary metabolite levels. The same plant species' secondary metabolite concentrations may vary depending on environmental conditions. The production of secondary plant metabolites depends on plant physiological needs, which are affected by climate. Geographic location and environmental conditions alter Nothapodytes nimmoniana's Camptothecin (mono-terpene indole alkaloid) content. The antibacterial potency of Mentha spicata varies by area, affecting yields. High-altitude flora is more antibacterial. Secondary metabolites like alkaloids, terpenoids, glycosides, flavonoids, flavonol, tannins, hydroxycinnamic acids, phytosterols, luteolin, phenolic compounds, anthocyanins and cyanogenic glycosides are affected by light, temperature, soil nutrients, and moisture.

5. Plant parts used [5]

There are often a variety of active components found in different plant sections. It is common for herbal components to have unused plant pieces that have been polluted. To raise the weight of the herbal remedy, adulteration happens when various plant components that have comparable physical qualities are put together. Plant structure is composed of several components. There are particular plant species that possess metabolites that are localized. This is due to a number of causal variables. As a result of plant adaptation, secondary metabolites concentrate in a variety of locations. The chemical compounds known as secondary metabolites are those that do not have an immediate impact on the development of plants. Through the use of particular procedures, they generate intermediates from primary metabolites in order to construct metabolites ranging from simple to complicated. Alkaloids, terpenes, phenolics, and vitamins are all examples of secondary metabolites that are of great significance. These metabolites are essential for the plants that generate them, despite the fact that the majority of them do not have an immediate impact on the growth and development of plants. The vast majority of secondary metabolites are poisonous and serve as a defense mechanism against infections, herbivores, allelopathy, and other harmful biotic stresses.

6. Time of harvesting of maximum herbal potency [6]

Specification of the optimal harvesting time is necessary when obtaining a specific botanical ingredient. During the growth cycle, the constituents, including a variety of concentrates derived from the secondary metabolites, undergo significant fluctuations. Therefore, the time of harvest is crucial in ensuring that the desirable constituent is present in the highest concentration.

7. Post harvesting factors

The quality of an ingredient made from herbs can be significantly impacted by how the gathered herbal raw materials are handled, including storage and transportation. Poor post-

collection storage can lead to microbial infection, and drying and other procedures can cause the thermolabile active ingredients to be lost.

8. Contamination in herbal ingredients

Herbs must be free of excreta, insects, and animal waste. Since herbal materials cannot be completely devoid of pollutants, specifications limit them. Calculate the ash value, the inorganic residue left after burning an herbal component. The acid insoluble ash formed by hydrochloric acid treatment is mostly silica. The dirt content may be determined. Organic compounds from beyond the vicinity are likewise affected. Almost all herbal remedies involve foreign organic materials, either plant pieces or other plants. Standards should minimize these unwanted plant contaminants. Plant material may contain aerobic bacteria and fungus from inappropriate cultivation, harvesting, storage, or processing. Herbal compounds often include 102–108 CFU/g aerobic microorganisms. Herbal products have been shown to include Shigella, Clostridium, Pseudomonas, Enterobacter, Entrococus, and Streptococcus. Herbal drugs with high starch content might stimulate microbe development, thus all contaminants should be limited.[7]

8.1 Environmental contaminants

Medicinal plants are wild or cultivated. They are exposed to heavy metals, herbicides, fertilizers, and microbial organisms. Thus, pollutants accumulate in plant tissues or secondary metabolite reservoirs including leaf, wood, barks, seeds, fruit material, rhizomes, fluids, resin ducts, and others. A common misconception is that "natural" or "herbal" means a drug is safe and reliable. Not so. In addition to compromising the medicinal ingredients of the herbal medicine, microbial contamination has caused serious illnesses in clinical case studies. Pharmaceutical limit tests determine the maximum allowed microbiological load in a certain amount of crude medication. Fungal or bacterial contamination may occur. They also cause chemical contamination via endotoxins, aflatoxins, and ochratoxin. A viable bacterial species can also survive as a spore, which can become viable under favourable conditions. Contaminants might be pesticides, fertilizers, fumigants, mycotoxins, or solvent residues. They may also include radioactive pollutants like Cs-134 and Cs-137. Adulteration, the intentional or unintentional addition of toxic plants, incorrect botanical species, synthetic products, and other substances like NSAIDs, corticosteroids and benzodiazepines to medicinal plant products, also lowers quality.

8.2 Hazardous metals, pesticides, fumigants:

Herbal materials may be contaminated with DDT, carbamates, organophosphates or polychlorinated biphenyls. Phosphine, methyl bromide, and ethylene oxide fumigants can control herbaria pests. Some herbs include lead, mercury, cadmium, thallium, and arsenic. Limit testing for pesticides, fumigants, and toxic metals is necessary to monitor plant material quality. Endotoxins, mycotoxins, and radionuclides should be restricted to improve botanical ingredient

quality. Such limits are important to ensure high-quality, therapeutic medicinal plants. [8,9]

9. Medicinal plant chemical composition variation

Environmental variables including humidity, rainfall, and altitude affect the chemical makeup of medicinal plants in addition to plant-specific elements. Processing processes affect the plant's chemical makeup. This needs batch-to-batch plant material uniformity. Unlike manufactured drugs, herbs are complex combinations of chemicals. They often work together. As herbal medicine becomes more popular worldwide, chemical ingredient diversity must be considered when assessing its quality (Xie *et al.,* 2009). A drug-approved isolated component is not the same as the complete plant extract. Examples include Artemisinin and A. annua Linn. Taking the whole plant extract increases bioavailability 45-fold. Isomers and conformations can modify markers, and solvents, temperature, and light can degrade and transmute pure components. The chemical makeup of medicinal plants depends on environmental variables, regional variances, intraspecies and interspecies variation, and plant part variation. Several variables affect metabolite content. This section will focus on metabolites and species variation. The plant's genetics, storage, and extraction will cause diversity.[10]

Conclusion:

It is possible that biotic and abiotic stressors, which can result in the production of secondary compounds, might act as tools to improve the health-related characteristics of plant material. Any increase in the accumulation of pharmaceutically helpful plant metabolites must be evaluated within the context of a comprehensive change in the metabolic profile, in which the levels of many chemicals vary simultaneously. This is necessary in order to guarantee the success of this one element. When it comes to the quantitative evaluation of these compounds, having a solid understanding of the elicitation of induced metabolites and the lifespan of these metabolites is absolutely necessary. Understanding these dynamics is likely the most important way for ensuring quality in phytomedicine, despite the fact that there are a number of intricacies and complicated relationships between the physiological and metabolic systems of the plant and the external factors that affect it. In reality, quality is something that is nurtured in the field. The responses of plants to environmental stimuli are species-specific, which is something that should be taken into consideration. The integration of ecology, biochemistry, and molecular physiology constitutes a multidisciplinary approach to this problem, which has tremendous potential for enhancing this field of study and shedding light on the degree to which interactions between plants and their environments contribute to phytomedicine. A considerable impact on the quality of secondary metabolites found in medicinal plant extracts is exerted by the timing of harvesting and the postharvest treatment of plant material.

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PROGRESSIVE RESISTANCE TRAINING IN PHYSIOTHERAPY: FUNDAMENTALS, CLINICAL APPLICATIONS AND EVIDENCE-BASED INSIGHTS

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Introduction:

Progressive resisted exercise (PRE) is a foundational intervention in the domain of physiotherapy, employed extensively to improve muscular strength, endurance, and functionality. It operates on the principle of gradually increasing resistance, thereby pushing muscles beyond their habitual load. This incremental challenge leads to muscle hypertrophy and neuromuscular adaptations. The aim of this chapter is to elucidate the scientific rationale behind PRE, discuss its practical applications in clinical settings, and review the supporting evidence across various patient populations.

Historical Background

Resistance training has ancient origins, with early forms traced back to Greek antiquity. However, the modern scientific basis of progressive overload was firmly established during the 1940s by DeLorme and Watkins. Their pioneering work, which focused on rehabilitating World War II soldiers, gave rise to the DeLorme technique. This method emphasized a structured progression of resistance and is considered a milestone in the evolution of therapeutic exercise (1).

Physiological Mechanisms Underpinning PRE

When muscles are subjected to higher-than-normal resistance, various physiological changes occur:

- **Hypertrophy**: Primarily involving the enlargement of fast-twitch (Type II) muscle fibers (2).
- **Neural Adaptations**: Enhanced recruitment and synchronization of motor units contribute to strength improvements (3).
- **Metabolic Adjustments**: Increases in enzymatic activity and mitochondrial density promote endurance (4). These physiological adaptations are pivotal in restoring or enhancing muscle function in both healthy individuals and those undergoing rehabilitation.

Key Principles of Progressive Resisted Exercise

For PRE to be effective, it must adhere to specific training principles:

- **Overload**: Muscles must be exposed to loads greater than they usually encounter.
- **Specificity**: Training must be tailored to the target muscles, contraction types, and energy systems.
- **Reversibility**: Gains in strength and function can diminish without continued use.
- **Progression**: Resistance must be increased progressively to sustain improvements (5).

Established Methods and Protocols

Several recognized methods are used in clinical practice:

- **DeLorme Method**: Involves three sets of 10 repetitions at 50%, 75%, and 100% of the 10-repetition maximum (RM), allowing a progressive increase in intensity.
- **Oxford Method**: A reverse approach with sets starting at 100% and tapering to 75% and 50% of the 10 RM to minimize fatigue (6).
- **Daily Adjustable Progressive Resistance Exercise (DAPRE)**: Adapts resistance based on performance in previous sessions, allowing for individualized progressions (7).

Equipment and Modes of Resistance

Progressive resisted exercise (PRE) can be implemented using a diverse array of equipment and resistance modalities, each tailored to match therapeutic objectives, patient capabilities, and clinical environments. These include:

- **Free Weights (Dumbbells and Barbells)**: Offer versatile and functional training options by engaging stabilizer muscles and permitting a full range of motion. These are suitable for individuals aiming to enhance balance, coordination, and compound strength.
- **Resistance Bands and Tubing**: Provide elastic resistance that changes throughout the range of motion. Lightweight, portable, and cost-effective, these are ideal for home-based rehabilitation and early-phase strengthening.
- **Weight Machines**: Deliver controlled movement patterns, making them beneficial for beginners, the elderly, or those recovering from injury. Machines reduce the demand for balance and are useful when isolating specific muscle groups.
- **Isokinetic Devices**: Maintain a constant speed regardless of the force applied by the user, allowing for accurate measurement of muscle strength and power throughout the joint range. These are frequently used for performance assessment and rehabilitation post-surgery (8).
- **Body Weight Exercises**: Push-ups, squats, and other self-resisted movements are accessible and modifiable, making them appropriate for diverse settings, including low-resource environments.

- **Aquatic Resistance Equipment**: Tools such as water paddles or resistance gloves used in hydrotherapy provide resistance with reduced joint stress, making them suitable for arthritic or post-operative conditions.
- **Smart Resistance Technologies**: Emerging tools like digital resistance machines and app-integrated devices offer real-time feedback, adjustable resistance, and personalized progression, supporting evidence-based practice.

Selecting appropriate equipment should be guided by the individual's health status, functional goals, and environmental constraints. Combining multiple resistance modes often enhances outcomes and maintains patient engagement.

- **Free Weights**: Enable natural movement patterns and engage stabilizing muscles.
- **Resistance Bands**: Offer variable resistance and are convenient for home-based exercises.
- **Machines**: Provide structured and controlled movements, ideal for beginners.
- **Isokinetic Devices**: Maintain constant angular velocity, allowing accurate assessment and safe resistance across the range of motion (8).

Clinical Applications in Different Populations

- **Musculoskeletal Conditions** PRE is effective in treating musculoskeletal disorders such as osteoarthritis, chronic back pain, and post-operative recovery. Strengthening the quadriceps has shown to decrease pain and enhance joint function in knee osteoarthritis (9,10).
- **Neurological Conditions** In individuals recovering from stroke or dealing with Parkinson's disease, PRE improves muscle strength and functional independence. However, careful regulation of intensity is necessary to avoid overexertion (11).
- **Cardiopulmonary Patients** When performed under supervision, PRE is beneficial even for individuals with stable cardiovascular diseases or chronic obstructive pulmonary disease (COPD). It enhances peripheral muscle strength and contributes positively to overall well-being (12).
- **Geriatric Population** PRE helps mitigate sarcopenia, preserving muscle mass, and reducing the risk of falls. Programs for older adults should emphasize gradual progression with moderate resistance levels (13).
- **Pediatric Applications** Although progressive resisted exercise (PRE) is not yet widely adopted in pediatric physiotherapy, growing evidence supports its safety and efficacy when structured in alignment with age-specific guidelines. Carefully designed programs can enhance muscular strength, coordination, and motor skill development in children without causing harm. Furthermore, engaging children in supervised resistance training can promote long-term physical activity habits and contribute to overall health and fitness (14).

Implementation Guidelines

Effective PRE programs typically include:

- **Frequency**: 2–3 sessions per week
- **Intensity**: 60–80% of one-repetition maximum (1 RM)
- **Repetitions**: 8–12 per set
- **Sets**: 2–4 per muscle group
- **Rest**: 1–3 minutes between sets
- **Progression**: 2–10% weekly load increase, as tolerated Including warm-up and cool-down phases is essential. Patient responses, such as signs of pain or fatigue, should be continuously monitored (15).

Safety Considerations and Contraindications

To maximize safety and effectiveness, programs must be tailored to the individual's medical status. Absolute contraindications include:

- Uncontrolled hypertension
- Acute injuries
- Severe cardiovascular or respiratory diseases
- Active inflammation or systemic infections (16) Professional supervision ensures correct technique and minimizes risks.

Evidence Supporting PRE

Research strongly supports the application of PRE:

- A meta-analysis by Peterson *et al.* reported significant improvements in muscle strength among older adults following PRE interventions (17).
- Hayes and colleagues demonstrated pain relief and enhanced function in patients with chronic low back pain undergoing resistance training (18).
- Clinical trials have validated the utility of PRE in post-stroke rehabilitation and cardiac care settings (19,20).

Challenges and Strategic Solutions

Despite its benefits, implementing PRE can be challenging. Common barriers include inadequate access to equipment, lack of professional supervision, and fear of injury. Practical solutions include:

- Use of portable resistance tools like bands
- Comprehensive patient education
- Utilization of mobile apps for monitoring and motivation

Future Perspectives

Emerging technologies such as smart resistance machines, virtual reality-enhanced training, and artificial intelligence-driven protocols are reshaping the landscape of therapeutic

exercise. These innovations promise to personalize and optimize PRE programs for better outcomes.

Conclusion:

Progressive resisted exercise (PRE) stands as a fundamental and evidence-based approach within physiotherapy, offering substantial benefits in improving muscle strength, endurance, and functional performance across a range of populations. Its versatility and adaptability make it suitable for musculoskeletal, neurological, cardiopulmonary, geriatric, and pediatric conditions. Successful implementation of PRE requires a personalized strategy that includes appropriate assessment, tailored load progression, and consistent monitoring. Adhering to scientifically grounded principles and ensuring patient education and safety are vital for maximizing outcomes and minimizing risks. As technological innovations continue to emerge, the future of PRE holds even greater promise in delivering optimized and individualized rehabilitation programs.

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TEMPO AND DURATION IN RESISTANCE TRAINING: FOUNDATIONS FOR STRENGTH AND POWER DEVELOPMENT

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1. Introduction:

With uses ranging from general health to competitive sports and therapeutic rehabilitation, resistance training is a crucial component of physical fitness and athletic performance. In order to produce physiological changes like muscular strength, power, hypertrophy, and endurance, resistance training fundamentally modifies a number of variables, such as intensity, volume, frequency, rest periods, exercise selection, and movement execution [(1)]. Although factors like load and volume have historically gotten a lot of attention, two often disregarded but crucial elements—exercise duration and tempo—have a big impact on training results, particularly when it comes to building strength and power [(2)].

The rate at which the repetition is performed, including the amount of time spent in the eccentric, isometric, and concentric phases of movement, is referred to as the exercise tempo. Conversely, exercise duration includes the time spent on each set or repetition, also referred to as time under tension (TUT), in addition to the duration of a training session [(3)]. Although these two factors are often discussed separately, they are inseparable: tempo affects TUT, which in turn affects hormonal responses, metabolic stress, neuromuscular recruitment, and, ultimately, performance outcomes.

Understanding and changing speed and time helps athletes, therapists, and coaches customize their training stimulus. A slower eccentric tempo, for instance, can lead to more muscular damage and hypertrophy; a fast, explosive concentric movement is needed to raise power output[(4)]. Longer session lengths might likewise enhance endurance but lower strength as more cortisol levels and neuromuscular exhaustion get higher.

It's important to carefully balance duration and tempo when strength and power training. Repetition speed and set duration have an impact on controlled movement patterns, maximal motor unit recruitment, and adequate recovery, all of which are critical components of strength development. Conversely, power training emphasizes timing and movement velocity while

requiring rapid force production [5]. If speed and length are not matched to training goals, adaptation may be hampered or injury risk may increase.

The goal of this chapter is to provide viewers with a comprehensive understanding of resistance training exercise duration and tempo. It will look into their physiological foundation, real-world uses, and evidence-based suggestions. It will also show how these variables interact in different training situations. By the end, readers will know how to combine duration and tempo in strength and power training to achieve the best results.

2. Foundations of Resistance Training Variables

Resistance training is guided by a number of factors that combine to cause specific physiological changes. They consist of training volume, intensity, frequency, rest periods, exercise order and selection, and—above all—tempo and duration. Program design in both general fitness and specific strength and power development is based on the adjustment of these variables. To get the best performance results, it is essential to comprehend how tempo and duration fit into this bigger framework.

2.1 Crucial Elements of Resistance Training

A resistance training session's primary components are: Volume, which is typically computed as sets × repetitions × load, is the total amount of work completed.

The weight or load in relation to the person's maximum capacity is called intensity. The number of weekly sessions or exposures for each muscle group or exercise is known as frequency.

Rest intervals: The amount of time that passes between sets and affects metabolic stress and recovery.

Exercise selection and order: These factors affect how muscles are activated and how fatigue is managed.

Tempo: The pace of movement during the concentric, isometric, and eccentric phases of each repetition.

Tempo and the general structure of the session have a significant impact on duration, which is the amount of time spent on each repetition, set, or session.

Although volume and intensity have long been the focus of research and practice, new evidence suggests that tempo and duration play an important role in shaping training outcomes, especially when goals shift toward maximal strength or power development [6,7].

2.2 Interactions Between Tempo and Duration

Tempo directly affects time under tension (TUT), which is a sub-component of duration. A slower tempo increases TUT, promoting hypertrophic and metabolic responses, while a faster tempo reduces TUT but enhances neural activation and rate of force development—crucial for power expression [7].

For example:

A set of 10 reps at a 2-0-2-0 tempo = 40 seconds TUT

A set of 10 reps at a 1-0-X-0 tempo = ~20 seconds TUT

Such differences affect not only muscle fatigue and adaptation but also hormonal response, perceived exertion, and session efficiency. In this context, tempo becomes a modifiable tool for controlling duration, which subsequently affects all other training variables.

2.3 Duration and Tempo: Load-Independent Instruments

Tempo and duration are unique because they let you alter the training stimulus while maintaining a steady external load. This has applications in a number of situations:

- Slower tempos that emphasize skill and control while avoiding excessive loads are beneficial for both beginners and clinical populations.
- Tempo contrast is a tool that advanced athletes can use to minimize joint stress and boost strength or power production.
- Tempo manipulation promotes tissue repair and motor control retraining by challenging muscles while lowering mechanical strain [8].
- Compared to load progression, which increases the risk of injury if used too rapidly, tempo progression is a safer way to manipulate internal workloads.

2.4 Connection to Power and Strength Results

By increasing TUT and motor unit recruitment, pace control, especially during the eccentric period (e.g., 3–4 seconds), can enhance maximal force output. In sports involving sprinting, jumping, or Olympic lifts, power can be maximized in a shorter amount of time by limiting duration and employing explosive tempos (e.g., X-0-X-0) [7, 9].

The SAID principle (Specific Adaptations to Imposed Demands), which states that training modifications should correspond with the type of stressor, is supported by these findings. Therefore, increasing power requires high-speed, low-duration repetition patterns, while increasing strength requires longer durations and controlled movement.

2.5 Periodization and Time/Duration's Role

Within periodized programs, the duration and tempo can be carefully adjusted to meet the objectives of each training phase.

- Hypertrophy Phase: Increased TUT at a slow pace (e.g., 4-1-2-1). Phase of strength: regulated tempo (e.g., 3-0-X-1) at increased loads.
- Power Phase: Low TUT, explosive rhythm (e.g., X-0-X-0).

In addition to improving particular results, strategic management of these variables enhances overall recovery and long-term performance sustainability [6].

3. Understanding Exercise Tempo

3.1 Definition

Exercise tempo is the specific speed at which a repetition is performed during resistance training, and it is typically represented by a four-digit number (3-1-2-0, for instance). Each digit represents the duration (in seconds) of the four phases of a repetition: concentric, pause at the top, pause at the bottom, and eccentric. For instance, a 3-1-2-0 tempo in a squat entails 3 seconds of descent, 1 second of holding at the bottom, 2 seconds of raising, and no rest at the top. This approach allows for the adjustment of internal training load without altering external resistance and offers precision in training prescription [(10)].

3.2 Tempo's Components and Their Functions

Every tempo phase has a distinct role in adaptation.

- **Eccentric Phase:** Significant force and muscle damage are caused during the eccentric phase, which promotes hypertrophy.
- **Isometric Pause:** Reduces dependency on elastic energy (SSC) while increasing joint stability.
- **Concentric Phase**: Vital for power and strength; higher speeds can increase neuromuscular effectiveness.
- **Pause at the Top:** Promotes recuperation, but if extended, lowers metabolic stress.

3.3 Types of Tempo

Tempo can be categorized based on training objectives.

- **Slow Tempo (e.g., 5-1-5-1):** Increases hypertrophy and control through prolonged time under tension (TUT).
- **A moderate pace (such as 3-1-2-1)** strikes a balance between motor acquisition and strength development.
- **Fast/Explosive Tempo (e.g., X-0-X-0):** Boosts power and velocity; great for athletes.

3.4 Tempo's Effects on the Physiology

Numerous physiological processes are impacted by tempo:

- **TUT and Hypertrophy:** TUT increases with longer tempos, leading to metabolic and mechanical stress [(11)].
- **Recruitment of Motor Units:** High-threshold units needed for strength and power are drawn in by explosive tempos.
- **Effects on the Neural and Mechanical Systems:** Slow eccentrics intensify muscle damage and adaptation, while fast tempos improve neuromuscular coordination.

3.5 The stretch-shortening cycle (SSC) and tempo

The SSC, a muscular activity that consists of a quick eccentric contraction followed by an instantaneous concentric phase, is influenced by tempo. While intentional pauses (e.g., 3-2-1-1) decrease SSC advantages by increasing concentric strength, which is beneficial in strength training and rehabilitation, eliminating the pause (e.g., X-0-X-0) increases elastic recoil and power [(10)].

4.Understanding Exercise Duration

4.1 What Exercise Is Resistance Training Duration

The term "exercise length" describes how much time is spent putting forth physical effort at various levels, such as during a set, session, repetition, or entire program cycle. Muscular adaptations in resistance training are more influenced by the quality and structure of time, including tempo, rest intervals, and set structure, than by the duration of a session [(12)].

4.2 Time Under Tension and Duration per Set (TUT).

The duration of a muscle contraction during a set is known as Time Under Tension (TUT). Tempo and repeat rate have a direct influence on it. Common TUT ranges strive for particular results:

- Maximum power and strength in less than 20 seconds.
- Hypertrophy: 30–60 seconds.
- Over 70 seconds: endurance of muscles
- According to research, hypertrophy works best when TUT lasts 30 to 60 seconds per set and the weights are between 60 and 80% of 1RM [(13)].

4.3 The Connection Between Duration and Rest Periods

The overall training effect is significantly impacted by rest periods.

- **Brief (30–60 seconds):** Promote hypertrophy and raise metabolic stress.
- **Moderate (60–120 seconds):** Give yourself time to recover and balance your goals.
- **Long (2–5 minutes):** Facilitate complete ATP-PCr recovery, which is essential for power and strength.

In order to maximize training adaptations, rest management is crucial and affects the overall length of the workout [(12)].

4.4 Total Length of Session and Hormonal Reaction

In order to balance training intensity and hormonal efficiency, resistance exercises should last 45 to 75 minutes. Sessions longer than ninety minutes may raise cortisol levels and make recovery more difficult [(14)]. Healthy testosterone and growth hormone responses are encouraged by high-quality, time-efficient sessions.

4.5 Periodization and Control of Time

To meet performance goals, training duration varies between phases.

- **Phase of accumulation:** increased volume and prolonged TUT for hypertrophy
- **Phase of intensification:** Increased load and decreased volume to increase strength.
- **Peaking phase:** The time and volume at which performance is at its lowest.

Over time, this pattern permits ongoing adaptation and recovery.

4.6 Customizing the Length

The following factors determine an effective training duration:

- **Experience level:** While more seasoned athletes can work out vigorously in less time, beginners may need longer sessions.
- **Goals:** Bodybuilders aim to maximize TUT, while powerlifters prioritize rest and intensity.

Shorter, more concentrated sessions are beneficial for older or recovering patients. Program adherence and effectiveness are increased when temporal variables are customized[(12)].

5. Sport-Specific Applications of Exercise Tempo and Duration

Exercise duration and tempo must be modified to fit the physiological requirements, movement patterns, and performance goals of each sport. Although power and strength are essential traits in many sports, how they are demonstrated depends on the demands of the particular sport. This section examines the strategic use of length and pace in training plans for a range of sports, including endurance events, football, basketball, weightlifting, sprinting, and combat sports.

5.1 Track events and sprints

The goal of sprinting is to exert the most force in the shortest amount of time, which calls for excellent neuromuscular coordination. To increase rate of force generation (RFD), resistance exercises often employ explosive concentric tempos (e.g., X-0-X-0), especially in motions like sled pushes, Olympic lifts, and plyometric drills [(15)]. In order to maximize peak power production, sprint-specific sessions combine short-duration efforts (5–10 seconds) with lengthy recovery periods (3-5 minutes) [(16)].

5.2 Lifting Olympic weights.

Olympic lifts such as the snatch, jerk, and clean require quick force application, strength, and technical accuracy. These motions are executed forcefully, typically at a tempo of X-0-X-0. In order to maintain neuromuscular quality, the eccentric phase is kept to a minimum to prevent excessive fatigue, and set lengths are brief (4-6 seconds) with 3-5 minutes of rest [(17)].

5.3 American football and soccer

Strength, power, and endurance are all necessary for football players. Slower eccentric tempos (such as 3-0-1-0) enhance time under tension and muscle growth during hypertrophy or general strength phases. In order to maintain power and reduce fatigue, in-season training is

conducted at faster tempos (1-0-X-0). Depending on the training goal, set durations vary from 8 to 15 seconds, with rest periods of 1-3 minutes [18].

5.4 Basketball

Basketball demands a great deal of movement variability, directional changes, and explosive vertical jumping. Power training exercises like medicine ball tosses and jump squats are done at fast tempos (X-0-X-0) to mimic game demands. During strength-building phases, controlled tempos (3-1-1-0) are used to enhance muscle balance and reduce injury risk [19]. Power set durations are typically short (4–8 seconds) with high rest-to-work ratios, whereas strength set durations are longer with moderate pauses.

5.5 Combat sports (wrestling, MMA, and boxing)

Both muscular endurance and explosive power are required for combat sports. Power training emphasizes rapid concentric motions (X-0-X-0) for rapid striking and grappling performance, while strength-focused phases use controlled tempos (e.g., 3-1-1-0) to enhance structural integrity and joint control. An explosive drill lasts 4 to 10 seconds, while a strength set lasts 20 to 30 seconds. Depending on the focus, rest periods could last anywhere from 30 seconds to 4 minutes [16,20].

5.6 Endurance Sports (Running, Cycling, and Triathlon).

Resistance training enhances neuromuscular control over maximal power output and injury prevention in endurance sports. Joint stability and fatigue resistance are enhanced by controlled tempos, such as 2-1-2-0. Depending on the training goals, sets are 20–40 seconds long and are executed at moderate intensities (40–60% 1RM), with rest periods varying from 30 to 2 minutes [18, 20].

The best results from strength and power training occur when the duration and tempo are modified based on the situation. Short, fast-paced, high-intensity fights are preferred by sprinters and Olympic lifters, but combat and team-sport athletes gain from a range of phase-specific techniques. In order to improve durability, endurance athletes employ longer sets and slower tempos. Improving performance in a particular sport and reducing the risk of injury depend on properly periodizing these variables.

6. Recommended Tempo and Duration for Strength and Power Training

6.1 Tempo for Strength Training

6.1.1 Tempo Tips for Increasing Strength

Through an increase in time under tension (TUT) and muscle fiber recruitment, slow eccentric tempos (2-4 seconds) contribute to strength development. Three-1-1-0, which stands for three seconds eccentric, one second pause, one second concentric, and no pause at the top, is a common pattern. This technique increases overall muscle strength, tendon adaptability, and neuronal activation [21].

6.1.2 Strength Training Protocols

Each set of strength training consists of 1–5 repetitions at 80–90% of one-rep maximum (1RM), with 2–5 minute recovery periods in between. High loads and brain adaptations are the main topics of these sessions. Depending on the rep tempo, the total set durations can vary from 10 to 20 seconds [22].

6.2 Tempo for Power Training

6.2.1 Essential Power Training Concepts: Tempo

The goal of power training is to generate force quickly by using explosive concentric tempos. In order to maximize rate of force development (RFD) and train fast-twitch fibers, cleans, snatches, and plyometrics are frequently executed at an X-0-X-0 tempo—explosive up, no pause[23]. To reduce fatigue during eccentric loading, certain drills use tempos that are only concentric [24].

6.2.2 Protocols for power training

To guarantee maximum velocity, power sets usually include three to five repetitions with lower loads (30–60% 1RM). To sustain power output, set lengths are short (4–10 seconds), followed by rest periods of 3–5 minutes [23, 25].

6.3 Duration per Set for Strength and Power

6.3.1 Set for Strength Training Time frame:

Depending on the tempo and number of repetitions, strength-focused sets typically last 10 to 20 seconds. This preserves technique while guaranteeing maximum mechanical tension. To enable almost full recovery, rest intervals between sets are prolonged from two to five minutes [21,22].

6.3.2 Duration of Power Training Set

Power training sets are shorter, lasting 4–10 seconds, due to the focus on velocity. To maintain explosive output throughout sets, high rest-to-work ratios (3-5 minutes) are employed [24,25].

6.4 Session Duration for Strength and Power

6.4.1 Length of Strength Training Sessions

Usually lasting 45 to 75 minutes, strength training consists of three to five exercises in three to five sets. Extended rest periods are necessary for heavy loads, which lengthens the session [21,22].

6.4.2 Duration of a Power Training Session

Power sessions, which last roughly 40 to 60 minutes, are a little shorter and concentrate on velocity-based movements with longer rest periods but lower volume. Maximal neuromuscular recovery is encouraged by this approach [23, 25].

Conclusion:

Strength and power training requires careful control of duration and tempo. While explosive concentric tempos boost power, slow eccentric tempos and extended TUT enhance strength. Effective programming across sports disciplines is made possible by matching set and session durations to training objectives. Performance results are improved and adaptations are optimized when these concepts are combined with appropriate load and recovery protocols.

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UNDERSTANDING EXTENSOR LAG AND EXTENSOR LACK: A CLINICAL PERSPECTIVE ON KNEE EXTENSION DEFICITS

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1. Introduction:

Knee extension is a critical component of lower limb function, enabling efficient gait, balance, and performance of daily activities. Full knee extension contributes to joint stability and energy-efficient locomotion. Any limitation in achieving full extension can lead to significant functional impairment, altered gait mechanics, and compensatory strategies that may predispose individuals to secondary musculoskeletal issues (1).

Within clinical and rehabilitative settings, two commonly encountered yet often misunderstood phenomena are extensor lag and extensor lack. Although these terms are frequently used interchangeably, they describe distinct pathomechanical conditions affecting knee extension. An extensor lag refers to an active limitation in knee extension despite passive capability, while extensor lack implies a passive restriction where full extension cannot be achieved, even with external assistance (2).

Understanding these distinctions is vital for clinicians in diagnosis, treatment planning, and rehabilitation. The prevalence of extension deficits is notable in post-operative populations, especially following anterior cruciate ligament reconstruction (ACLR), total knee arthroplasty (TKA), and meniscal surgeries (3,4). This chapter explores the anatomical and biomechanical underpinnings, clinical presentation, etiology, and evidence-based management of extensor lag and extensor lack.

2. Anatomy and Biomechanics of Knee Extension

The knee joint is a complex synovial hinge joint comprising the femur, tibia, and patella. It involves three compartments: medial tibiofemoral, lateral tibiofemoral, and patellofemoral. Knee extension primarily involves the quadriceps femoris muscle group, particularly the vastus medialis obliquus (VMO), vastus lateralis, vastus intermedius, and rectus femoris. The patella acts as a fulcrum, increasing the mechanical advantage of the quadriceps tendon during extension (5).

The joint capsule, cruciate ligaments, and collateral ligaments contribute to joint stability and guide motion. Terminal knee extension, or the final 15 degrees of motion, is crucial for joint

locking through the screw-home mechanism. This motion involves external rotation of the tibia on the femur, contributing to stability in standing (6).

Arthrokinematically, extension involves anterior rolling and gliding of the femur on the tibial plateau, and patellar tracking within the femoral trochlear groove. Any disruption in this biomechanical interplay—due to muscle weakness, joint effusion, fibrosis, or proprioceptive deficits—can impede full extension (7).

3. Defining Extensor Lag and Extensor Lack

- **Extensor Lag:** Extensor lag is defined as an active deficit in knee extension wherein the individual cannot actively extend the knee to its full range, although passive range of motion (PROM) is intact. It is often a consequence of quadriceps weakness, particularly of the VMO, or neuromuscular inhibition due to pain, swelling, or reflex inhibition (8,9). Extensor lag is commonly observed following ACLR, TKA, patellar tendon ruptures, or prolonged immobilization (10).
- **Extensor Lack:** Extensor lack, in contrast, refers to a passive restriction of knee extension. Even with external help, the knee cannot reach its full extension range. This condition is typically associated with joint pathology such as arthrofibrosis, joint effusion, capsular contracture, or mechanical blocks like meniscal entrapment (11,12). It is often more challenging to manage and may require surgical intervention if conservative approaches fail (13).

While both conditions result in a loss of terminal extension, their pathophysiological origins differ significantly, necessitating different clinical approaches (14).

4. Clinical Presentation and Assessment

Patients with extensor lag or lack typically present with complaints of difficulty in walking, ascending or descending stairs, or standing from a seated position. Visual observation may reveal an inability to fully straighten the knee during gait, resulting in a flexed-knee posture and compensatory hip or ankle motions (15).

Assessment Tools:

- Goniometry: Quantifies active and passive range of motion.
- Straight Leg Raise (SLR): Assesses quadriceps strength and active extension.
- Prone Knee Hang Test: Evaluates passive extension in a gravity-assisted position.
- Functional Tests: Sit-to-stand test, step-down test, and gait analysis offer insights into functional impact.
- Manual Muscle Testing (MMT): Determines quadriceps strength.
- Palpation: Identifies tenderness, swelling, or mechanical blocks (16,17).

Differentiating between extensor lag and lack is crucial, as treatment approaches vary depending on whether the limitation is active or passive (18).

5. Etiology and Risk Factors

Extensor Lag:

- Quadriceps weakness (especially VMO)
- Pain-induced inhibition
- Joint effusion
- Prolonged immobilization
- Neuromuscular dysfunction (9,10)

Extensor Lack:

- Arthrofibrosis and capsular contracture
- Post-surgical adhesions
- Meniscal entrapment or loose bodies
- Joint effusion or hemarthrosis
- Poor surgical technique or hardware impingement (11,12,13)

Post-operative factors such as delayed rehabilitation, insufficient quadriceps activation, and early joint stiffness significantly contribute to the development of extension deficits (2,3).

6. Management Strategies

Management of Extensor Lag:

- Strengthening Exercises: Emphasis on quadriceps strengthening through open kinetic chain (OKC) and closed kinetic chain (CKC) exercises.
- Neuromuscular Electrical Stimulation (NMES): Enhances voluntary muscle activation, particularly after surgery (19).
- Biofeedback: Visual and auditory feedback helps improve muscle control.
- Task-Specific Training: Functional exercises like sit-to-stand, squats, and step-ups (20).

Management of Extensor Lack:

- Manual Therapy: Joint mobilizations (Grade III-IV) targeting posterior capsule and anterior glide of tibia.
- Stretching: Prolonged low-load stretching using braces or weights.
- Modalities: Cryotherapy, ultrasound, and interferential therapy to reduce inflammation.
- Passive Range of Motion (PROM): Continuous passive motion (CPM) machines can aid early post-operative phase.
- Soft Tissue Mobilization: Myofascial release and deep tissue massage (21,22).

Surgical options such as arthroscopic lysis of adhesions or manipulation under anesthesia may be considered for refractory cases (13).

7. Evidence-Based Rehabilitation Approaches

Recent literature emphasizes early mobilization and quadriceps reactivation as critical components in preventing extension deficits post-ACL and TKA. Rehabilitation protocols integrating NMES, cryotherapy, and progressive loading demonstrate superior outcomes (23).

A study by Palmieri-Smith *et al.* (2008) highlighted the importance of early quadriceps activation in reducing extensor lag post-ACL reconstruction (1). Similarly, Shelbourne *et al.* (2011) advocated for immediate extension restoration in ACL rehab protocols to prevent long-term stiffness and lack (2).

Functional bracing, proprioceptive training, and gait retraining also play essential roles in comprehensive rehabilitation (24).

8. Prognosis and Clinical Implications

Untreated extension deficits can result in chronic knee dysfunction, increased joint loading, and accelerated osteoarthritis progression (25). Gait abnormalities, reduced functional independence, and secondary hip or back issues are common consequences.

Clinicians must prioritize early detection and intervention to ensure optimal outcomes. A structured, individualized rehabilitation program addressing the specific type of extension deficit is critical for recovery (26).

Conclusion:

Extensor lag and extensor lack are distinct clinical entities affecting knee extension. Accurate diagnosis, rooted in understanding anatomical and biomechanical foundations, is essential for effective management. With timely and targeted intervention, most patients can regain functional extension, improve mobility, and enhance quality of life.

Future research should explore novel therapeutic approaches, long-term outcomes, and preventive strategies to minimize the burden of knee extension deficits in diverse patient populations.

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CHARACTERIZATION AND ISOLATION OF LACTIC ACID BACTERIA FROM DAIRY SOURCES

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Abstract:

Lactic acid bacteria (LAB) are a pivotal group of microorganisms in dairy, influencing fermentation, flavor, texture, and potential health benefits. This chapter details the methodologies employed for their isolation and comprehensive characterization from diverse dairy matrices. Selective enrichment and plating techniques on media like MRS and M17 agar are described for obtaining pure LAB cultures. Initial phenotypic characterization, including Gram staining and catalase testing, is followed by detailed physiological and biochemical assays focusing on carbohydrate fermentation, volatile compound production, enzymatic activities (proteolytic, lipolytic), and potential antibacterial properties. A significant portion of the chapter is dedicated to advanced molecular characterization techniques, encompassing 16S rRNA gene sequencing for identification, whole-genome sequencing for in-depth genomic analysis, and DNA fingerprinting methods (RAPD-PCR, AFLP, PFGE) for strain typing. The integration of data from these multifaceted approaches is crucial for accurate identification, determination of functional properties relevant to dairy applications (starter cultures, probiotics), and understanding the diversity of LAB within dairy ecosystems. This chapter provides a comprehensive guide for researchers and professionals seeking to isolate and characterize LAB from dairy sources for various scientific and industrial purposes.

Keywords: Isolation, Characterization, Antibacterial Properties, Dairy Sources.

1. Introduction:

Lactic acid bacteria (LAB) are a diverse group of Gram-positive, catalase-negative, acid-tolerant, and generally non-spore-forming bacteria that produce lactic acid as the major end product of carbohydrate fermentation. They play a crucial role in the fermentation of milk and the development of various dairy products, contributing to flavor, texture, preservation, and potential health benefits. This chapter will detail the methods used for the isolation and comprehensive characterization of LAB specifically from dairy sources.

The Importance of LAB in Dairy:

Lactic acid bacteria are ubiquitous in dairy environments, originating from raw milk microbiota, the processing environment, and intentionally added starter cultures. Understanding the diversity and specific traits of LAB isolated from dairy is essential for:

- **Starter Culture Development:** Identifying novel strains with superior technological properties (e.g., rapid acidification, specific flavor production, bacteriophage resistance).
- **Probiotic Selection:** Discovering strains with potential health-promoting effects for use in functional dairy foods.
- **Spoilage Prevention:** Characterizing LAB that may contribute to spoilage or defects in certain dairy products.
- **Understanding Traditional Fermentations:** Identifying the key LAB responsible for the unique characteristics of artisanal dairy products.
- **Quality Control:** Monitoring the presence and characteristics of LAB in dairy products during production and storage.

2. Isolation of Lactic Acid Bacteria from Dairy Samples

Isolating pure cultures of LAB from complex dairy matrices requires selective enrichment and plating techniques:

2.1 Sample Collection and Preparation

- **Representative Sampling:** Obtaining samples that accurately represent the microbial population of the dairy source (e.g., raw milk, fermented milk, cheese, whey).
- **Serial Dilution:** Preparing a series of tenfold dilutions of the sample in a sterile diluent (e.g., physiological saline, buffered peptone water) to obtain a countable number of colonies on agar plates.

2.2 Selective Enrichment (Optional)

- For samples with low LAB populations or high levels of background microbiota, selective enrichment can be employed. This involves incubating the diluted sample in a liquid medium that favors the growth of LAB while inhibiting others. Examples include:
    - **MRS Broth (de Man, Rogosa and Sharpe):** A widely used medium rich in nutrients and with a slightly acidic pH (around 6.2-6.5) that favors the growth of most LAB.
    - **M17 Broth:** Often used for the selective enrichment of *Streptococcus* and *Lactococcus* species, especially when supplemented with specific carbohydrates.
    - Incubation is typically carried out anaerobically or microaerobically at temperatures relevant to the dairy source (e.g., 30-37°C).

2.3 Selective Plating

- Following serial dilution (or enrichment), aliquots are plated onto selective or differential agar media for LAB:
    - **MRS Agar:** The solid counterpart of MRS broth, often supplemented with calcium carbonate ($CaCO_3$) to visualize acid production (LAB colonies will be surrounded by a clear zone where the $CaCO_3$ has dissolved).
    - **M17 Agar:** Selective for *Streptococcus* and *Lactococcus*, often used with specific carbohydrate supplements to aid differentiation.
    - **Rogosa SL Agar:** Selective for *Lactobacillus* due to its low pH and the presence of acetic acid.
    - **Tomato Juice Agar:** Enriched medium suitable for the growth of many LAB, particularly *Lactobacillus*.
- Plates are incubated anaerobically or microaerobically at appropriate temperatures for 24-72 hours.
- **Colony Morphology:** Observe and record different colony morphologies (size, shape, color, surface texture) to aid in distinguishing potential LAB isolates. Colonies exhibiting characteristics typical of LAB (e.g., small, round, white or cream-colored) are selected for further purification.

2.4 Purification of Isolates

- To obtain pure cultures, well-isolated colonies are streaked onto fresh selective agar plates and incubated under the same conditions. This process is repeated until single, morphologically uniform colonies are obtained.
- Pure cultures are then transferred to appropriate maintenance media (e.g., MRS broth with glycerol for cryopreservation at -80°C, or slant agar for short-term storage at 4°C).

3. Initial Characterization of LAB Isolates

Once pure cultures are obtained, initial characterization helps to confirm their identity as LAB and provides preliminary phenotypic information:

3.1 Gram Staining: A fundamental step to confirm that the isolates are Gram-positive, a key characteristic of LAB. LAB typically appear as purple or blue under a microscope after Gram staining.

3.2 Catalase Test: LAB are generally catalase-negative, meaning they do not produce the enzyme catalase to break down hydrogen peroxide. This test involves adding a drop of hydrogen peroxide solution to a bacterial colony; the absence of immediate bubbling indicates a negative result.

3.3 Morphology and Motility: Microscopic observation of cell morphology (cocci, rods, arrangement) and testing for motility (e.g., using motility agar or wet mount) can provide further clues about the genus of the isolate. LAB are typically non-motile.

3.4 Growth at Different Temperatures: Testing the ability of isolates to grow at various temperatures (e.g., 10°C, 30°C, 45°C) can help differentiate between species adapted to different environments or processing conditions.

3.5 Growth in Different NaCl Concentrations: Assessing growth in media containing different concentrations of sodium chloride (e.g., 2%, 4%, 6.5%) can provide information about the salt tolerance of the isolates, which is relevant for certain dairy products like cheese.

4. Physiological and Biochemical Characterization

Further characterization involves a range of physiological and biochemical tests to identify the genus and species of the LAB isolates and to determine their functional properties:

4.1 Carbohydrate Fermentation Tests: Determining the ability of isolates to ferment various carbohydrates (e.g., glucose, lactose, sucrose, maltose, mannitol, sorbitol) with the production of acid. This is typically done using carbohydrate fermentation broths containing a pH indicator (e.g., bromocresol purple), where a color change indicates acid production. The fermentation patterns are crucial for species identification.

4.2 Production of Volatile Compounds: Testing for the production of specific volatile compounds, such as diacetyl (using the Voges-Proskauer test), which contributes to the buttery flavor in some dairy products.

4.3 Production of Ammonia from Arginine: The arginine deiminase pathway is present in some LAB and results in the production of ammonia, which can affect the pH and flavor of dairy products. This can be tested using specific media containing arginine and a pH indicator.

4.4 Production of Exopolysaccharides (EPS): Screening isolates for their ability to produce EPS, which can influence the texture and rheological properties of fermented dairy products. This can be observed as ropy or slimy growth on agar media or by biochemical assays.

4.5 Proteolytic and Lipolytic Activities: Assessing the ability of isolates to break down proteins (proteolysis) and fats (lipolysis), which are important for flavor development during cheese ripening. This can be done using agar plates supplemented with casein or tributyrin, respectively, where a clear halo around the colony indicates enzymatic activity.

4.6 Antibacterial Activity: Screening isolates for their ability to inhibit the growth of spoilage bacteria or pathogens, often through the production of bacteriocins or organic acids. This can be tested using agar overlay assays or well diffusion assays.

5. Molecular Characterization

Molecular techniques provide more definitive identification and allow for strain typing and phylogenetic analysis of LAB isolates:

5.1 16S rRNA Gene Sequencing: Amplification of the 16S rRNA gene (a component of the small subunit of prokaryotic ribosomes) using universal primers, followed by sequencing of the PCR product. The resulting sequence is compared to databases of known 16S rRNA gene sequences (e.g., NCBI GenBank, Ribosomal Database Project) to identify the genus and often the species of the isolate. This is a widely used "gold standard" for bacterial identification (1).

5.2 Whole-Genome Sequencing (WGS): Determining the entire genomic sequence of the isolate provides the highest resolution for identification, strain typing, and functional analysis. WGS can reveal genes involved in specific metabolic pathways, virulence factors (if any), antibiotic resistance, and adaptation to the dairy environment (e.g., [Reference 2]).

5.3 DNA Fingerprinting Techniques: Techniques like RAPD-PCR (Random Amplified Polymorphic DNA PCR) and AFLP (Amplified Fragment Length Polymorphism) generate unique DNA fingerprints for each strain, allowing for differentiation at the subspecies or strain level. These are useful for tracking strains in industrial settings or studying the diversity within a dairy product.

5.4 Pulsed-Field Gel Electrophoresis (PFGE): PFGE separates large DNA fragments based on their size after digestion with restriction enzymes. The resulting banding patterns can be used for high-resolution strain typing, particularly in epidemiological investigations of foodborne outbreaks.

6. Data Analysis and Interpretation

The data obtained from phenotypic, biochemical, and molecular characterization methods are integrated to:

- **Identify the Genus and Species:** Using taxonomic keys, databases, and phylogenetic analysis based on molecular data.
- **Determine Functional Properties:** Assessing the potential of the isolates for specific applications in the dairy industry (e.g., probiotic potential based on survival in simulated gut conditions, technological properties for starter culture use).
- **Compare Isolates:** Identifying unique strains or clusters of related strains within a dairy environment.
- **Document and Store Information:** Maintaining detailed records of the isolation source, characterization data, and storage conditions of each isolate in a culture collection.

Conclusion:

The isolation and comprehensive characterization of lactic acid bacteria from dairy sources are crucial for advancing our understanding of the microbial ecology of dairy products and for harnessing the beneficial potential of these microorganisms. A combination of selective isolation techniques and a tiered approach to phenotypic, biochemical, and molecular characterization allows for accurate identification and the determination of key functional

properties, ultimately contributing to the development of improved starter cultures, novel probiotic strains, and enhanced quality and safety in the dairy industry.

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PROXIMATE ELEMENTAL COMPOSITION OF COMMERCIALLY IMPORTANT FISH SPECIES OF GANESHGURI FISH MARKET, GUWAHATI, ASSAM

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Introduction:

Fish are known to be a very healthy food item and are an excellent source of various minerals and vitamins necessary for good health. Scientists reported that societies with high fish intake have considerably lower chances of acute myocardial infarctions, atherosclerosis and other ischemic heart diseases. The present availability of protein is much below the minimum daily requirements and the livestock sector alone will not be able to meet the protein requirements of ever-increasing human population. Fish is an excellent and relatively cheaper source of high biological value. Fish protein contains all essential amino acids in the required proportion and hence has a high nutritional value, which contribute to their high biological value. (Deshmukh and Shilleswar, 2018). Fishes are the wide spread organism across the world. So, they stand as a better source of food. Even the regions those are covered with ice throughout the year, where land culture is impossible, there also fishes are being used a cheap source of food. According to marketing perspective, fishes can be divided into two categories: Wet fish and Dry fish.

Wet fishes are having the high-water content in their body, if they are stored for long, enzymatic reactions inside decays the body. They have a very low preservation rate. In contrast, dry fishes are those, which are having high-protein content. If they are stored for long, they don't show such body decomposition.

Fresh water fishes play a vital role for animal proteins in the world. (Flowra *et al.*, 2012). Approximately 16% of animal proteins consumed by the world's population are derived from fishes and over one billion people depend on fish as their source of animal proteins. (Fisheries and Aquaculture, 2012, 2013). Fishes are easily digestible nature of proteins and are important sources of minerals. Biochemical composition of fish shows very wide variation from one species to another species, within the same species in different portions of the body, from season to season according to age, size, growth etc. (Yesmin & Khanum, 2019). Fish is a major source

of food for mankind with a significant amount of the animal protein diet, excellent dietary sources of highly unsaturated fatty acid (HUFA) and polyunsaturated fatty acid (PUFA), especially the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoicacid (DHA) (Dhaneesh *et al.,* 2012). Fishes contains good quality, balanced and digestible protein (Mohanty *et al.,* 2012). Fish protein considered as a high nutritional value (Sargent, 1997).

Fish is recommended as a part of healthy diet and it is considered to be a key component of a cardio-protective diet. Furthermore, fish is an important source of various nutrients such as protein, n-3 fatty acid, vitamin D, iodine and selenium which may contribute to healthier metabolic profile (Tørris *et al.,* 2018). The increase in the population that led to shortage of animal protein sources all over the world directed to the attention to fish as rapid and healthy compensatory source of good quality animal protein. The increase in the human population in India has simultaneously led to decrease of animal protein (Weatherley and Gill, 1987).

Proteins have a major role in the growth and maintenance of the human body and are, along with carbohydrates and lipids, the energy giving nutrients in diet. In addition, proteins also pose a wide range of other functions in the body, such as enzymatic activity and transport of nutrients and other biochemical compounds across cellular membranes (Maehre H.K. *et al.,* 2018). In order to maintain these important functions, it is essential to provide the body with good quality proteins through diet. Inadequate intake of dietary proteins containing essential amino acids results in increased turnover of muscular proteins, leading to reduced growth and loss of muscle mass, impaired immunity, as well as reduced hormonal and enzymatic activity may subsequently follow (Wu *et al.,* 2014). The term protein is derived from a Greek word "*proteios*", meaning holding the first place. Berzelius (Swedish chemist) suggested the name proteins to the group of organic compounds that are utmost important to life. Mulder (Dutch chemist) in 1838 used the term proteins for the high molecular weight nitrogen-rich and most abundant substances present in animals and plants. Proteins perform a great variety of specialized and essential functions in the living cells. These functions may be broadly grouped as static (structural) and dynamic.

- **Structural functions:** Certain proteins perform brick and mortar roles and are primarily responsible for structure and strength of body. These include collagen and elastin found in bone matrix, vascular system and other organs and D-keratin present in epidermal tissues.
- **Dynamic functions:** The dynamic functions of proteins are more diversified in nature. These include proteins acting as enzymes, hormones, blood clotting factors, immunoglobulins, membrane receptors, storage proteins, besides their function in genetic control, muscle contraction, respiration etc. Proteins performing dynamic functions are appropriately regarded as the working horses of cell. (Satyanarayan and Chakrapani, 1999).

Fish protein is composed of 20-30 % sarcoplasmic composed of mainly albumins besides hemoproteins, 66-77 % myofibrillar such as myosin, actin, actomyosin and troponin and 3-5 % stromal proteins including collagens (Gates, 2016).

The myofibrillar proteins play an important role in determining the functional properties. The gelling properties of fish meat and the rheological characteristics of the gel depend on the properties of myofibrillar fraction. Connective tissues are made up of stroma proteins. The characteristics texture of fish muscle is due to the low content of stroma proteins in it. Collagen, present in skin, air bladder, etc. is another form of protein, similar to the stroma proteins.

The chief components of fish tissue include water, protein and lipid. The amount or percentage of each within a fish body is termed as proximate composition. Proximate composition is often determined in studies of fish physiology, growth and nutrition. Extensive work was done to evaluate the proximate composition of freshwater fishes with the assistant of government in various areas. There is always variation in their nutritive value. To ensure nutritional security, increased availability of diverse types of foods of animal origin such as milk, meat and fish besides cereals are essential. Fishes are also a valuable source of vitamin A and D. In addition to proteins, vitamins and minerals, fish oils contain polyunsaturated fats. In spite of high preferences for fish and fishery products, the per-capita consumption of fish in India is still very low. Globally, fish and shellfish accounts for about 16 % of animal protein consumed. In some countries figure is as high as 50%. Protein content of raw fish flesh is 18-22% Therefore; aquatic food can in some ways be the medical food of 21st century. Fish is an excellent source of protein and other elements for the maintenance of healthy body In addition, it is very good source of polyunsaturated fatty acid (PUFA) (Conner, 2000; Gocke, 2004).

Fish provides essential nutrients especially proteins of high biological 6 values and fats, so it is often referred to as ‘rich food for poor people’ and the micro and macronutrients present in the fish make it better than other animal protein sources (Sujatha *et al.,* 2013). Fish species had received tremendous attention from researchers due to the excellence in their nutritional aspects. Apart from being a food source, fish also functions to prevent human beings from a variety of diseases in the world (Abraha *et al.,* 2018). A portion of 140gm of fish can provide about 50-60% of the daily protein required by an adult human (FAO, 2012). As compared to land-living animals, fishes are a rich source of protein and have a high content of omega-3 long-chain fatty acids. Intake of animal source foods (ASF) in young children has been associated with improved dietary quality and growth outcomes (Murphy and Allen 2003, Neumann *et al.,* 2003).

Review of Literature

Nanda and Sahu, 2005 worked on lipid and lipid-fraction of some freshwater fishes and their inter-relationship they found that total lipid (TL) contents of all class of fishes ranged from 2.05 to 3.83 %. These fishes may be categorized as non-fatty fishes as Arroya (1974) reported

that fatty fishes are those containing more than 4-5% fat but higher lipid content was noted in carnivore fishes which may be attributed to the major sources of their food, rich in lipid (Love, 1974).

Flowra *et al.*, 2012 worked out the biochemical composition of dried fish samples of *Mystus vittatus, Channa punctatus, Chanda nama, Corica soborna* and *Trichuirus haumela* and found net moisture content ranged from 14.06% to 24.58%, protein varied between 44.08% to 65.65% and 53.45% to 76.399% (dry matter basis), lipid content of the selected dried fish ranged from 1.91% to 17.76% (moisture busis) and 11.21% to 28.15% (dry matter basis).

Binukumari and Vasanthi, 2013 conducted a study on changes in protein content of the freshwater fish. *Labeo rohita* due to the effects of an insecticide Encounter (Herbal Plant Extract) and found that their liver tissue showed 2.89, 2.01, 1.07 and 1.03 mg/g of protein in 0.11 ppm of insecticide Encounter and 3.51 mg/g of protein in control after 24, 48, 72 and 96 hours exposure. Decreased value of protein content in kidneys as 5.46, 3.78, 2.35 and 6.10 mg/g in 0.11 ppm of Encounter exposure and 2.89 mg/g in control after 24, 48, 72 and 96 hours exposure. In muscle tissues 2.12, 1.61, 1.30 and 0.69 mg/g of protein in 0.11 ppm of Encounter exposure and 2.89 mg/g in control after 24, 48, 72 and 96 hours respectively.

Debnath *et al.* (2014) studied on eight fish species namely, *A. mola*, *E. danricus*, *P. sophore, C. fasciata, L. bata, C. catla, L. rohita* and *C. mrigala* from Tripura were analysed for protein and mineral compositions in their dried form. Protein and mineral contents showed significant variations among the species. Protein content varied from 39.37% (*C. lalia*) to 75.43% (*L. bata*). Phosphorus content varied from 0.09% (*P. sophore*) to 0.54% (*C. fasciata*), potassium from 0.28% (*C. fasciata*) to 0.95% (*P. sophore*) and calcium from 0.06% (*Labeo*species) to 1.66% (*C. fasciata*). Copper content was found from 16.1 mg/kg (*P. sophore*) to 273.9 mg/kg (*C. mrigala*), zinc from 23.5 mg/kg (*L. rohita*) to 154.4 mg/kg (*E. danricus*), iron from 91.8 mg/kg (*C. catla*) to 997.7 mg/kg (*C. fasciata*) and manganese from 3.9 mg/kg (*L. rohita*) to 51.4 mg/kg (*C. fasciata*). Moisture content was the highest in *C. fasciata* (14.17%) whereas, *L. bata*and *C. catla*showed the least amount (11.17%).

Rath and Parida *et al.*, (2018) studied on Protein Estimation in Different Fresh Water Fishes fish consumption has a positive effect on human health due to the biochemical composition like lipid, Carbohydrates and protein. Many of the mechanisms are not fully explored and more research is still needed to completely understand the effect of fish protein. It is concluded that the excellent and healthier part is the fish muscle to fulfil the protein deficiency in human body.

Shabir *et al.*, (2018) studied proximate composition (moisture and ash content) of some economically important fishes of the valley. The two major components of fish biochemical composition i.e., moisture and ash content of three selected important food fishes of the valley has been determined. Lowest moisture content (77.68 ± 3.10) was reported in *Cyprinus carpio*

specularis. While no difference in moisture content was reported among the remaining two species *Cyprinus carpio communis* and *Schizothorax labiatus*, both species showed overall higher moisture contents. However a numerically lower value of body moisture content (79.26 ± 0.29) was recorded in *Schizothorax labiatus* when compared to *Cyprinus carpio communis* (79.78 ± 0.83). The body ash content also produced significant differences among each fish species with highest body ash content (16.47 ± 1.5) was recorded in *C. carpio specularis* followed by C. carpiocommunis (14.79 plus/minus 0.83) Lowest body ash content (12.62 ± 0.53) was registered in *S. labiatus*.

Yesmin and Khanum, 2019 conducted a study on Biochemical Analysis of Different Nutritional Components of *Clarias batrachus* (Linnaeus) and *C. gariepinus* (Burchell) in Relation to Parasitic Infestation which two catfish: *Clarias batrachus* (Linnaeus) and *C. gariepinus* (Burchell) were selected for examination during June, 2014 to May, 2016. The percentage (g/100 g) of nutrients such as moisture, ash, fat, protein, carbohydrate contents (mg/100 g) and energy (K cal) in *C. batrachus* and *C. gariepinus* was determined. Analyses were done to measure the nutrition value of edible body parts (flesh) of *C. batrachus* and *C. gariepinus*. The effects of modifying factors such as, season, sex, length, and diet of the hosts on the abundance of parasites were also studied. Present observation on biochemical analysis showed small variation in nutrient contents between *C. batrachus* and *C. gariepinus.* Protein, fat, moisture, carbohydrate levels were higher in non-infected *C. batrachus* (moisture 78.25 ± 0.06 g/100 g, ash 1.42 ± 0.09 g/100 g, fat 1.20 ± 0.04 g/100 g, protein15.05 ± 0.19 g/100 g, carbohydrate 5.53%) and non-infected *C. gariepinus* (moisture 78.62 ± 0.01 g/100 g, ash 1.22 ± 0.10 g/100 g, fat 1.19 ± 0.03 g/100 g, protein 14.69 ± 0.07 g/100 g, carbohydrate 4.95% than those of infected *C. batrachus* and *C. gariepinus*. In both species the highest presence of most of the nutrient components was observed in winter.

Bezbaruah and Deka, 2021 carried out research on moisture and protein content in the muscle of three catfishes and they found that the moisture content was in range of 71.20% to 79.41%. In *Mystus vittatus,* 79.41%, in *Clarius batrachus,* 78.58%, in *Heteropneustus fossilis* 71.20%. The protein content lies in between 14.49% to 18.14%. In *Mystus vittatus* 14.49%, in *Clarius batrachus* 15.70%, in *Heteropneustus fossilis* 18.14%

Deori and Baruah*et al.,* 2020 conducted an assessment of physico-chemical characteristics of Charon Beel, a freshwater wetland in Morigaon District, Assam, India. The study revealed that the water quality is rich in total dissolved solids, nitrogen, phosphorous and potassium content which indicates that the wetland is moderately eutrophicated.

Objective

To estimate the proximate composition (protein and moisture content) of commercially available fishes sold in Ganeshguri Fish Market, Guwahati and assessment of the nutritional qualities of the fishes

Materials and Methods:

Study Area and Time Period:

Guwahati, the largest metropolitan hub in Assam, India, is home to several prominent fish markets dispersed throughout the city. These markets have historically served as crucial centres for the fish trade, providing an extensive array of both freshwater and seafood products to meet the demands of local residents, businesses, and restaurants alike. The research was conducted from 20 February 2023 to 24 May 2023 at the Department of Zoology, University of Science and Technology, Meghalaya, India (26.0997° N, 91.8451° E).

Geographical Information:

- Location: The fish markets of Guwahati are strategically distributed across several key locations within the city, including well-known areas such as Chandmari, Beltola, Ganeshguri and Uzan Bazar. These markets are centrally situated to ensure convenient access for both vendors and consumers.
- Address: The various fish markets can be found in high-traffic areas such as Ganeshguri Fish Market, Beltola Fish Market, Uzan Bazar Fish Market, among others, all within the heart of Guwahati.
- Coordinates: While the exact geographical coordinates of each individual market may differ, the general coordinates for Guwahati are approximately 26.1445° N latitude and 91.7362° E longitude.

Collection of Sample:

- The fishes were collected from the fish market.
- They were photographed to determine their morphological characters.
- They were incapacitated using chloroform.
- Then washed under running water to remove the secretory mucus.
- The specimens were then identified up to species level using keys.
- The total length was noted down.
- The fish was then dissected and the muscle was taken out and dipped in absolute (100%) alcohol. (*if necessary*)
- The required amount of muscle was weighted with the help of digital weight balance.
- After extraction of muscle, biochemical analysis was carried out.

Estimation of Protein:

The isolation of protein has been done after that estimation is carried out by the Lowry's method.

Isolation of Protein:

- 2 gm. of tissue weighted from the fish.

- 8 ml of ice-cold distilled water was added to this 2gm of tissue in mortar and then homogenized.
- 2ml of homogenate was added in 4 centrifuged test tubes and was centrifuged at 5000 rpm for 5-7 minutes and the supernatant was discarded.
- To the homogenate 2.5ml of 1% TCA was added in each test tube and centrifuged for 5-7 minutes and supernatant was then discarded.
- To the ppt. 10% of TCA 3ml in each test tube was added and stirred with a glass rod and centrifuge for 5-7 minute, the supernatant was discarded.
- Again to the ppt. 3ml of 5% TCA was added in each test tube, stirred with the glass rod centrifuge for 3-5 minutes. The supernatant was discarded.
- To the ppt. again alcohol 3ml in each test tube was added stirred with glass rod, centrifuged and supernatant was discarded.
- Again to the ppt. 3ml of chloroform was added in each test tube stirred with a glass rod, centrifuge and supernatant were discarded.
- Further to the ppt. 3ml of diethyl ether or ether was added to each test tube, stirred with a glass rod, centrifuged and the ppt. was kept for further analysis.
- The ppt. was allowed to air dry and the dried ppt. now in powdered form was dissolved in 0.1 NaOH (Sodium Hydroxide) i.e., 3ml in each test tube. If the powder did not dissolve then it was kept in incubator at 37 °C overnight.
- New the dissolve protein is proceeded to protein estimation i.e., Lowry's Method.

Lowry's Method

Principle:

This method was introduced by O.H Lowry and his co-workers in 1951. The Phenolic group of tyrosine and tryptophan residues in a protein will produce a blue colour complex, with maximum absorption in the region of 620nm wavelength, with Folin-Ciacaltaeu reagent which consist of Phosphomolybdic complex which is a mixture of sodium tungstate, Sodium Molybdate and phosphate along with copper sulphate solution due to the reduction of phosphomolybdotungstate to hetero- polymolybdenum blue by copper catalyzed oxidation of amino acid and its intensity depends on the amount of aromatic amino acid presence and will thus vary for different protein.This method is sensitive down to about 10 microgram per ml and is probably the most widely used protein assay despite it has been only a relative method.

Reagent and Chemical Required:

- BSA
- Folin Phenol reagent
- Sodium Carbonate
- Sodium hydroxide
- Sodium potassium tartarate

- Sulphate Pentahydroxide

Preparation of Reagent:

- 2% Sodium Carbonate in 0.1 N Sodium Hydroxide.
- 1% Sodium Potassium tartarate in distilled water
- 0.5% Copper sulphate Pentahydroxide in water
- Reagent 1:48 ml of A,1 ml of B and 1 ml of C
- Reagent II: One part of Folin Phenol and 1 part water
- Standard BSA: 0.1 gram in 100 ml distilled water

Preparation of Calibration Line:

- BSA working standard was taken in 5 test tubes in increasing order i.e., 0.2, 0.4, 0.6, 0.8 and 1 ml making the volume up to 1 ml using distilled water.
- Test tube with 1ml distilled water serves as blank.
- 1 ml of sample was taken in test tube and marked as unknown.
- 4.5 ml of Reagent I was added to all the test tube and incubate for 10 minutes.
- After incubation 0.5 ml of Reagent II was added to all the test tubes and incubated for 30 minutes.
- Measurement was taken at the absorbance at 660nm and the standard graph was plotted.
- The amount of protein present was estimated in the given sample from the standard graph.
- The protein concentration was calibrated following the different concentration of Protein solution.

Determination of Moisture content - (AOAC. 2020):

For determination of total moisture content of the whole body of fish, the viscera, fins and tail were removed from the body of the fish and then the eatable portion of the fish was divided into several parts for making three-four uniform samples from all the parts of fish. The wet samples were put in the pre- weight dry petri dishes and then weighted again. The petri dishes with wet samples were kept in digital hot air oven for drying at 105°C for about 24 hours or until the constant weight was obtained. Then dry samples were taken out from oven and put in desiccators, after 30 minutes the weight was taken, the difference in weight (wet and dry sample) was calculated and expressed as percentage moisture content of the sample.

Preparation:

- Dry the empty dish and lid in the oven at 105°C for 3 hour and transfer to desiccator to cool.
- Weigh the empty dish and lid.
- Weigh about 3 gram of sample to the dish; spread the sample to the uniformity.
- Place the dish with simple in the oven. Dry for 2-3 hour at 105°C

- After drying, transfer the dish with partially covered lid to the desiccator to cool
- Reweigh the dish and its dried sample.

Calculation:

Moisture (%) $=\frac{W1-W2}{W1}\times 100$

Where,

W1= weight (g) of sample before drying.

W2= weight (g) of sample after drying

Results:

For Estimation of Protein:

The amount of protein in the fish bought from the market (Guwahati Fish Market) was calculated in order to study the comparative analysis of protein. Firstly the optical densities (OD) were estimated against the specific concentration of the solution using colorimeter at 660nm in a table and plotted a graph which gave the Calibration line from which we can get the concentration of the fish solution (assumed as unknown) of protein.

Table 1: Protein content in 100gm of commercially available fishes in Ganeshguri fish market

Sl. No.	Scientific Name	Moisture Content (%)
1.	*Labeo rohita*	75.66
2.	*Clarias batrachus*	80
3.	*Heteropneutes fossilis*	79.33
4.	*Anabas testudineus*	82
5.	*Channa punctate*	78.33
6.	*Puntius sophore*	82.66
7.	*Sperata seenghala*	82.33
8.	*Cyprinus carpio*	79
9.	*Piaractus brachypomus*	80.33
10.	*Pangasius pangasius*	76.33
11.	*Ompok pabda*	78.66
12.	*Mystus vitatus*	78.33
13.	*Labeo gonius*	80.33
14.	*Labeo bata*	78.33
15.	*Labeo calbasu*	76.66
16.	*Mystus tengera*	84.33

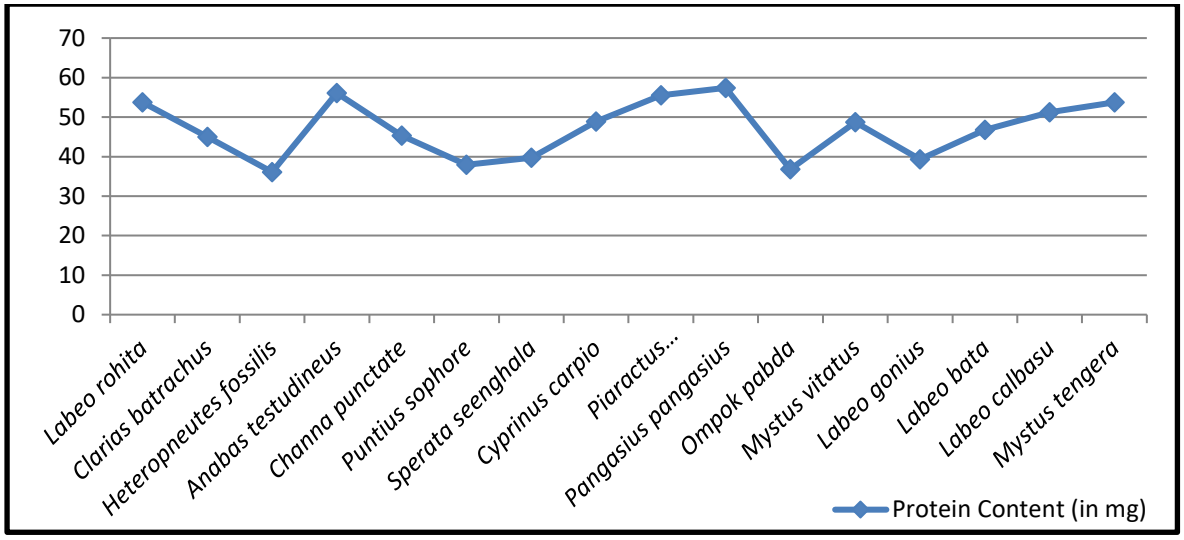

Figure 1: Graphical representation of protein content in different fish specimens

Moisture Content:

Table 2: Moisture content (%) of commercially available fishes in Ganeshguri market

Sl. No.	Scientific Name	Moisture Content (%)
1.	*Labeo rohita*	75.66
2.	*Clarias batrachus*	80
3.	*Heteropneutes fossilis*	79.33
4.	*Anabas testudineus*	82
5.	*Channa punctate*	78.33
6.	*Puntius sophore*	82.66
7.	*Sperata seenghala*	82.33
8.	*Cyprinus carpio*	79
9.	*Piaractus brachypomus*	80.33
10.	*Pangasius pangasius*	76.33
11.	*Ompok pabda*	78.66
12.	*Mystus vitatus*	78.33
13.	*Labeo gonius*	80.33
14.	*Labeo bata*	78.33
15.	*Labeo calbasu*	76.66
16.	*Mystus tengera*	84.33

The amount of moisture was calculated from the collected fishes to study the comparative analysis of moisture. At first the viscera, fins and tail were removed from the body of the fish and then the eatable portion of the fish were taken. The wet samples were put in the pre- weight dry petri dishes and then weighted again. The petri dishes with wet samples were kept in digital hot air oven for drying at 105°C for about 2-3 hours or until the constant weight was obtained.

Then dry samples were taken out from oven and put in desiccators, after 30 minutes the weight was taken, the difference in weight (wet and dry sample) was calculated and expressed as percentage moisture content of the sample.

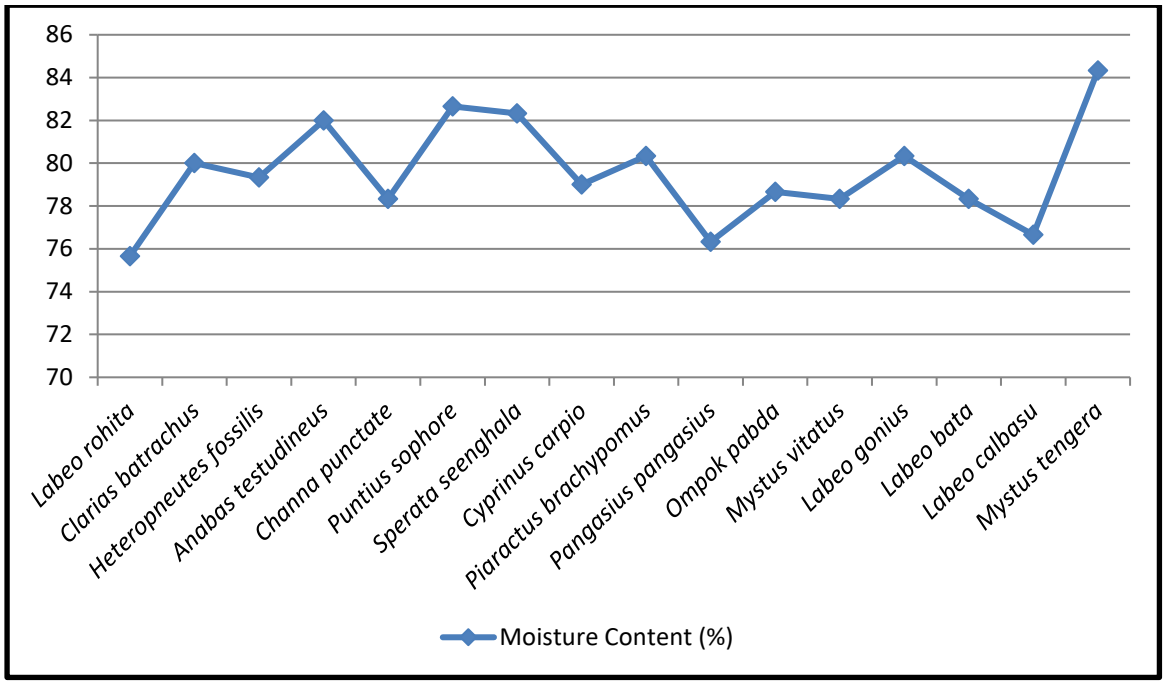

Figure 2: Graphical representation of moisture content in different fish specimens

Discussion:

Fish is a high-quality animal protein source with a higher satiety effect than other animal protein sources such as beef and chicken (Uhe *et al.*, 1992, Mahanty *et al.*, 2014). In comparison to the other dietary animal proteins sources, consumers have a vast choice for fish as far as affordability is concerned as there are many varieties of fish species available in tropical countries. Fish protein is easily digestible. Additionally, it is an important source of both essential and non-essential amino acid (Astawan, 2004). Proteins are predominantly constituted by five major elements – Carbon (50-55%), Hydrogen (6-7.3%), Oxygen (19-24%), Nitrogen (13-19%) and Sulphur (0-14%). Besides these, protein may also contain other elements such as Phosphorus, Iron, Copper, Iodine, Magnesium, Manganese, Zinc, etc. (Satyanarayan, 1999).

The present study demonstrated the protein and moisture content of some of the fish species available in Guwahati fish markets. The result from the study elucidates the levels of protein and moisture and showed variations among the 10 fish species collected. The protein content ranges between 36.1 mg/gm to 57.4 mg/gm. The highest protein content was observed in *Pangasius pangasius, Anabas testudineus* and *Piaractus brachypomus* with 57.4mg/gm, 56.1mg/gm and 55.5mg/gm respectively. And the lowest were *Heteropneustes fossilis, Ompok pabda* and *Puntius sophore* with 36.1mg/gm, 36.8mg/gm and 37.9mg/gm respectively. According to Ahmed *et al.* (2012), protein content in fish species in *Channa punctatus, Puntius sophore, Anabus testudineus, Labeo rohita, Pangasius pangasius, Clarias batrachus*and

Heteropneustes fossilis ranged from 15 to 20 % which were in contrast to our present findings. The variation in nutrients of fish species often appears to vary from season to season, probably the basic causes of change in composition are usually variations in amount and quality of food the fish eats and the amount of movement it makes as well as physical activities.

The moisture content of different fish species shows wide variation among each other in the present study and the values are within the range of 75.66% – 84.33% with the highest in *Mystus tengra* and *Puntius sophore* with 84.33% and 82.66% respectively. The fish species with lowest moisture content are and *Labeo rohita* and *Pangasius pangasius* with 75.66% and 76.33% respectively. It has been evident from the earlier research works that the moisture content found in in *Heteropneustus fossilis* is 71.20% and in *Clarius batrachus* 78.58% (Bezbaruah and Deka, 2021). The moisture content in *Channa punctata*, *Puntius sophore*, *Anabus testudineus*, *Labeo rohita* and *Pangasius pangasius* are 81.93%, 75.63%, 76.60%, 77.91% and 82.76% respectively. The results obtained from the study are more or less similar as reported in other works.

There is a strong inverse correlation between protein and moisture content in fish. This means that as the moisture content of fish increases, the protein content decreases. This is because the water molecules in the fish displace the protein molecules, resulting in lower protein content.

Conclusion:

Present study was carried to find out the nutritional quality of sixteen commercially available fish species found in Guwahati Fish Markets. Results showed that these fish species are source of high Protein and Moisture Content. The nutritional composition of studied fish samples confirmed variation among the fish species. Since the present work elucidated more on the importance of commercially available fishes so consumption of these species are highly recommended. In this study, we focused only on selected elements in fish, and their high levels indicated that they also would be rich in other nutrients which can be expected to be high in fish.

The study revealed that different fish species in Assam exhibited varying protein contents. Some species, such as Kosh (*Pangasius pangasius*), Kawoi (*Anabas testudineus*) and Roop Chanda (*Piaractus brachypomus*) were found to have relatively higher protein contents compared to other species. These species are known for their nutritional value and are rich sources of dietary protein. On the other hand, species like Puthi (*Puntius sophore*) and Pabo (*Ompok pabda*) were found to have comparatively lower protein contents. The study indicated that the moisture content of fish species generally available in the Guwahati Fish Markets also varied. Generally, fresh fish has higher moisture content, but it decreases as the fish is processed or dried. Some species, like Puthi (*Puntius sophore*) and Tingorah (*Mystus tengera*) were observed to have a relatively higher moisture content, while others, such as Rohu (*Labeo rohita*) and Kosh (*Pangasius pangasius*), had a lower moisture content.

Additionally, understanding the protein and moisture content of commercially available fish in Assam has several practical implications. For consumers, this knowledge allows them to make informed decisions when selecting fish for their dietary needs. Individuals seeking to increase their protein intake can prioritize species like Kosh, Kawoi and Roop Chanda, which offer relatively higher protein contents. On the other hand, those who prefer fish with lower moisture content for certain culinary preparations, such as grilling or frying, can choose species like Rohu and Kosh. Traders and fish vendors can benefit from this information by catering to the market demand for specific fish species. By prioritizing species with higher protein content, they can attract health-conscious consumers who value nutritional quality. Moreover, understanding the moisture content of different fish species enables traders to plan their storage and distribution strategies accordingly. Species with lower moisture content may have longer shelf lives, making them suitable for transport and storage over longer distances.

From a fisheries management perspective, this comparative study provides insights into the nutritional profiles of commercially important fish species. It highlights the significance of sustainable aquaculture practices that enhance the protein content of cultured species. Fisheries managers can focus on optimizing feeding regimes, water quality, and growth conditions to maximize the protein content in farmed fish. Additionally, post-harvest handling practices such as proper storage, freezing, and packaging can help maintain optimal moisture levels, ensuring that the fish reaches consumers in the best possible condition. Overall, the knowledge of protein and moisture content in commercially available fishes empowers consumers to make healthy choices, aids traders in meeting market demands, and guides fisheries management towards sustainable practices. This information contributes to the overall development and growth of the fish industry in Assam, promoting both economic prosperity and consumer satisfaction.

In conclusion, the comparative study on the protein and moisture content of commercially available fish in Guwahati Fish Markets highlights the variations that exist among different species. Kosh, Kawoi and Roop Chanda are generally known for their higher protein content, making them desirable from a nutritional standpoint. These findings contribute to the understanding of the nutritional composition of fish can inform decisions related to consumer preferences, marketing strategies, and fisheries management practices. Hence, an overall study has helped to generate and document comprehensive information on the nutritional component of several commonly consumed fish species.

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TRANSFORMATIVE RESEARCH TRENDS IN LIFE SCIENCES

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Abstract:

This chapter presents a detailed overview of the transforming landscape of life sciences research, focusing on its ecological, technical, computational, biomedical, agricultural, ethical, and multidisciplinary aspects. It discusses advances in biodiversity conservation, climate change impact research, biotechnology, bioinformatics, precision medicine, sustainable agriculture, and ethical issues. The chapter underlines the need to bring together multiple scientific fields to tackle complicated biological concerns and global challenges. Life sciences may make a significant contribution to sustainable development and human well-being by encouraging collaborative, data-driven, and ethically responsible research.

Keywords: Biodiversity, Biotechnology, Bioinformatics, Precision Medicine, Sustainable Agriculture, Ethics

Introduction:

The life sciences have long been at the forefront of scientific investigation, with an emphasis on understanding living organisms' structure, function, development, and interactions. Traditionally, research in this field was based on descriptive and experimental biology, with an emphasis on individual biological processes like metabolism, reproduction, and inheritance. However, the landscape of life sciences research has shifted dramatically in recent decades, owing to rapid technological advancements, the rise of computational biology, and an increased emphasis on interdisciplinary and systems-level approaches (National Research Council, 2009).

With the introduction of high-throughput technologies such as Next-Generation Sequencing (NGS), CRISPR **(**Clustered Regularly Interspaced Short Palindromic Repeats**)**-based genome editing, sophisticated imaging techniques, and omics-based analysis (genomics, proteomics, transcriptomics, and metabolomics), life sciences have extended outside traditional laboratory boundaries.

These techniques have allowed researchers to examine biological concerns on unprecedented scales and resolutions (Goodwin *et al.,* 2016). For example, NGS technologies have transformed our understanding of genetic diversity and evolutionary biology, whereas CRISPR-Cas9 has opened up new possibilities in gene therapy and functional genomics.

In parallel, computer tools and bioinformatics have become essential for managing, analysing, and interpreting the massive quantities created by current biological investigations. Big data analytics, artificial intelligence, and machine learning are increasingly being employed for disease prediction, drug discovery, protein structure modelling, and ecological predictions (Libbrecht& Noble, 2015). Simulations and mathematical models increasingly supplement empirical research by allowing in silico experimentation, lowering the cost and time required for hypothesis testing.

Field-based ecological research has also changed with the integration of satellite data, remote sensing technology, and global biodiversity databases, allowing scientists to monitor ecosystem dynamics and climatic impacts on a worldwide scale (Pettorelli *et al.*, 2014). These advancements have made life science research more predictive, integrative, and solution-focused.

Furthermore, ethical, legal, and social considerations have become critical components of life sciences, particularly in genetic engineering, synthetic biology, and personalized medicine. As research has an expanding impact on public health, agriculture, and environmental policy, there is a greater need to examine social values, equal access to technology, and bioethical principles (Jasanoff, 2011).

2. Molecular and Cellular Dimensions

2.1 Genomics and Proteomics

To better comprehend the fundamental processes that regulate biological systems, modern life sciences have increasingly shifted their focus to molecular research. Two of the most transformational subfields in this regard are genomics (the study of complete genomes) and proteomics (the large-scale study of protein structures and functions).

The completion of the Human Genome Project in 2003 was an important turning point in molecular biology, enabling the shift from gene-specific investigations to genome-wide analyses. Whole-genome sequencing (WGS), genome-wide association studies (GWAS), and RNA sequencing (RNA-seq) technologies have revolutionized our understanding of gene expression patterns, genetic variation, and molecular evolution (Collins *et al.*, 2003).

Parallel advancements in proteomics have enabled the simultaneous identification and quantification of hundreds of proteins, providing insights into how gene products function under various physiological and pathological situations (Aebersold& Mann, 2016). Proteomic data, when combined with genomic and the transcriptome data, make significant contributions to systems biology, allowing researchers to map out complex systems of biological regulation.

The development of CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9) genome-editing technology marked a significant milestone in molecular research. This technology enables scientists to make exact changes to living species' DNA, with applications including gene therapy, crop improvement, and functional

genomics (Jinek *et al.*, 2012). Its simplicity, efficiency, and adaptability have transformed the fields of genetics and molecular medicine.

2.2 Cell Signalling and Metabolic Pathways

Understanding cellular biology requires elucidating the methods by which cells communicate and regulate their internal surroundings. Cell signalling pathways transmit molecular signals from the cell surface to the nucleus, coordinating processes like growth, differentiation, death, and immunological responses (Lodish *et al.*, 2021). Key signalling cascades, such as the MAPK/ERK, PI3K/AKT, and JAK/STAT pathways, play critical roles in cellular homeostasis and are frequently linked to disease development, including cancer. Understanding these signalling pathways has resulted in the discovery of specific molecular targets for therapeutic intervention (Hanahan & Weinberg, 2011).

In tandem, the study of metabolic pathways—such as glycolysis, the Krebs cycle, and oxidative phosphorylation—provides insights into how cells generate and use energy. Metabolomics, a rapidly expanding area, comprises the profiling of metabolites in biological systems and provides a snapshot of cellular metabolism under different conditions (Patti *et al.*, 2012). These findings are critical to the development of tailored treatments for metabolic disorders, neurological diseases, and infectious diseases. For example, inhibitors of tyrosine kinases in cancer cells have been created based on a thorough comprehension of abnormal signalling (such as imatinib in chronic myeloid leukaemia).

3. Ecological and Environmental Dimensions

3.1 Biodiversity and Conservation Biology

Biodiversity—the diversity and variability of life on Earth—is critical to ecological stability, resilience, and productivity. The alarming rate of biodiversity loss caused by anthropogenic pressures such as habitat fragmentation, pollution, overexploitation, and invasive species has brought conservation biology to the forefront of life science research. This subject aims to understand the ecological and evolutionary mechanisms that conserve biodiversity, as well as devise solutions to reduce species extinction (Primack, 2014).

Modern conservation biology is becoming more interdisciplinary, using ecological modeling, evolutionary theory, genetics, and climate research to evaluate species viability and habitat integrity. Tools such as Species Distribution Models (SDMs), remote sensing, and environmental DNA (eDNA) analysis are increasingly commonly used to monitor biodiversity and track elusive or endangered species across habitats (Bálint *et al.*, 2018).

The National Ecological Observatory Network (NEON) in the United States is an excellent example of large-scale ecological monitoring. NEON provides long-term, open-access data on temperature, land use, biodiversity, and ecosystem functioning across many biomes, assisting scientists in detecting and forecasting ecological changes over time (Thorpe *et al.* 2016).

3.2 Climate Change and Ecosystem Health

Climate change is one of the most serious threats to world biodiversity and ecosystem health. Rising temperatures, changed precipitation patterns, ocean acidification, and the increased frequency of extreme weather events are all affecting ecosystems on a local and global scale. Climate biology, a burgeoning field in biological sciences, studies how organisms and ecosystems respond to environmental stressors (Pecl *et al.*, 2017).

This field's research focuses on species' physiological, behavioral, and genetic adaptations to climate stress, as well as flora and fauna's shifting ranges. Many species, for example, are migrating poleward or to higher elevations to avoid rising temperatures, a behavior observed in terrestrial, freshwater, and marine systems (Parmesan &Yohe, 2003). Furthermore, phenological changes—shifts in the timing of life cycle events like as flowering or migration—are being detected across species, which could lead to mismatches in ecological interactions such as pollination or predator-prey dynamics.

In addition to understanding biological reactions, life sciences help to measure ecosystem health—ecosystems' ability to retain structure and function in the face of environmental change. Indicators such as species richness, functional diversity, and biogeochemical cycling are used to evaluate ecosystem resilience and inform conservation and restoration practices (Díaz *et al.*, 2006).

4. Technological and Engineering Dimensions

4.1 Biotechnology and Synthetic Biology

Biotechnology, which involves manipulating live organisms or their components to make useful products, has emerged as a key component of applied life sciences. It applies molecular biology, genetic engineering, and systems biology to address global health, agriculture, and environmental issues. Its most apparent uses include genetically modified organisms (GMOs), biofuels, biopharmaceuticals, and bioremediation technologies (Singh *et al.*, 2016).

The rise of synthetic biology, which seeks to design and build unique biological entities such as synthetic genes, pathways, and even complete genomes, represents a tremendous advancement in this discipline. Unlike traditional genetic engineering, which alters existing DNA, synthetic biology use engineering concepts to construct new biological systems from standardized components known as "biobricks" (Chandran *et al.*, 2014). These advancements make it possible to develop custom microorganisms for a variety of applications, including medication production and environmental detoxification. The Sc2.0 project, which involves the synthesis of a fully modified yeast genome, is an important turning point in this field. This endeavor strives to expand our understanding of genomic organization, gene function, and chromosomal design, paving the way for programmable biological systems. (Richardson *et al.*, 2017).

4.2 Biomedical Engineering

Biomedical engineering applies engineering principles to medicine and biology, resulting in ground-breaking improvements in healthcare diagnostics, treatments, and rehabilitation technology. This interdisciplinary domain includes advancements in tissue engineering, regenerative medicine, biosensors, medical imaging systems, and prosthetic devices (Phelps & García, 2010). Scaffolds implanted with stem cells and growth factors promote tissue regeneration. 3D bioprinting, a cutting-edge technology, enables the accurate production of complex tissue structures such as skin, cartilage, and even simple organ models (Murphy & Atala, 2014).

Similarly, biosensors—devices that detect biological molecules and transform them into measurable signals—are critical for early illness detection, environmental monitoring, and personalized therapy. Nanotechnology and microfluidics advancements have made portable and very sensitive biosensing platforms possible (Justino *et al.*, 2017).

5. Computational and Data-Driven Dimensions

5.1 Bioinformatics and Systems Biology

The post-genomic age has seen an unparalleled collection of biological data, owing to improvements in high-throughput technologies such as next-generation sequencing (NGS), mass spectrometry, and microarrays. The bioinformatics revolution arose to address the crucial demand for data storage, management, and analysis. It combines biology, computer science, and statistics to understand large datasets and provide important biological insights (Mount, 2004).

Bioinformatics makes significant contributions in genomic annotation, gene expression analysis, phylogenetic investigations, and protein structure prediction. GenBank, Ensembl, and UniProt are examples of public repositories that have made genomic and proteomic information more accessible. BLAST (Basic Local Alignment Search Tool) allows for rapid sequence comparisons and homology detection, considerably expediting genetic research and evolutionary studies (Altschul *et al.*, 1990).

In parallel, systems biology takes a comprehensive approach, modeling the dynamic interactions of biological components (genes, proteins, and metabolites) within cells or organisms. Systems biology uses network analysis, mathematical modeling, and simulations to better understand how these interactions result in complex biological behavior and phenotypes (Kitano, 2002). This method is critical for understanding disease causes, developing synthetic gene circuits, and predicting the outcome of pharmacological interventions. The use of GenBank in comparative genomics enables researchers to follow alterations in viral genomes such as SARS-CoV-2, enabling the development of diagnostics and vaccines.

5.2 Artificial Intelligence in Life Sciences

Artificial intelligence (AI) is rapidly changing the face of life sciences by allowing robots to learn from data, spot patterns, and make judgments with little human participation. Machine

learning (ML) and deep learning (DL) techniques are now widely used in a variety of fields, including genomics, proteomics, pharmacology, and medical diagnostics (Esteva *et al.*, 2019).

AI-powered drug discovery tools can examine chemical libraries, anticipate drug-target interactions, and optimize lead compounds. This significantly reduces the time and expense of introducing novel treatments to the market. In medical diagnostics, AI algorithms interpret medical pictures (e.g., radiography, MRIs) with the same accuracy as professional doctors. Natural language processing (NLP) is also utilized to extract and synthesize clinical data from unstructured medical records.

AlphaFold, an AI system built by DeepMind, is a game changer since it can accurately predict protein 3D structures from amino acid sequences. This achievement solves a major barrier in molecular biology, paving the way for advances in drug development, enzyme engineering, and disease understanding (Jumper *et al.,* 2021). AI algorithms are now being utilized in personalized medicine to examine individual genomic profiles and prescribe targeted medicines, particularly in oncology and uncommon genetic illnesses.

6. Health and Biomedical Research Dimensions

6.1 Precision and Personalized Medicine

Precision medicine is a revolutionary approach to healthcare in which disease prevention and treatment are adjusted to individual variations in genes, environment, and lifestyle. Personalized medicine, in contrast to the traditional "one-size-fits-all" concept, personalizes patient care using advanced molecular diagnostics, genomic profiling, and data analytics (Collins & Varmus, 2015). Next-generation sequencing (NGS), biomarker discovery, and pharmacogenomics all play important roles in enabling this paradigm change. These techniques help with early diagnosis, risk prediction, and drug response tracking. Precision medicine is used in oncology to discover specific genetic alterations in malignancies (for example, BRCA1/2 in breast cancer) and prescribe tailored therapy such as PARP inhibitors (Turner *et al.,* 2004). Emerging programs, such as the National Institutes of Health's All of Us Research Program, are building vast databases of different genetic and health information to enhance health equity and accelerate personalized medical solutions.

6.2 Infectious Diseases and Immunology

Infectious diseases have significantly altered the global health landscape, as evidenced by pandemics such as COVID-19, Ebola, and Zika virus epidemics. As a result, research in virology, immunology, vaccinology, and epidemiology has become more critical and multidisciplinary (Morens *et al.,* 2020). Modern immunological research focuses on the complex relationships between infections and the human immune system. This encompasses innate and adaptive immunological responses, cytokine signalling, and immune memory. Novel tools like single-cell RNA sequencing, T-cell repertoire analysis, and monoclonal antibody production are speeding up our understanding of host-pathogen interactions and vaccine development.

The development of mRNA-based vaccines against SARS-CoV-2 is an important turning point in this field. These vaccines build on decades of foundational research in RNA biology, lipid nanoparticle delivery, and immune regulation. Their rapid development and great efficacy demonstrate the potential of platform-based vaccination technologies (Sahin *et al.,* 2014; Pollack *et al.,* 2020). The effectiveness of mRNA vaccines (e.g., Pfizer-BioNTech and Moderna) in combating COVID-19 highlights how interdisciplinary research—spanning molecular biology, immunology, and nanotechnology—can quickly address global health concerns.

7. Agricultural and Food Science Dimensions

7.1 Sustainable Agriculture

Sustainable agriculture is essential for attaining global food security while protecting natural resources for future generations. This domain's life sciences research combines plant genetics, soil biology, agroecology, and climate science to create ecologically friendly and economically sustainable farming practices. Recent research on the soil microbiome has shown that beneficial microorganisms play important roles in nutrient cycling, disease suppression, and stress tolerance (Fierer, 2017). These discoveries have resulted in the development of biofertilizers and biopesticides, which reduce dependency on synthetic agrochemicals.

Another cornerstone is the development of pest-resistant and climate-resilient crops through marker-assisted selection, genetic modification, and genome editing techniques such as CRISPR-Cas9. These measures help to reduce crop losses and increase yields in response to changing climatic circumstances (Qaim, 2020).

7.2 Food Biotechnology

Food biotechnology combines molecular biology and genetic engineering to improve food quality, nutritional value, and shelf life. Innovations in this sector seek to alleviate global malnutrition, reduce food spoilage, and increase food processing efficiency. One of the most significant discoveries is crop genetic modification to increase important micronutrients, often known as biofortification. Golden Rice, genetically altered to produce β-carotene (a precursor of Vitamin A), deals with Vitamin A insufficiency, which is prevalent in developing nations (Tang *et al.,* 2009).

Furthermore, fermentation technologies have been enhanced to produce probiotics, functional foods, and biopreservatives by utilizing genetically modified microbial strains. These technologies promote intestinal health, reduce food waste, and increase shelf life. Food fortification is a complementary method that enhances popular foods with vital vitamins and minerals to prevent nutrient deficits on a large scale.

8. Ethical, Legal, and Social Implications (ELSI)

As life sciences research develops rapidly due to sophisticated technologies like CRISPR gene editing, synthetic biology, and large-scale data analytics, ethical, legal, and social concerns (ELSI) have emerged. The proliferation of genomic databases and personalized medicine has

raised severe questions about data privacy, ownership, and informed permission. Initiatives such as the Global Alliance for Genomics and Health (GA4GH) are increasingly pushing for ethical data-sharing standards. Gene editing, particularly germline alteration, sparks intense discussions about human enhancement and long-term social consequences, whereas synthetic biology raises biosafety and dual-use concerns. Animal testing, while important in scientific research, must conform to the 3Rs—Replacement, Reduction, and Refinement—to reduce suffering and justify need.

Equally crucial is providing fair access to biotechnological developments, as demonstrated by the COVID-19 pandemic, which revealed substantial differences in worldwide vaccine distribution between rich and poor countries. Addressing these ELSI concerns is crucial for developing responsible innovation that upholds human rights, promotes environmental safety, and secures social justice. Ethical monitoring, inclusive public involvement, and global regulatory frameworks are critical for steering the life sciences toward a more egalitarian and sustainable future (Knoppers, 2014; Jasanoff *et al.*, 2015; Wouters *et al.*, 2021).

9. Interdisciplinary and Transdisciplinary Research

The evolution of life sciences has been characterized by increasing integration with other fields, resulting in dynamic multidisciplinary and transdisciplinary research methods. Biophysics, for example, uses principles from physics to better comprehend molecular structures and cellular mechanics, whereas chemical biology combines chemistry and biology to investigate biochemical relationships and generate new therapeutic medicines. The combination of science and engineering has resulted in biomedical engineering, which has enabled advances in prosthetics, diagnostic gadgets, and regenerative medicine. Computational collaborations, notably with computer science, have resulted in improvements in bioinformatics, systems biology, and artificial intelligence applications for genomics, drug development, and epidemiological modelling. Furthermore, the involvement of social sciences improves public health research, health policy design, and behavioural studies, guaranteeing that scientific solutions are both socially relevant and ethical.

These cross-disciplinary collaborations not only extend the area of investigation, but also encourage creative problem-solving, particularly when dealing with complicated issues like pandemics, climate change, and food poverty. Transdisciplinary frameworks go beyond academia, bringing together policymakers, industries, and communities to co-create knowledge and long-term solutions (Ledford, 2015; Choi & Pak, 2006).

Conclusion:

Life sciences now span a vast, multidimensional framework that includes molecular biology, ecology, technology, data science, ethics, and interdisciplinary collaboration. This comprehensive approach allows researchers to address complex global concerns like illness, climate change, and food shortages more precisely and effectively. Advances in genetics,

artificial intelligence, biotechnology, and public health are altering scientific understanding and real-world applications. At the same time, ethical and fair procedures are critical to ensuring responsible innovation. As fields converge and collaborative research expands, the bio sciences are ideally positioned to drive sustainable and inclusive growth. This growing paradigm emphasizes the necessity of integrating knowledge from several fields in order to comprehend, protect, and improve life in all of its forms.

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ETHNOBOTANICAL EXPLORATION AND PHYTOCHEMICAL SCREENING OF *MURRAYA KOENIGII* (LINN.) SPRENG.: A CASE STUDY FROM BARGARH DISTRICT, ODISHA, INDIA

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Abstract:

Murraya koenigii (Curry Leaf) is a versatile multi-potential medicinal plant known for its rich phytochemical composition, including alkaloids, flavonoids, tannins, glycosides, saponins, and phenolic compounds. These bioactive compounds contribute to its wide range of pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and hepatoprotective effects. The plant is traditionally used to treat various ailments, such as diabetes, digestive disorders, skin infections, and hair loss. Various phytochemical tests, including the Wagner's test for alkaloids, Lead acetate test for flavonoids, Ferric chloride test for phenols and tannins, Foam test for saponins, and the Keller-Killiani test for glycosides, confirm the presence of these bioactive compounds in *Murraya koenigii*. Additionally, tests for carbohydrates, proteins, and steroids also reveal the plant's diverse chemical makeup. These findings support the plant's medicinal use in local and traditional healing practices, particularly in regions like Odisha, where it plays an integral role in managing health conditions. Further scientific research, including clinical studies, is necessary to validate its therapeutic potential and establish standardized usage in modern medicine.

Keywords: Curry Leaf, Rutaceae, Medicinal Plant, Phytochemical Screening

Introduction:

Murraya koenigii (Linn.) Spreng., commonly known as curry leaf, is a tropical plant belonging to the Rutaceae family. It is native to the Indian subcontinent and is widely cultivated

across Southeast Asia for its aromatic leaves, which are a common ingredient in traditional cooking practices (Mohanty, 2018). It has been noted by the World Health Organisation that 80% of the population in the developing countries rely on plant based natural products for their primary health care needs (Sahu *et al.,* 2010; Sahu *et al.,* 2013; Gahlawat *et al,* 2014). It is a medicinal herb, which is well known in the Ayurvedic system of medicine, and has been widely used in India and other Asian countries as a spice and condiment for culinary purposes. It has been known to possess a varied range of biological activities too (Bhandari, 2012). In the traditional medicinal system, it is widely used as a cure in various lifestyle diseases and disorders. It is used as a febrifuge, tonic, antidiabetic, antidiarrheal, anti-obesity and as a flavor enhancer in varied culineries (Kumari, 2018). Varied mineral elements such as iron, calcium, magnesium, Zinc, Sodium had been found to be present along with varied vitamins such as Vitamin A, Vitamin B1, Vitamin B2, Vitamin B3 and Vitamin E which suggests that the leaves can be used as a supplement for nutrient scarcity (Igara *et al.,* 2016). The oil is used externally for bruises, eruption, in soap and perfume industry (Prajapati *et al.,* 2003). The phytoconstituents isolated so far from the leaves are alkaloids viz., mahanine, koenine, koenigine, koenidine (Narasimhan *et al.,* 1975), girinimbiol, girinimibine (Adebajo *et al.,* 2006), koenimbine, O-methyl murrayamine A, Omethyl mahanine (Tachibana *et al.,* 2003), isomahanine, bismahanine, bispyrayafoline and other phytoconstituents such as coumarin glycoside viz., scopotin, murrayanine (Adebajo and Johannes, 2000), calcium, phosphorus, iron, thiamine, riboflavin, niacin, vitamin C, carotene and oxalic acid. The essential oil from leaves yielded di- α-phellandrene, D-sabinene, D-α-pinene, dipentene, D-α-terpineol and caryophyllene (Gopalan *et al.,* 1984).

M. koenigii has been widely used in Indian cookery for centuries and has a versatile role to play in traditional medicine. The plant is credited with tonic and stomachic properties. Bark and roots are used as stimulants and externally to cure eruptions and bites of poisonous animals. Green leaves are eaten raw for cure of dysentery, diarrhoea and for checking vomiting. Leaves and roots are also used traditionally as bitter, anthelmintic, analgesic, curing piles, inflammation, itching and are useful in leucoderma and blood disorders (Kirtikar and Basu, 1981). Mahanimbine and koenigine, two carbazole alkaloids, isolated from the leaves of *M. koenigii* showed antioxidant activity. Koenigine also showed a high degree of radical scavenging properties (Rao *et al.,* 2006). The possible mechanism by which the mahanimbine decreases blood sugar level may be by potentiating of insulin effect either by increasing the pancreatic secretion of insulin from beta cells of islets of langerhans or by increasing the peripheral glucose uptake. Mahanimbine showed the appreciable alpha-amylase inhibitory effect as compared with acarbose (Dineshkumar *et al.,* 2010). The methanolic extract of *M. koenigii* showed a significant increase in phagocytic index by rapid removal of carbon particles from the bloodstream. The

extract also increased the antibody titre against ovalbumin and protection towards cyclophosphamide induced myelosuppression in albino mice.

Oral administration of the aqueous extract of leaves at doses of 250 and 500 mg/kg significantly enhanced the delayed-type hypersensitivity reaction induced by ovalbumin. The extract also potentiated the production of circulating antibody titre significantly in response to ovalbumin (Shah and Juvekar, 2010). The secondary metabolites found in *M. koenigii* that can kill or impede the proliferation of cancer cells add to the cytotoxic effect of the extract from the plant's leaves. This outcome indicated a possible *M. koenigii* natural product that may be developed as an anticancer drug. Cytotoxic Function Koenoline, an isolated carbazole alkaloid from the root bark of *M. koenigii*, was found to be cytotoxic to the KB cell culture system (Manfred *et al.*, 1985). In comparison to petroleum ether and hexane extracts, which do not reduce inflammation, it was discovered that *Murraya koenigii* leaf extract in methanol and aqueous form is effective against carrageenan-induced edoema in male albino rats at a concentration of 400 mg/kg. When compared to the aqueous extract, the methanol extract was determined to have the strongest anti-inflammatory properties (Prasad and Dua, 2011). The petroleum ether extract of the dried plant was subjected to column chromatography in order to isolate mahanimbine, a chemical component of *M. koenigii*. On streptozotocin-induced Wistar rats, the anti-diabetic action was tested using the pure substance at doses of 50 mg/kg and 100 mg/kg. Mahanimbine may lower blood sugar levels by potentiating the effects of insulin, either by boosting pancreatic insulin secretion from islets of langerhans beta cells or by enhancing peripheral glucose uptake. When compared to acarbose, mahanimbine significantly inhibited alpha-amylase (Dineshkumar *et al.*, 2010). In HepG2 cells, carbazole girinimbine, which is isolated from *Murraya koenigii* bark, causes extensively, programmed cell death. The findings of the investigation carried out in 2010 provided proof that mahanine was involved in the death receptor arbitrated extrinsic route of apoptosis. Although it strangely failed to develop in K562 cells, it demonstrated anti-cancer action in MOLT-3 cells. In addition, pyrayafoline, murrafoline, and three carbazole alkaloids, including mahanine, have significant efficacy against HL-60 cells, also validated mahanine as the main anti-cancerous bioactive chemical in *M. koenigii* (Samantaa *et al.*, 2018). There was no literature available regarding the phytochemical screening of this essential plant available from Bargarh district, Odisha. Hence, keeping this importance in mind the present study was conducted to document the medicinal use and phytochemical screening of the *Morraya koenigii* from Bargarh district, Odisha.

Materials and Methods:

Study Area

Bargarh District is located between latitudes 20.15°N and 21.05°N and longitudes 82.45°E and 83.40°E in the western part of Odisha, India. The district is bordered by Sambalpur

to the north east, Jharsuguda to the north, and the state of Chhattisgarh to the west. The field survey was carried out in Bargarh district, Odisha and literature survey was carried out using several authenticated scientific websites. The data presented in the current chapter is accumulated from various electronic catalogues like PubMed, Scopus, ScienceDirect, Web of Science, Google Scholar and library searches of scientific journals and books. The Plant List, International Plant Name Index and Kew Botanical Garden Plant name databases were some of the important websites used for literature review. Relevant articles and books published were also reviewed for the same study site (Sahu *et al.*, 2010; Sahu *et al.*, 2013, Sahu and Sahu, 2017, 2020).

Sampling and Collection of Leaves

The twigs of *Murraya koenigii* (Linn.) Spreng. were collected from various parts of Bargarh district, Odisha and brought to the Laboratory of Department of Botany, Vikash Degree College, Bargarh (Figure 1).

Figure 1: Photograph of entire *plant (a), leaves (b), flowers (c) and fruits (d) of Murraya koenigii.* (Linn.) Spreng.

After proper identification by Dr. Alok Ranjan Sahu, Assistant Professor, Department of Botany, Vikash Degree College, Bargarh, the plant materials were washed 2-3 times with distilled water and air dried in open air. The dried leaves were powdered in a mixer grinder and were kept in a plastic bag until it was analysed. All the Apparatus used was first washed with distilled water. The preliminary phytochemical analyses were done by using water extraction, methanol extract, Chloroform extract and petroleum ether extract followed by the standard procedures.

Preliminary Phytochemical Screening:

For preliminary phytochemical screening of all the four extracts *i.e.* aqueous, methanol, chloroform and petroleum ether were carried out by using following protocols (Rani *et al.,* 2025; Sahu *et al.,* 2024; Sharma *et al.,* 2024; Nayak *et al.,* 2024):

a) Test for alkaloids:

Wagner's test: Prepare the plant extract by dissolving a small amount of the dried plant material in a solvent like ethanol or water and few drops of Wagner's reagent were added to the extracts and the formation of a reddish-brown precipitate indicates the presence of alkaloids.

b) Test for Flavonoids:

Shinoda Test: Add 1-2 pieces of magnesium ribbon and a few drops of concentrated hydrochloric acid to the extract. A pink to red color indicates flavonoids.

Lead acetate test: 10mg of all extracts were taken and 0.5 ml of 1% lead acetate solution was added and the formation of a yellow precipitate or cloudy solution precipitate indicates the presence of flavonoids.

c) Test for Phenols and Tannins:

Ferric chloride test**:** 5mg of all extracts were taken and 0.5 ml of 5% ferric chloride was added. The development of dark bluish black colour indicates the presence of tannins in all the extracts.

d) Test for steroids and sterols:

Salkowski's test: 5mg of extracts were dissolved in 2 ml of chloroform and an equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and the lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound in the extracts.

e) Test for carbohydrate:

Molisch's test**:** Adding a few drops of Molisch's reagent to the extracts and then carefully layering concentrated sulfuric acid will result in a purple ring appearing at the interface this indicates a positive test for presence carbohydrates in aqueous, methanol and pet ether extracts but there in absence of carbohydrate in chloroform extract.

f) Test for Saponins:

***Foam test*:** 0.5 mg of extracts were diluted with 20 ml distilled water and shaken well in a graduated cylinder for15 min. The formation of foam to a length of 1cm indicated the presence of saponins in chloroform, aqueous and pet ether extracts but absence in methanol extract.

g) Test for Glycosides:

Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. Formation of yellow colour indicates the presence of glycosides in chloroform, aqueous and methanol extracts but absent in pet ether extract.

h) Test for Protein and amino acids:

***Biuret test*:** To 0.5 mg of extracts equal volume of 40% NaOH solution and two drops of 1% copper sulphate solution were added. The appearance of violet colour indicates the presence of protein in chloroform extract and absence in rest of the extracts.

***Ninhydrin test*:** About 0.5 mg of extracts was taken and 2 drops of freshly prepared 0.2% Ninhydrin reagent were added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids in chloroform, methanol and aqueous extract and absent in pet ether extract.

i)Test for Anthraquinone:

***Borntrager's test*:** About 0.5 gm of the extracts were taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate was shaken with equal volume of 10% ammonia solution. A pink violet or red colour in the lower layer indicates the presence of anthraquinone in all extracts except petroleum ether.

Results:

Medicinal use of *Morraya koenigii*

The ethnomedicinal usage of *M. koenigii* from the Bargarh district was enumerated as follows:

- The plant has significant anti-inflammatory effects, making it useful in treating conditions like arthritis, joint pain, and inflammation-related disorders.
- The leaves have shown effectiveness against a variety of pathogens, including bacteria and fungi, making them useful in treating infections, wounds, and skin conditions.
- Curry leaves are known to help regulate blood sugar levels and improve insulin sensitivity, making them beneficial in managing type 2 diabetes**.**
- Curry leaves have been used to treat digestive disorders such as indigestion, diarrhea, and constipation. They promote healthy bowel movements and alleviate gastric problems.
- *M. koenigii* has hepatoprotective properties, aiding in the detoxification and protection of the liver from damage caused by toxins and alcohol consumption.

- The leaves are used in hair oils and treatments to prevent hair loss, promote hair growth, and improve scalp health due to their nourishing properties.
- Curry leaves are believed to aid in weight loss by boosting metabolism and promoting fat breakdown, making them a popular ingredient in weight management diets.
- Curry leaves are used in traditional medicine to treat eye conditions such as conjunctivitis and cataracts, due to their anti-inflammatory and antioxidant properties.
- Regular consumption of curry leaves has been shown to help in lowering blood pressure by improving circulation and reducing hypertension.
- The leaves are used in traditional skincare preparations due to their ability to fight aging, wrinkles, and pigmentation, providing a youthful appearance to the skin.
- Curry leaves are used in traditional remedies to treat respiratory conditions such as coughs, colds, and flu, as they have antibacterial and anti-inflammatory effects.

Phytochemical Screening

Table 1: Preliminary phytochemical screening from the leaves of *Murraya koenigii* (Linn.) Spreng

Plant Constituent	Name	Extracts			
	Name of Test	Chloroform Extract	Methanol Extract	Aqueous Extract	Petroleum Ether Extract
Alkaloids	Wagner's test	+	+	+	+
Flavonoids	Lead acetate test	+	+	+	+
	Shinoda test	+	+	+	-
Test of Phenols & Tannins	Ferric chloride test	+	+	+	+
Steroids & Sterols	Salkowski's test	+	+	+	+
Carbohydrates	Molisch's test	-	+	+	+
Saponis test	Foam test	+	-	+	+
Glycosides	Keller-Kiliani test	+	+	+	-
Proteins & Amino acid	Biuret test	+	-	-	-
	Ninhydrin test	+	+	+	-
Anthraquinone Test	Borntrager's test	+	+	+	-

*Positive: +, Negative: -

The phytochemical screening of *M. koenigii* (Curry Leaf) revealed the presence of several bioactive compounds with potential medicinal properties in Table 1. Alkaloids were detected using Wagner's test, indicated by the formation of a reddish-brown precipitate in all the four extracts, suggesting the plant's potential antimicrobial and analgesic activities. Flavonoids were identified through the Shinoda and alkaline reagent tests, showing a positive result with a

red or yellow colour change in three extracts i.e. chloroform, methanol and aqueous extracts while not found in petroleum ether extract, Presence of flavonoids supports the plant's antioxidant and anti-inflammatory properties. Phenols and tannins were confirmed by the Ferric chloride test, producing a green or black colour, indicating their role in antioxidation and antimicrobial action. Steroids were detected using the Salkowski's test, giving a red color, suggesting the plant's possible anti-inflammatory and anticancer effects. The presence of carbohydrates in three extracts (Methanol, Aqueous and Petroleum ether extract) and absence in chloroform extract was confirmed by Molisch's tests, the presence of carbohydrates indicate its potential role in energy production. Saponins were detected via the foam test, implying their antimicrobial and cholesterol-lowering effects. Glycosides were found using the Keller-Kiliani test, supporting the plant's potential use in heart-related treatments. Proteins and amino acids were detected through the Biuret and Ninhydrin test, which suggests the plant's nutritional and enzyme-related benefits. Lastly, anthraquinones were identified through Borntrager's test, indicating possible laxative and antimicrobial properties. Overall, the phytochemical screening of *M. koenigii* confirms the presence of a wide range of bioactive compounds with diverse therapeutic potentials (Table 1).

Discussions:

The traditional community played an important role in conserving traditional knowledge of this plant which was used for the treatment of several disorders. *Murraya koenigii* (Curry Leaf), known for its cooking and medicinal applications, has long been a staple in the traditional healing practices of Odisha, including Bargarh district. Rich in bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and glycosides, *M. koenigii* exhibits a wide range of pharmacological properties. Sahu and Sahu (2019) reported the use of leaves of *Murraya koenigii* as chutney, also used to give good smell to curry, further used for medicinal purposes like Indigestion, anthelmintic, curing piles, inflammation, itching and are useful in blood disorders by the native of Bargarh district, Odisha. Sahu and Ekka (2021) reported that leaves of *M. koenigii* are used as spice in curry and give it a specific scent. Further roasted leaf pieces are used in various food materials for batter taste by the four dominant tribes of Bargarh district, western Odisha, India. Dash and Sahu (2023) reported that the leaves and roots of the *M. koenigii* were used by the native of Balangir district, Western Odisha, India as aromatic food and also used for treatment of night blindness, Dysentery, diarrhea, vomiting, and bites of poisonous animals' bruises eruption. Sahu and Sahu (2025) reported that the leaves of *M. koenigii* were used as green leafy vegetables by the tribal peoples of Jharigaon Block of Nabarangpur district, Odisha, India.

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suggesting the plant's potential antimicrobial and analgesic activities. Flavonoids were identified through the Shinoda and alkaline reagent tests, showing a positive result with a red or yellow colour change in three extracts i.e. chloroform, methanol and aqueous extracts while not found in petroleum ether extract, Presence of flavonoids supports the plant's antioxidant and anti-inflammatory properties. Phenols and tannins were confirmed by the Ferric chloride test, producing a green or black colour, indicating their role in antioxidation and antimicrobial action. Steroids were detected using the Salkowski's test, giving a red color, suggesting the plant's possible anti-inflammatory and anticancer effects. The presence of carbohydrates in three extracts (Methanol, Aqueous and Petroleum ether extract) and absence in chloroform extract was confirmed by Molisch's tests, the presence of carbohydrates indicates its potential role in energy production. Saponins were detected via the foam test, implying their antimicrobial and cholesterol-lowering effects. Glycosides were found using the Keller-Kiliani test, supporting the plant's potential use in heart-related treatments. Proteins and amino acids were detected through the Biuret and Ninhydrin test, which suggests the plant's nutritional and enzyme-related benefits. Lastly, anthraquinones were identified through Borntrager's test, indicating possible laxative and antimicrobial properties. Similar kinds of photochemical screenings were done in the same plants by various authors (Handral *et al.,* 2010; Uraku and Nwankwo 2015; Igara *et al.,* 2016).

Acknowledgment:

The authors (ARS and SM) are thankful and obliged to the staff members, Principal, Chairman of Laxmi's Vikash Group of Institutions for providing us with the entire necessary infrastructure for the study.

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HUTCHINSON-GILFORD PROGERIA (HGPS) – A RARE SYNDROME

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Abstract:

Clinical characteristics of progeria resemble premature aging. The mutation that causes this condition has been identified, but its exact mode of action is yet unknown. The last two decades have seen a significant increase in the study of progeria due to the potential for uncovering information about the aging process in both healthy and pathological settings. Premature aging syndromes, also known as progeroid syndromes, share many clinical traits with normal aging and offer a unique opportunity to shed light on the fundamental mechanisms that cause human aging. Individuals diagnosed with HGPS exhibit clinical manifestations of early aging, including hair loss, uneven skin tone, loss of subcutaneous fat, and early-life atherosclerosis and cardiovascular mortality in their adolescent years. HGPS is quite concerning due to its many crippling effects. HGPS is an autosomal dominant disorder mostly caused by de novo point mutations in the LMNA gene. Missense mutations affecting the head, coil 1B, coil 2, or tail domains of lamin A in the LMNA gene cause mandibuloacral dysplasia with lipodystrophy type A (MADA). Numerous cellular and molecular alterations are brought about by progerin; however, little is known about the cascade of adverse effects that lead to the deterioration of tissues and organs and, ultimately, premature aging. Hutchison Gilford progeria syndrome (HGPS) can be identified by performing a genetic testing for the changes in the LMNA gene which is a confirmative diagnosis for the progeria. Despite the continuous hunt for a workable cure, there isn't a diagnostic test available for the early diagnosis of HGPS. In clinical practice, a clinical assessment usually starts with phenotypical data and the child's medical history.

Keywords: Syndrome, Gene, Mutation, Aging, Human

Introduction:

The natural effect of human life is aging, which raises the chance of developing chronic illnesses and causes a slow decline in cell, tissue, and organismal function. Progeroid syndromes, another name for premature aging syndromes, resembles many clinical characteristics of typical aging and present a rare chance to clarify the underlying processes that drive human aging. Patients with HGPS show clinical signs of premature aging, such as baldness, irregular pigmentation, loss of subcutaneous fat, and early-life cardiovascular and atherosclerotic deaths in

their teens. Progeria children have a small jaw, a short nasal bridge and tip, an abnormally protruding eyeball, crowding and malformation of the teeth, and a disproportionately small face in relation to the head. By the time childern are one and a half to two years old, they are underweight, have a small face and an unusually large jaw in relation to their head size, a high-pitched voice, uneven dentition, a pinched nose, and noticeably large, open eyes. They also lack sexual development. The skin becomes extremely thin, fragile, and translucent, allowing veins to be seen. Angina, hypertension, joint stiffness, and hip dislocation are other typical complaints. These kids age by around ten years every year on a biological level. It is remarkable that their IQ and intelligence are average. However, cardiovascular problems, such as left ventricular and vascular smooth muscle cell (VSMC) dysfunction, ultimately cause their deterioration and death at an average age of 14.6 years.

Common symptoms include baldness, nail dystrophy, coxa valga, uneven skin with little outpouching across the abdomen and upper thighs, gradual joint contractures, loss of subcutaneous fat, which delayed the eruption and loss of primary teeth. Later findings included dental crowding, a partial lack of secondary teeth eruption, and low-frequency conductive hearing loss.

HGPS occurs as a result of a de novo point mutation in the DNA. Hutchinson-Gilford One of the most well-researched laminopathies, progeria (HGPS) is an accelerated aging condition brought on by a mutation in lamin A. An inner nuclear membrane protein called lamin A has impacts on cell signaling as well as structure. Rather than altering the translated amino acid (Gly608Gly), the single C to T transition at nucleotide 1824 of LMNA activates a cryptic splice site that causes the deletion of 150 base pairs from the 3' section of exon 11. A shorter aberrant prelamin A protein with a 50 amino acid deletion is produced by translation and post-translational processing of this modified mRNA, and it is referred to as "progerin." The probably chronic farnesylation of progerin, which causes it to become permanently intercalated into the inner nuclear membrane where it can accumulate and cause ever greater damage to cells with aging, is a major contributing factor to the disease in HGPS. The nucleus experiences structural stress as a result of the incapacity to release progerin from the nuclear membrane. Prelamin A, or progerin, is a continuously farnesylated mutant form, and it is thought to be the cause of the gradual abnormalities in nuclear architecture observed in HGPS. Despite being uncommon, HGPS is nevertheless quite concerning due to its many crippling effects.

This condition currently affects one in four to eight million newborns. Because the prevalence of progeria is consistent worldwide and does not correlate with gender, geography, or ethnicity, it is typically regarded as sporadic. There are roughly 114 youngsters at this time, In 39 different nations with an HGPS diagnosis.

Epidemiology

A single HGPS case out of every four to eight million live births is an uncommon genetic condition. There are now 46 nations and 148 recognized progeria patients. This ratio is 1:8, meaning that there is one case of progeria for every eight million births in the United States. The female to male ratio, which stands at 1:1.5, is concerning in this particular condition. 97% of patients strongly suggest that tribal vulnerability favors white people, whereas just 3% of patients identify as black Americans. The cause of this tribal disparity is unknown, though. As of April 2013, 103 children with progeria had been found, out of an estimated 200–250 globally, according to the Progeria Research Foundation database. Twenty cases have been reported from North America, sixteen from Central and Southern America, twenty-four from Europe and the Mediterranean area, four from Africa, and eighteen from Asia.This illness is typically not inherited since the patient passes away before the reproductive period. According to genetic research, the most likely mode of inheritance is a periodic autosomal dominant mutation of the sperm or fertilizing ovum. Given that it is genetically dominant, healthy patients never pass it on to their children.

The de novo point mutation in exon 11 of the LMNA gene (c.1824C.T, p.G608G) is responsible for most cases of classical HGPS.5. A-type lamins, which are intermediate filament proteins of the inner nuclear lamina, are encoded by the LMNA gene. The activation of a cryptic splice donor site that eliminates 150 nucleotides from exon 11 is caused by the c.1824C.T mutation. The ensuing lamin AΔ150 messenger ribonucleic acid produces progerin, a lamin A isoform with an internal 50 amino acid deletion that is incapable of undergoing full maturation. The majority of the nuclear lamina is made up of lamins, which organize various processes such as chromatin organization, deoxyribonucleic acid (DNA) replication, transcription, DNA methylation and epigenetic regulation, and DNA repair. Lamins also give the nucleus structure and shape. These processes may be linked to the progeria phenotype in HGPS due to their impairment. In fact, when grown in culture, cells derived from HGPS patients exhibit a significantly shorter lifespan. Additionally, as the cells age, they develop flaws in nuclear architecture and structure, such as lobulation of the nuclear envelope, thickening of the nuclear lamina, loss of peripheral heterochromatin, and clustering of nuclear pores. These defects are accompanied by an increase in progerin levels in the cells.

Clinical Manifestations

Numerous cellular and molecular alterations are brought about by progerin; however, little is known about the cascade of adverse effects that lead to the deterioration of tissues and organs and, ultimately, premature aging.

With the exception of rare inheritable variations like Werner's disease, spontaneous autosomal dominant mutations are typically the source of classical HGPS.6. A few unusual

forms of progeria, also known as non-classical progeria, are characterized by less retarded growth, slow-moving hair loss on the scalp, delayed lipodystrophy progression, evident osteolysis (save in the face), and survival that is typically documented until maturity.

At birth, these children appear normal and healthy, but because of development failure, they gain relatively little weight over time (usually within a year).

When these childern reach the ages of 1.5 to 2 years, their faces are small, their jaws are abnormally large compared to their heads, they have a high-pitched voice, irregular teeth, a pinched nose, large, wide-open eyes, undersized dystrophic clavicles, and they have not yet reached sexual maturity. Alopecia is the gradual loss of body fat and eyelashes, along with thinning and falling hair that eventually results in total baldness. Veins can be visible through the skin, which becomes extremely thin, sensitive, and translucent. Angina, hypertension, joint stiffness, and hip dislocation are other typical complaints.

Pathophysiology

The LMNA gene's de novo point mutations are the primary cause of HGPS, an autosomal dominant condition. Mandibuloacral dysplasia with lipodystrophy type A (MADA) is caused by missense mutations in the LMNA gene that impact the head, coil 1B, coil 2, or tail domains of lamin A. A single-base variation at codon 608 in exon 11 of the gene, c.1824C>T, is the source of the characteristic HGPS phenotype. The LMNA gene encodes three proteins located inside the inner nuclear membrane called lamin A (LA), lamin C (LC), and lamin 10. Constituting the nuclear laminae are these proteins. Lamina function in transcription, nuclear structure, DNA replication, chromatin architecture, and cell division has now been proven. The usual form of LMNA mutation in HGPS is the C-T nucleotide substitution, which results in a cryptic splice donor site at position 1824 and no change in the encoded amino acids. During activity, this region generates mRNA that is missing 150 nucleotides. An internal loss of 50 amino acids near the C terminus results in the production of "progerin," a mutant protein, once this mRNA is translated.

Progerin is unable to complete the cleavage phase because it lacks the Zmptse24 recognition site. As a result, it remains farnesylated and abnormally tethered to the nuclear envelope, changing the nuclear lamina's function. The c-terminal cysteine group experiences methyl esterification after farnesylation, and the three-terminal amino acids (AAX) are eliminated. Remarkably, the complete protein undergoes a second cleavage within the nucleus to remove additional 15 C-terminal amino acids, including the farnesylated cysteine. Following this final stage of cleavage and the removal of the farnesyl anchor, prelamin A is presumably freed from the nuclear membrane and integrated into the nuclear lamina. The last cleavage phase requires the endoprotease recognition segment, which is eliminated by the internal loss of amino

acids 606–656. Since LMNA G608G cannot undergo the final cleavage, progerin retains its farnesyl group, maintaining progerin contacts with the inner nuclear membrane.

In this scenario, progerin will induce the nuclear envelope (NE) to grow inward, increasing the surface area that can hold the buildup of excess progerin in HGPS.

Progerin induces a wide range of cellular and molecular changes, but little is known about the series of negative consequences that result in the degradation of tissues and organs and, eventually, premature aging.

Diagnosis

Hutchison Gilford progeria syndrome (HGPS) can be identified by performing a genetic testing for the changes in the LMNA gene which is a confirmative diagnosis for the progeria [1].
Procedure for LMNA Gene testing:

The patient blood is sent to a lab, for DNA analysis. The laboratory employs methods like polymerase chain reaction (PCR) and DNA sequencing to detect the c.1824C>T mutation, in the LMNA gene leading to the creation of progerin [2].

Other diagnostic consideration such as a thorough physical examination which includes:

1) Measuring height and weight
2) Putting the measurements on a growth curve chart
3) Testing hearing and vision
4) Measuring the vital signs
5) Observing for visible symptoms of progeria

Furthermore, there are various other physical changes observed such as, Growth Defects, ectodermal defects, musculoskeletal deformities, and facial features changes, cardiovascular and cerebrovascular disease, ophthalmological and hearing defects.

The above-mentioned defects can be identified by following characteristics:

Growth Defects [3]:

- Short height
- Poor weight gain
- Low subcutaneous body fat

Facial features [3]:

- Head disproportionately large than face
- Long narrow nose
- Thin vermilion of the upper and lower lip
- Retrognathia and micrognathia

Ectodermal defects:

- Delayed eruption and delayed loss of primary teeth, partial secondary tooth eruption, dental mislocation

- Skin may consist of pigments, sclerodermatous, skin outpouchings over lower abdomen and proximal thighs
- Hair alopecia and sometimes loss of eyebrows
- Dystrophic nails

Musculoskeletal changes

- Improper gait which may be sometimes accompanied by avascular necrosis of the femoral Head
- Distance phalanges osteolysis
- Short clavicles
- Pear shaped thorax [3].

Cardiovascular changes

- Increasing stiffness of blood vessels
- Progressive age can lead to hardening and calcification of arterial blood vessels and left sided heart valves

These cardiovascular changes are life threatening and can be diagnosed by monitoring:

i. Resting heart rate and blood pressure
ii. A 12 Lead electrocardiogram at least annually
iii. Measuring the fasting blood lipids including cholesterol and blood sugar
iv. And educating the family regarding maintaining appropriate fluid intake and heart healthy diet.
v. Carotid duplex ultrasound [4]

Computerized tomography (CT scan) and magnetic resonance imaging (MRI) are diagnostic tests that can be performed to identify stroke and transient ischemic episodes (TIAs). Arterial constriction and stroke can be identified with magnetic resonance angiography (MRA) of the main arteries in the brain and neck [5].

Treatment

Hutchinson-Gilford Progeria Syndrome (HGPS) patient may suffer from various complications like dermatologic changes, neurologic deformities/cerebrovascular changes, bone and cartilaginous abnormalities and cardiovascular disease. The treatment options are described as follows:

- **Farnesyltransferase Inhibitors:**

HGPS is caused by a gene product called progerin, which lacks 50 amino acids and is persistently farnesylated, unlike the normal lamin A. Progerin binds to a cell's inner nuclear membrane, maybe permanently. Protein farnesyltransferase inhibitors (FTIs) are tiny compounds that attach to the farnesyltransferase binding site and prevent progerin from being farnesylated

and inserted into the nuclear membrane [1]. Lonafarnib, or Zokinvy, is a farnesyltransferase inhibitor. The FDA authorized it in November 2020 to treat Hutchinson-Gilford Progeria Syndrome (HGPS) and processing-deficient progeroid laminopathies. Lonafarnib is a CYP3A and CYP2C9 substrate, a strong CYP3A inhibitor, and a moderate CYP2C19 inhibitor [2].

Oral farnesyltransferase inhibitor (FTI); farnesyltransferase is an enzyme that modifies proteins through a process known as prenylation [3]. A mutation in the LMNA gene causes excessive production of progerin, a farnesylated-aberrant protein; prolonged farnesylation promotes progerin buildup in the inner nuclear membrane and is, at least partly, responsible for HGPS. Accumulation of faulty lamin A protein causes the nucleus to become unstable, resulting in rapid aging in children with progeria.

Dosing:

Adults and children 12 months and older with a body surface area (BSA) of 0.39 meter squared (m2)—Initially, 115 mg per square meter (mg/m2), taken twice daily with morning and evening meals [4].

Adverse effects of Lonafarnib:

Diarrhoea, nausea, vomiting, abdominal pain, and loss of appetite, hyperkalaemia, elevated liver enzymes, upper respiratory infection, arthralgia, myalgia, headache, rashes [5-6].

- **Statins and Aminobisphosphonates:**

Statins and aminobisphosphonates suppress farnesyl pyrophosphate production. These medications impede farnesylation and geranylgeranylation, perhaps preventing geranylgeranylation, an alternate mechanism for progerin prenylation [7-8].

Statins:

Statins, like pravastatin and atorvastatin function by blocking the activity of the enzyme HMG CoA reductase, which plays a role in the process of forming cholesterol. As a result, there is a reduction in cholesterol levels in the bloodstream, in low density lipoprotein (LDL) cholesterol. Progeria is a condition where statins are believed to help decrease the formation of farnesylated proteins, such, as progerin. This decrease could potentially ease cell abnormalities linked to the buildup of progerin, a factor, in progerias development. Statins play a role, in safeguarding heart health by addressing atherosclerosis a condition characterized by artery hardening that poses risks for children with progeria. Through their ability to lower cholesterol and combat inflammation statins can effectively stall the advancement of ailments, in these individuals. Additionally ongoing research is exploring the potential of statins to mitigate the impact of progerin although further investigation is required to confirm this effect [9].

Side Effects:

Myalgia, elevated liver enzymes, and rhabdomyolysis (rare)

Bisphosphonates:

Bisphosphonates, such, as alendronate and zoledronic acid are medications that work to prevent the breakdown of bone tissue by osteoclasts. Osteoclasts are responsible for breaking down bones and bisphosphonates aid in maintaining bone strength by slowing down this process. In individuals, with progeria bisphosphonates are utilized to address symptoms resembling osteoporosis. This condition often results in decreased bone density increasing the likelihood of fractures. By impeding bone resorption bisphosphonates assist in fortifying bones decreasing risks [9-10].

Maintaining bones; Bisphosphonates have shown to be successful, in enhancing bone mineral density (BMD) and lowering the risk of bone fractures in individuals with progeria.

Supporting Joint and Bone Strength; Additionally, these medications play a role, in enhancing wellbeing, which is essential considering the joint rigidity and other skeletal irregularities linked to progeria.

Adverse Effects:

- Gastrointestinal discomfort
- Heart burns
- Oesophageal irritation
- Osteonecrosis (rare)

Combined Use of Statins and Bisphosphonates in Progeria:

Studies conducted on animals have indicated that when statins and bisphosphonates are used together, they might work effectively in reducing characteristics of progeria. This joint treatment has demonstrated an ability to prolong lifespan and enhance health indicators, in mouse models of progeria. By combining the benefits of statins for issues and bisphosphonates, for bone density improvement a holistic strategy is adopted to address the impacts of progeria [11].

Supportive Care

Cardiovascular Management:

- Blood Pressure Control: Medications are prescribed to manage blood pressure a concern, for individuals with progeria due to the potential for heart related issues.
- Aspirin Use: Low dose aspirin is typically advised to lower the chances of stroke and heart attack by preventing blood clot formation [7].

Dietary Assistance:

- Calorie Rich Diets: Children with progeria may face challenges, in achieving growth and weight gain. Providing high calorie diets and dietary supplements can aid in promoting development.

- Vitamin D and Calcium Intake: Taking supplements is often recommended to maintain bone strength and reduce the risk of fractures.

Occupational Therapy:

- Maintaining mobility and muscle strength is essential, through physical therapy especially as the disease progresses. Engaging in stretching exercises can enhance flexibility. Alleviate stiffness. Additionally orthopaedic devices like braces may be necessary to provide support and prevent dislocations while assistive technologies can aid in tasks and promote independence, for the patient [12].

Cardiac Surgery [7]

- Coronary Artery Bypass Surgery (CABG); This operation might be needed for patients dealing with artery disease, a frequent issue, in individuals, with progeria.
- Percutaneous Coronary Interventions (PCI); Doctors may use stent placement to unblock arteries that are narrowed or blocked.

Orthopedic Surgery [7]

- Hip Surgery: due to the risk of hip dislocation and associated issues surgery may be necessary to secure the joint.

Psychological Counseling [6]:

- Mental Wellbeing Assistance: Acknowledging the weight of coping with a life altering ailment, therapy and mental health resources play a vital role, for individuals and their loved ones.
- Community Support: Engaging in community support networks offers solace and practical guidance from individuals who can relate to the difficulties of managing progeria.

Recent Advances

There is currently no diagnostic kit available for the early diagnosis of HGPS, despite the ongoing search for a viable treatment. In clinical practice, phenotypical data and the child's medical history are typically the basis for a clinical assessment.

Farnesyltransferase is an enzyme that, in the case of normal cells, essentially farnesylates the amino acid cystein, which is linked to the carboxy-terminal end of the protein known as prelamin-A and contains a pattern called CaaX. The accurate formation of the entire protein is highly dependent on the farnesyl group present at the carboxy-terminal of the protein prelamin-A, which fundamentally stimulates the attachment of the nuclear sheath to the protein.

However, a splice location inside the protein Lamin A ultimately results in the loss of roughly 50 amino acids, which becomes the primary cause of this uncommon premature aging illness in the case of Hutchinson-Gilford Progeria illness. However, this type of elimination does

not interfere with progerin's typical farnesylation process or negatively impact the CaaX motif in any way.

Yet, this removal method leaves the progerin in a farnesylated state because it eliminates the ZMPSTE24-FACE1 detection site, which was essential for the entire cutting process.

Therefore, it was discovered in the current study that the medication known as farnesyltransferase inhibitors, or FTIs, was in charge of preventing the farnesyl group from attaching to the protein because they bind to the CaaX domain in an irreversible conformation.

Prevastatin, lonsafarnib, and zoledronic acid are the three "drugs of hope" that the Progeria Research Foundation, National Institutes of Health, Children's Hospital Boston, and Dana-Farber Cancer Institute are evaluating for their potential to help treat Hutchison-Gilford Progeria Syndrome. Therefore, as a result of the testing done to find a treatment for the disorder, it was reported that the medication Lonafarnib was helpful in treating a number of cardiovascular conditions associated with the syndrome, it improved disorders related to bones, and it also assisted patients suffering from severe cases of Hutchinson-Gilford Syndrome in becoming more obese. In addition to Lonafarnib, Prevastatin has also been shown to be beneficial in evaluating cardiovascular issues and in lowering cholesterol levels in the bodies of people with progeria. Furthermore, zoledronate, the third medication that had also been subjected to a scientific trial, was discovered to be a biphosphonate and to be effective in improving osteoporosis, thereby preventing skeletal fractures or wounds.

Additionally, it was looked into whether the aggregation of farnesylated prelmin A, an accessory process of the wild type progerin, degrades the cell's phenotypic. Accordingly, it was reported that in this instance, an antibiotic known as rapamycin (belonging to the macrolides class) was discovered to cause autophagy in cells by upsetting the distortive path, or the mTOR dependent pathway, which could ultimately lead to the stimulation of the progerin degeneration process. Consequently, a study shows that the application of the antibiotic rapamycin to cells affected by Hutchinson-Progeria Syndrome significantly reduced the levels of progerin and prelamin-A. Additionally, it induced the recovery of trimethylated H3K9 arrangement, leading to the development of chromatin epigenetic prominence.

The decrease in methyltransferase Suv39h1 that results in DNA reformation and increased longevity in a Progeria murine model. According to the research done in this instance, progerin, also known as prelamin-A, has a greater ability to link with SUV39H1, and the lamin protein's A form has a relationship with SUV39H1, which enhances the H3K9me3 content by protecting it from proteosomal degradation.

Increased SUV39h1 constancy in the presence of preLamin A/progerin, which also causes an increase in the H3K9me3 content, essentially stimulates an adjustment in genome preservation that results in an increased agedness in laminopathy-established immature elderliness. Therefore, by concentrating on heterochromatin remodeling, which is typically

driven by SUV39h1, the current study offers a viable method for interruption as well as an interpretation for the epigenetic abnormalities seen in the instance of Hutchinson-Progeria syndrome.

The prelamin-A protein aggregates in zmpste24 mosaic murine, which decreases the incidence of penetrating oral cancer but does not prevent cyst induction or development. In addition, the inhibited Zmpste24 reduces the invasion of cancer cells in humans.

As contemporary farnesylation regulators, aminopyrimidines were studied pharmacologically in pluripotent stem cells afflicted with Hutchinson-Gilford Progeria Syndrome. Since it is commonly known that the process of farnesylation regulates the virulent behavior of progerin, a method for examining sophisticated medicinal interrupters for the process of farnesylation was developed and tested in the current experiment. Therefore, in order to complete the task, the pluripotent stem cells' unique capabilities were used. Through a research, it was ultimately determined that components such as monoaminopyrimidines typically identify two significant biological catalysts, namely farnesyl transferase and farnesyl pyrophosphate synthase, which are primarily involved in the farnesylation process. In addition, the in vitro investigation revealed significant physical characteristics that were primarily associated with Hutchinson-Gilford Progeria Syndrome.

Conclusion:

De novo point mutations in the LMNA gene are primarily responsible for the autosomal dominant disorder known as HGPS. Mandibuloacral dysplasia with lipodystrophy type A (MADA) is caused by missense mutations in the LMNA gene that impact the head, coil 1B, coil 2, or tail domains of lamin A. Progerin causes a variety of cellular and molecular changes, but little is known about the series of negative consequences that eventually cause tissues and organs to deteriorate and premature aging.Aging is a natural consequence of human existence; it increases the risk of chronic diseases and results in a gradual deterioration of cell, tissue, and organismal function. Individuals diagnosed with HGPS exhibit clinical manifestations of early aging, including hair loss, uneven skin tone, loss of subcutaneous fat, and early-life atherosclerosis and cardiovascular mortality in their adolescent years. tiny jaws, short nasal bridges and tips, unusually projecting eyeballs, crowding and malformation of the teeth, and disproportionately tiny faces relative to heads are all characteristics of progeria children.

A single HGPS case out of every four to eight million live births is an uncommon genetic condition. There are now 46 nations and 148 recognized progeria patients. This ratio is 1:8, meaning that there is one case of progeria for every eight million births in the United States. The female to male ratio, which stands at 1:1.5, is concerning in this particular condition. 97% of patients strongly suggest that tribal vulnerability favors white people, whereas just 3% of patients identify as black Americans.

In essence, the enzyme known as farnesyltransferase farnesylates the amino acid cystein, which is connected to the carboxy-terminal end of the protein known as prelamin-A in the case of normal cells and includes a pattern known as CaaX. As a result of the removal method's elimination of the ZMPSTE24-FACE1 detecting site, which was necessary for the whole cutting process, the progerin remains farnesylated. As a result, the present study found that when farnesyltransferase inhibitors, or FTIs, bind to the CaaX domain in an irreversible conformation, they are responsible for blocking the farnesyl group from adhering to the protein. The Progeria Research Foundation, National Institutes of Health, Children's Hospital Boston, and Dana-Farber Cancer Institute are assessing three "drugs of hope" for the treatment of Hutchison-Gilford Progeria Syndrome: zoledronic acid, lonsafarnib, and prevastatin. Prevastatin and lonsafarnib have also been demonstrated to be helpful in assessing cardiovascular problems and reducing cholesterol levels in the bodies of individuals with progeria. Moreover, zoledronate—the third drug that had also undergone scientific testing—was shown to be a biphosphonate that effectively improved osteoporosis, avoiding bone fractures or wounds. In addition to providing an explanation for the epigenetic anomalies observed in the case of Hutchinson-Progeria syndrome, the current study provides a workable strategy for disruption. The in vitro study has uncovered important physical traits that were mostly connected to Hutchinson-Gilford Progeria Syndrome.

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RECRYSTALLISATION: A FUNDAMENTAL TECHNIQUE FOR THE PURIFICATION OF ORGANIC COMPOUNDS

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Abstract:

Recrystallisation is a fundamental and widely adopted purification method in organic chemistry, particularly effective for solid compounds. This technique is grounded in the principles of differential solubility and controlled crystallisation, wherein an impure solid is dissolved in a hot solvent and then allowed to recrystallise upon cooling, leaving most impurities behind. The process is highly valued for its simplicity, cost-effectiveness, and ability to yield pure products essential for accurate analytical characterization, reproducible reactions, and pharmaceutical development. By providing compounds of high purity, recrystallisation supports consistency in research, education, and industrial manufacturing. This chapter explores the scientific basis, procedural steps, and broad applications of recrystallisation, highlighting its indispensable role in chemical purification.

Keywords: Recrystallisation, Organic Compound Purification, Differential Solubility, Crystallisation Process, Solid-State Purification, Analytical Purity, Impurity Removal, Pharmaceutical Chemistry, Laboratory Techniques, Organic Synthesis.

1. Introduction:

Recrystallisation is a widely used purification technique in organic chemistry, especially effective for the purification of solid compounds. This technique relies on the scientific principles of differential solubility and the process of crystallisation. It is based on the fact that the solubility of most compounds increases with temperature—compounds tend to dissolve more readily in hot solvents than in cold ones [1].

When a solid organic compound is contaminated with impurities, recrystallisation provides an effective means of separating and removing these unwanted substances. The basic process involves dissolving the impure compound in a suitable hot solvent to form a saturated solution. As the solution cools, the solubility of the desired compound decreases, causing it to crystallise out of the solution in a purer form, while the impurities either remain dissolved in the solvent or are filtered out beforehand if they are insoluble.

This method is not only simple and cost-effective but also yields highly pure compounds, making it a staple technique in both educational and industrial laboratories. The ultimate goal of recrystallisation is to isolate a single, pure substance from a heterogeneous mixture [2]. This purified substance is crucial for several downstream applications, such as:

- **Accurate characterisation** of compounds through analytical techniques (e.g., melting point analysis, spectroscopy).
- **Monitoring chemical reactions**, where the presence of impurities could interfere with reaction outcomes.
- **Pharmaceutical development**, where purity is directly related to the efficacy and safety of a drug.

Additionally, recrystallisation contributes to the repeatability and reliability of scientific research and industrial manufacturing by providing pure materials that behave consistently under experimental or production conditions [3].

In summary, recrystallisation is a cornerstone of purification in organic chemistry that blends scientific principles with practical utility, ensuring the integrity of chemical substances across various fields.

2. Principle of Recrystallisation

Recrystallisation operates on the fundamental principle that the solubility of a solid compound typically increases with temperature. This principle allows chemists to selectively dissolve a mixture of compounds in a hot solvent and then induce crystallisation of the desired compound by cooling the solution. When a saturated hot solution is slowly cooled, the solubility of the primary compound decreases, leading to its gradual precipitation in the form of pure crystals. Ideally, impurities either remain dissolved in the solvent (if they are more soluble) or are filtered out prior to crystallisation (if they are insoluble).

The process involves two critical stages:

1. **Dissolution at High Temperature** – The impure solid is dissolved in a minimal amount of hot solvent to create a saturated solution.
2. **Crystallisation During Cooling** – As the temperature of the solution decreases, the solubility of the compound drops, and it begins to crystallise out.

This difference in solubility forms the scientific basis of recrystallisation. A successful recrystallisation hinges on choosing the correct solvent, which should ideally dissolve the target compound well at elevated temperatures but poorly at lower temperatures. Conversely, impurities should either remain fully soluble across the temperature range or be insoluble so they can be removed during filtration. Thermodynamically, crystallisation is a process of moving from a higher-energy disordered solution to a lower-energy ordered solid state. The kinetic aspect, such as the rate of cooling, also plays a crucial role slower cooling generally results in the

formation of well-formed, larger, and purer crystals. Thus, understanding and applying the principle of differential solubility underlies every successful recrystallisation procedure. This makes it not only a practical method but also a conceptually elegant one, embodying both the physical and chemical foundations of separation science [4].

3. Selection of Solvent

The selection of an appropriate solvent is a pivotal step in the recrystallisation process. The success of the technique largely hinges on finding a solvent or a combination of solvents that maximizes the differential solubility between the desired compound and its impurities. A suitable solvent should meet several essential criteria to ensure efficient and effective purification:

- **Dissolve the compound when hot but not (or only slightly) when cold:** This property ensures that the target compound fully dissolves upon heating, forming a saturated solution. Upon cooling, it should crystallise out because of its reduced solubility. This solubility differential is the core of the recrystallisation process.
- **Either not dissolve impurities at all or dissolve them completely:** If impurities are insoluble in the chosen solvent, they can be removed via hot filtration. If they are soluble, they should remain in the solution even after the pure compound has crystallised out. This prevents co-precipitation and enhances the purity of the final product.
- **Be chemically inert with respect to the solute:** The solvent must not react with the compound being purified. Chemical interactions could degrade the compound or form unwanted byproducts, compromising both yield and purity.
- **Be volatile enough for easy removal after recrystallisation:** Once the crystals are formed and isolated, the residual solvent should evaporate easily, either at room temperature or with mild heating. This ensures that the final product is dry and free from solvent residues.
- **Be non-toxic and cost-effective whenever possible:** Especially in large-scale operations or educational settings, the environmental impact, toxicity, and cost of the solvent are important considerations. Safer, greener solvents like water and ethanol are preferred when they meet other selection criteria.

Commonly used solvents include:

- **Water:** Ideal for polar compounds; safe, cheap, and environmentally friendly.
- **Ethanol and Methanol:** Good for a wide range of organic compounds; moderately polar.
- **Ethyl Acetate:** Useful for non-polar to moderately polar compounds.
- **Acetone:** Excellent for polar compounds; volatile and fast-evaporating.
- **Toluene:** Suitable for non-polar substances; high boiling point.

In many cases, a binary solvent system is employed, where the solute is soluble in one solvent and insoluble in the other. The solute is first dissolved in the hot primary solvent, and

then the secondary solvent (in which the compound is poorly soluble) is added dropwise until crystallisation begins. This approach helps in fine-tuning the solubility characteristics and can improve both yield and purity [5].

4. Steps of Recrystallisation

4. Steps of Recrystallisation

Recrystallisation is carried out through a systematic series of steps that must be executed carefully to ensure maximum purity and yield. These steps include:

4.1 Dissolution

The dissolution step is the foundation of the recrystallisation process and must be performed with precision. The impure compound is first placed in a clean, dry beaker or Erlenmeyer flask. A suitable solvent, chosen based on the compound's solubility profile, is then added gradually while the mixture is gently heated using a hot plate or water bath. The amount of solvent used should be just enough to completely dissolve the solid when hot-typically the minimum required to produce a saturated solution. Adding too much solvent can reduce the yield by keeping the compound in solution even after cooling. To promote faster dissolution, the solution is stirred continuously using a glass rod or magnetic stirrer. Gentle heating ensures that the solvent does not evaporate excessively or decompose sensitive compounds. Visual cues play a crucial role during this step. The solution should become clear, indicating complete dissolution of the desired compound. Any cloudiness or undissolved particles suggest incomplete dissolution, necessitating the addition of more hot solvent in small increments. Care should be taken to avoid overheating, which may lead to the decomposition of the compound or the formation of byproducts. In cases where coloured impurities are present, activated charcoal may be added to adsorb them, followed by hot filtration to remove the charcoal. Ultimately, a successful dissolution results in a clear, homogeneous solution devoid of suspended solids an ideal starting point for the subsequent steps of recrystallisation [6].

4.2 Hot Filtration

After complete dissolution, the solution may still contain insoluble impurities. These are removed by performing hot filtration. A fluted filter paper and a stemless funnel (or a Büchner funnel under vacuum) are typically used. Importantly, the filtration apparatus should be preheated to prevent premature crystallisation during filtration, which can cause clogging and product loss. This step ensures that only the dissolved compound and soluble impurities remain in the filtrate [7].

4.3 Crystallisation

Following filtration, the hot, clear solution is allowed to cool undisturbed at room temperature. As the solution cools, the solubility of the compound decreases, and pure crystals begin to form. After some crystallisation occurs, placing the container in an ice bath can enhance

the yield by further reducing the solubility of the compound. The rate of cooling is crucial—slow cooling favors the formation of larger, well-defined crystals that are easier to filter and wash, while rapid cooling may trap impurities within the crystal lattice [8].

4.4 Filtration of Crystals

Once crystallisation is complete, the solid crystals are separated from the mother liquor using vacuum filtration. A Büchner funnel and side-arm flask with a filter paper are commonly used. The crystals are collected on the filter paper and rinsed with a small amount of ice-cold solvent to remove any residual impurities clinging to the surface. The wash solvent should be chosen carefully to ensure it does not dissolve the product.

4.5 Drying of Crystals

The final step involves drying the isolated crystals. This can be achieved by leaving them in the open air, placing them in a drying oven, or storing them under reduced pressure in a desiccator. Proper drying is vital for accurate melting point determination, analytical measurements, and yield calculation. Residual solvent can distort these results and affect the chemical stability of the compound [9].

Each of these steps contributes critically to the efficiency and effectiveness of the recrystallisation process. Mastery of these steps ensures reproducibility and high-quality results, making recrystallisation a reliable technique for both academic and industrial applications.

5. Applications of Recrystallisation

Recrystallisation is an indispensable technique in various scientific and industrial contexts due to its ability to purify solid compounds effectively. Its widespread applications span several fields:

- **Pharmaceutical Industry:** Recrystallisation is extensively employed for purifying active pharmaceutical ingredients (APIs). Pharmaceutical products require a high degree of purity for safety and efficacy. Even trace impurities can cause significant adverse effects or alter the drug's therapeutic properties. Recrystallisation ensures that APIs meet the stringent purity standards set by regulatory authorities.
- **Research Laboratories:** In both academic and industrial research settings, pure compounds are essential for accurate experimental results. Recrystallisation allows chemists to obtain clean samples necessary for structure elucidation, spectral analysis, kinetic studies, and further synthetic transformations. The method is often used to purify intermediates and final products in synthetic organic chemistry.
- **Quality Control Processes:** In chemical manufacturing, maintaining product consistency and compliance with standards is vital. Recrystallisation serves as a critical step in quality control procedures, ensuring that the products meet desired purity levels. It is used to

validate the chemical identity and confirm the absence of unwanted byproducts or contaminants.

- **Recovery of Valuable Materials:** During chemical reactions, crude mixtures often contain significant amounts of the desired product alongside impurities and side products. Recrystallisation helps in recovering valuable substances from these mixtures in a pure, usable form, enhancing both yield and cost-efficiency. This is particularly important in large-scale industrial processes where maximizing recovery translates into significant economic benefits.
- **Educational Demonstrations:** Recrystallisation is a fundamental experiment taught in undergraduate chemistry courses. It helps students understand principles such as solubility, saturation, and crystallisation while developing practical lab skills.
- **Environmental and Forensic Chemistry:** Recrystallisation is sometimes used in environmental testing and forensic investigations to purify and isolate specific substances from complex mixtures for further analysis [10].

6. Examples of Recrystallisation

Recrystallisation is a practical method frequently demonstrated through specific examples that illustrate its effectiveness in purifying organic compounds. Two commonly studied compounds in both academic and industrial laboratories are benzoic acid and acetanilide. These examples provide a clear understanding of the process and reinforce the key principles of solubility, crystallisation, and impurity removal.

6.1 Purification of Benzoic Acid: Benzoic acid is a simple aromatic carboxylic acid and serves as a classic example for recrystallisation using water as the solvent. Water is ideal in this case because benzoic acid has low solubility in cold water and high solubility in boiling water.

Procedure:

1. Weighing and Dissolution: A known quantity of crude benzoic acid is weighed and placed in a beaker. A minimal amount of boiling distilled water is added gradually while stirring and heating to dissolve the solid. The goal is to form a saturated solution at the elevated temperature.
2. Hot Filtration: If the crude sample contains insoluble impurities (e.g., dirt or polymers), the hot solution is filtered using a fluted filter paper and funnel. This step ensures the removal of particulate matter before crystallisation begins.
3. Crystallisation: The hot, clear filtrate is left undisturbed to cool slowly at room temperature. As the temperature drops, pure benzoic acid begins to crystallise out of the solution. To maximise yield, the mixture may be further cooled in an ice bath.

4. Filtration of Crystals: The formed crystals are collected via vacuum filtration using a Büchner funnel. The crystals are then rinsed with a small amount of cold distilled water to remove any residual soluble impurities.
5. Drying: The purified benzoic acid is dried in air or in a desiccator. Once dry, its purity can be assessed by determining its melting point, which should be close to 122°C for pure benzoic acid [11].

6.2 Recrystallisation of Acetanilide: Acetanilide, an aromatic amide, is another compound commonly used to demonstrate recrystallisation. It can be purified using either hot water or ethanol as the solvent, depending on its solubility characteristics and the nature of the impurities.

Procedure:

1. Dissolution: A sample of crude acetanilide is placed in a beaker, and a suitable hot solvent (typically hot water or ethanol) is added gradually while stirring. The mixture is gently heated until all of the solid dissolves, forming a clear solution.
2. Hot Filtration: If any insoluble impurities remain, the solution is filtered while still hot to remove them. This prevents crystallisation during the filtration process.
3. Crystallisation: The hot filtrate is allowed to cool slowly at room temperature. As the solution cools, pure acetanilide crystallises out. Further cooling in an ice bath may be used to increase the yield.
4. Collection and Washing: The crystals are collected via vacuum filtration and washed with a small amount of cold solvent to remove residual impurities from their surfaces.
5. Drying: The purified crystals are dried by air-drying, oven drying at low temperatures, or in a desiccator under reduced pressure [12].

7. Factors Affecting Recrystallisation

7.1 Solubility

Solubility is the cornerstone of recrystallisation. For the process to succeed, the compound must exhibit high solubility in the chosen solvent at elevated temperatures and low solubility at lower temperatures. This temperature-dependent solubility allows the substance to dissolve completely when hot and to crystallise as the solution cools.

- **Ideal Scenario:** The target compound should dissolve in the minimum volume of hot solvent to create a saturated solution, which then becomes supersaturated upon cooling, promoting crystal growth.
- **Impurities:** Should either remain soluble in the cold solvent or be completely insoluble (and thus removable during hot filtration).
- **Effect:** Poor solubility behaviour (e.g., high solubility at all temperatures) will hinder effective crystallisation and reduce yield [13].

7.2 Rate of Cooling

The cooling rate of the hot saturated solution plays a pivotal role in determining the size and purity of the crystals.

- **Slow Cooling:** Encourages the gradual formation of a regular crystal lattice, leading to larger, well-formed, and purer crystals. This is because impurities are less likely to be incorporated into the crystal structure.
- **Rapid Cooling:** May result in small, irregular crystals or amorphous precipitates. It can also lead to entrapment of impurities within the growing crystals, reducing overall purity.
- **Best Practice:** Allow the solution to cool at room temperature first, followed by ice-bath cooling to increase crystal yield without compromising quality [14].

7.3 Solvent Purity

The chemical purity of the solvent used is equally important in ensuring the success of the recrystallisation process.

- **Pure Solvent:** Prevents introduction of new contaminants that could co-crystallise with the target compound or interfere with crystal formation.
- **Contaminated Solvent:** May introduce unknown substances that alter solubility profiles or crystallisation behavior, leading to mixed or impure crystals.
- **Recommendation:** Always use freshly distilled or analytical-grade solvents for sensitive or high-purity applications [15].

7.4 Concentration

The concentration of the compound in the hot solvent affects how easily and cleanly it recrystallises.

- **Optimal Concentration:** A saturated solution at high temperature ensures sufficient driving force for crystallisation upon cooling.
- **Overly Concentrated Solution:** May result in premature precipitation or supersaturation, leading to rapid, uncontrolled crystallisation and trapping of impurities.
- **Too Dilute Solution:** May reduce yield significantly as only a small amount of compound crystallises out upon cooling.
- **Tip:** Add the solvent gradually and only as much as needed to achieve complete dissolution at the boiling point of the solvent [16].

8. Characterization of Recrystallised Compound

After recrystallisation, the purity of the product is verified using techniques like:

- **Melting Point Determination**: A pure compound has a sharp, characteristic melting point.
- **Thin Layer Chromatography (TLC)**: Assesses purity by comparing Rf values.

- **Spectroscopic Methods**: FTIR, NMR, and UV-visible spectroscopy may be employed for structural confirmation [17].

9. Limitations of Recrystallisation

- Not suitable for compounds that decompose upon heating.
- Some impurities may co-crystallise.
- Requires careful solvent selection.
- Not effective for very small quantities [18].

10. Modifications and Advanced Techniques

10.1 Multiple Solvent Systems

Sometimes a single solvent does not provide ideal solubility characteristics. A mixture of solvents (e.g., ethanol-water) may be used. The compound should be soluble in one and insoluble in the other.

10.2 Seeding

Seeding involves adding a small crystal of the pure compound to the saturated solution to initiate crystallisation. This is helpful when nucleation is slow or doesn't occur spontaneously.

10.3 Use of Activated Charcoal

If the solution contains colored impurities, activated charcoal can be added before filtration. It adsorbs the colored substances and is removed by hot filtration [19].

11. Troubleshooting Recrystallisation Problems

11.1 No Crystals Form

- Solution may be too dilute.
- Use scratching or seeding to initiate crystallisation.
- Evaporate some solvent to concentrate the solution.

11.2 Oily Precipitate Forms

- Cooling too quickly.
- Impure solvent or compound.
- Try a different solvent or solvent mixture.

11.3 Crystals Are Coloured or Impure

- Use activated charcoal to remove colour.
- Ensure hot filtration is done properly [20].

12. Industrial Applications of Recrystallisation

In industry, recrystallisation is scaled up for:

- Bulk drug purification
- Production of high-purity reagents
- Isolation of natural products
- Manufacture of specialty chemicals

Techniques such as continuous crystallisation and automated crystallisers are used to optimize yield and purity while minimizing energy and solvent usage [21].

13. Green Chemistry and Recrystallisation

Recrystallisation processes can be adapted to align with green chemistry principles by:

- Using environmentally benign solvents (e.g., water, ethanol)
- Recycling solvents
- Minimizing solvent volume
- Improving energy efficiency by crystallising at ambient temperature [22]

Conclusion:

Recrystallisation remains one of the most effective and accessible methods for purifying solid compounds. Its success depends heavily on understanding the principles of solubility, proper solvent selection, and careful execution of each step. While it has limitations, modifications and innovations continue to expand its scope in both academic and industrial contexts. For students and professionals alike, mastering this technique is fundamental to the practice of synthetic and analytical chemistry.

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THE INVISIBLE ARCHITECTS OF THE BRAIN: EPIGENETICS AND NEUROLOGICAL DISORDERS

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Abstract:

The brain, with its intricate architecture and complex functions, is not solely sculpted by genetic blueprints but also profoundly influenced by "invisible architects"—epigenetic mechanisms. These dynamic processes study the heritable gene expression changes that don't modify the DNA sequence, and play a pivotal role in neurodevelopment, neuronal plasticity, and cognitive function. Their dysregulation can lead to synaptic dysfunction and neurodegeneration. Environmental factors (e.g., diet, stress) also influence the brain's epigenome. This chapter explores how epigenetics contributes to neurological disorders like autism, schizophrenia, Alzheimer's, and Parkinson's disease highlighting its potential for new biomarkers. Understanding these epigenetic landscapes opens up new possibilities for developing innovative diagnostic biomarkers and therapeutic strategies, paving the way for a new era in the prevention and treatment of neurological disorders by targeting the brain's hidden regulatory mechanisms.

Keywords: Epigenetics; Neurological Disorders; DNA Methylation; Histone Modifications; Non-coding RNA; Neurodegeneration; Therapeutic Targets.

Introduction:

The Brain: The Command Centre of the human body

The intricate complexity of the human brain, with its billions of neurons and trillions of synapses, relies on exquisitely fine-tuned gene expression for its development, function, and remarkable resilience. Genetics is concerned with the actual sequence of DNA, while epigenetics examines changes in gene expression that can be inherited without modifying the DNA sequence itself. The word "epigenetics" translates to "on top of" or "in addition to" genetics.[1] The. term introduced by British biologist Conrad Waddington in the mid-20th century, determine which genes are turned "on" or "off" in different cell types or under different conditions, even though every cell contains virtually the same DNA [2,3]. Epigenetic mechanisms, like DNA methylation and histone modifications, can modulate gene expression, impacting neuronal signalling, synaptic plasticity, and overall brain circuitry [4] These modifications are key to long-term memory formation and can significantly influence memory performance. Epigenetics also shapes the brain's response to stress by regulating gene expression within the central nervous system [5]

Specific sets of epigenetic modifications (epigenomes) can lead to distinct molecular changes involved in epigenetic processes such as gene silencing, X-chromosome inactivation or imprinting. The gene sequence remains unchanged throughout life; however, environmental factors such as stress [6] diet [7] or maternal care [8] act through certain chemical reactions to influence the chromatin state. These reactions can unravel the chromatin and cause stretches of DNA containing a gene to be exposed for longer or shorter periods, essentially turning the gene on or off and allowing for changes in protein production. This change in protein production, in turn, can affect physiological and behavioural traits and can be passed from one cell to the next as the cells multiply within an organism and can even be passed from parents to children [9]

This chapter explores the role of epigenetics in the etiology and progression of a diverse spectrum of neurological disorders, ranging from neurodevelopmental conditions like autism spectrum disorder and schizophrenia to debilitating neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Figure 1 conveys those epigenetic modifications are crucial regulators of gene expression in the brain.

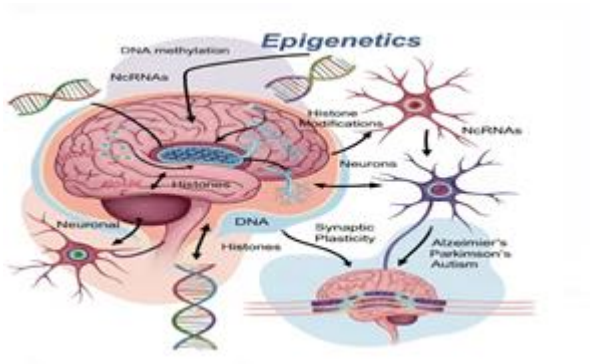

Figure 1

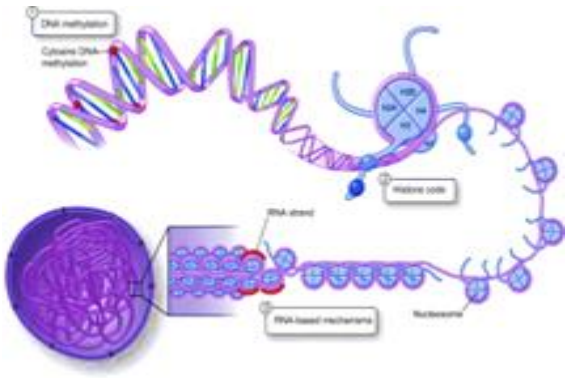

Figure 2

Fundamental Epigenetic Mechanisms: The Molecular Orchestra of Gene Expression

Epigenetic mechanisms control gene expression by modifying DNA structure and chromatin accessibility. DNA is wrapped around histone proteins (H2A, H2B, H3, H4) forming nucleosomes, with histone H1 stabilizing the structure., Linker histone H1 stabilizes these structures, with multiple nucleosomes making up chromatin. Chromatin can be in an inactive form (heterochromatin) or active form (euchromatin), affecting transcription. Studying epigenetic mechanisms has enabled the mapping of key epigenetic marks that regulate gene expression [10-11]. Figure 2 shows the schematic representation of fundamental mechanisms.

1. **DNA Methylation: The Molecular Dimmer Switch**

DNA methylation is a common chemical modification where methyl groups are added to DNA, mainly at CpG islands found in regions like telomeres and inactive X chromosomes. It plays key roles in genomic imprinting, gene regulation, genome stability, and transposon silencing. Various types exist, including 5mC—the most studied form. DNA methylation is

carried out by DNA methyltransferases (DNMTs), which are divided into two groups based on their function DNA methyltransferase 1 (DNMT1) maintains DNA methylation by adding methyl groups to existing patterns during DNA replication, while DNMT3a and DNMT3b perform de novo methylation by adding new methyl groups to previously unmethylated cytosines [12-13]

The chemistry of DNA methylation

In mammals, DNA methylation mainly occurs by adding a methyl group to the fifth carbon of cytosine, forming 5-methylcytosine (5mC), known as the "fifth base" of DNA. This methylation happens primarily at CpG dinucleotides, often clustered in CpG islands located in gene promoter regions which are the regulatory sequences that influence a gene's transcription. Unmethylated CpG islands are associated with active gene expression, while methylation usually silences genes. About 70% of human gene promoters are within CpG islands [14]. Figure 3-4 shows the molecular process of DNA methylation, a key epigenetic mechanism and CpG island.

Impact on Gene Expression

The presence of methyl groups on DNA primarily leads to gene silencing or repression through several mechanisms:

- Blocking transcription factors from binding DNA, preventing gene activation.
- Recruiting methyl-CpG binding proteins (e.g., MeCP2) that attract complexes to condense chromatin.
- Promoting chromatin remodelling by recruiting enzymes like histone deacetylases (HDACs) and histone methyltransferases (HMTs) which create a compact, inactive chromatin state (heterochromatin).

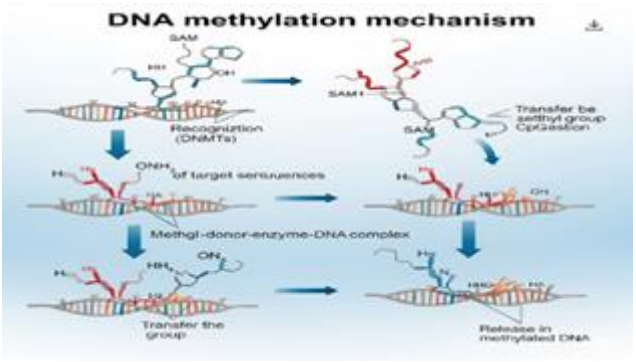

Figure 3

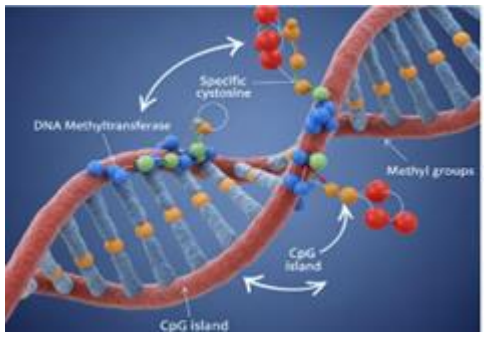

Figure 4

2. The Chromatin Choreographers

DNA is tightly wrapped around histone protein complexes called nucleosomes, which are further compacted into chromatin. The chromatin's structure controls DNA accessibility to transcription machinery. Histone modifications—chemical tags on histone tails—regulate chromatin structure and gene accessibility [15]

Histone Modification

Histone modifications are another crucial epigenetic mechanism that influences gene expression. DNA is tightly wrapped around positively charged histone octamers, compacting it and preventing transcription. For gene expression to occur, DNA must unwind, which happens when histones undergo modifications like acetylation or methylation on lysine residues. These acetylation reactions are catalysed by enzymes referred to as histone acetyltransferases (HATs) (which acetylates the lysine amino acid by transferring a molecule of acetyl group from acetyl-COA to convert the lysine into ε-N-acetyl lysine)[16].

Aberrant histone modifications are linked to cancer development and clinical outcomes. Over 20 histone acetyltransferases (HATs) have been identified and grouped into five families: GNAT1, MYST, TAFII250, P300/CBP, and nuclear receptor coactivators like ACTR. Histone deacetylases (HDACs) play a key role in removing acetyl groups from lysine residues in histones through hydrolysis and are classified into four classes (I–IV). Disruptions in HDAC activity and other histone modifications like phosphorylation, methylation, acetylation, and ubiquitination can alter gene expression and contribute to cancer development [17].

Histone methylation primarily takes place on the sides of lysine and arginine residues. Unlike acetylation, this process does not change the charge of the histone protein [18] It involves the addition of one, two, or three methyl groups from S-adenosyl-L-methionine to the lysine or arginine sites of histone proteins, facilitated by histone methyltransferases (HMTs). These modifications play a crucial role in regulating DNA methylation, affecting transcription either by suppressing or promoting gene expression. Histone acetylation adds acetyl groups (-COCH) and affects chromatin structure, transcription, cell cycle, differentiation, and DNA replication. Unlike DNA methylation, histone modifications can activate or inhibit genes, with specific patterns defining cell identity—for example, embryonic stem cells have both active (H3K4me3) and repressive (H3K27me3) marks. Histone phosphorylation, controlled by kinases and phosphatases, plays roles in DNA repair, chromatin compaction, and transcription often interacting with other histone modifications [19-20].

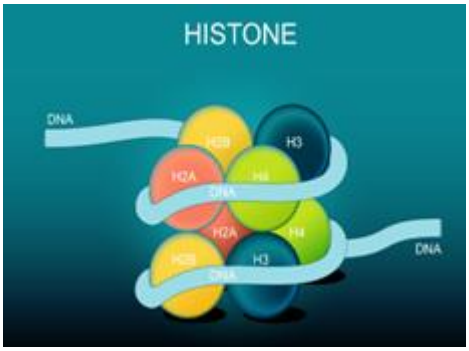

Figure 5

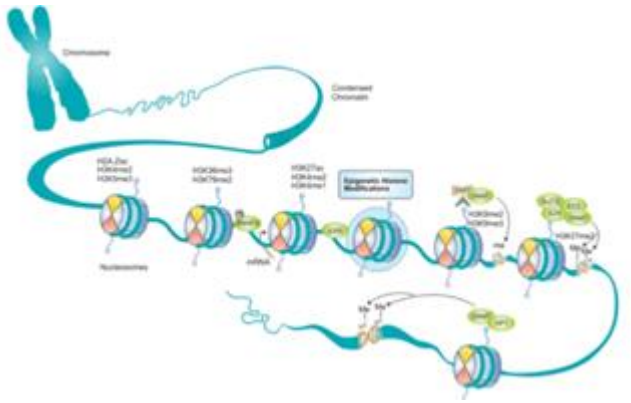

Figure 6

3. Non-coding RNAs (ncRNAs): The Regulatory Maestros

Non-coding RNAs (ncRNAs) regulate gene expression without encoding proteins. MicroRNAs (miRNAs), about 20–25 nucleotides long, bind to 3′ untranslated regions (UTRs) of target mRNAs to degrade them or inhibit translation, thereby influencing multiple genes. Long non-coding RNAs (lncRNAs), over 200 nucleotides, act as scaffolds, decoys, or guides for chromatin-modifying enzymes, affecting DNA methylation and histone modifications. Abundant in the brain, ncRNAs are essential for neurodevelopment, synaptic plasticity, and neuronal function. Dysregulation of miRNAs and lncRNAs is associated with neurological disorders [21]. The dysregulation of certain miRNAs and lncRNAs has been linked to a range of neurological disorders. Figure 5-7 shows the image that illustrates histones and modification, RNA-based mechanisms – Noncoding RNA-mediated modulation of gene expression and histones.

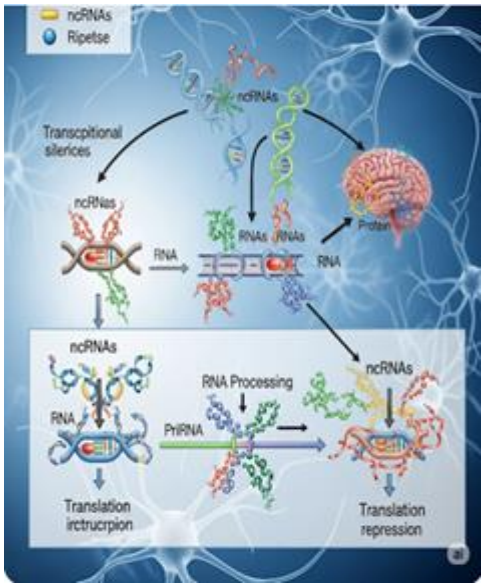

Figure 7

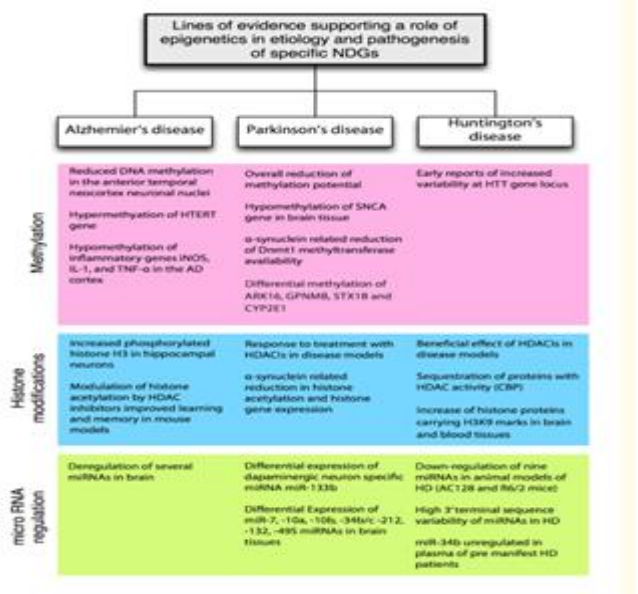

Figure 8

Epigenetics and its role in neurodegeneration

Proper differentiation and function of the central nervous system cells are subject to significant influence of a variety of epigenetic modifications. Epigenetic modifications play a crucial role in the development and function of the central nervous system. Dynamic changes in DNA methylation and histone modifications occur from early brain development through ageing[22].

Monogenic neurodevelopmental disorders, such as Rett syndrome caused by MECP2 coding for methyl-CpG-binding protein mutation highlight the link between epigenetic dysfunction and neurological disease. Similar mutations in genes regulating methylation and histone modification have also been associated with neurological conditions across age groups. Only recently has the role of epigenetic alterations been explored in common neurodegenerative diseases such as Huntington's, Parkinson's, and Alzheimer's disease, where distinct epigenetic disruptions have been identified and shown in Figure 8.

Alzheimer's Disease (AD): The Forgotten Memories

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder that primarily affects the hippocampus, temporal, and frontal lobes, resulting in memory loss, cognitive decline, mood disturbances, and reduced functional ability. Pathologically, AD is characterized by the accumulation of amyloid-beta (Aβ) plaques and tau-based neurofibrillary tangles (23)

Epigenetic Mechanisms in Alzheimer's Disease (AD):

- **DNA methylation**: Abnormal DNA methylation is a central epigenetic feature in AD. Research shows altered methylation in several AD-related genes, such as **TMEM59** (involved in APP processing) and **HTERT** (related to telomerase activity) (24). Hypomethylation of pro-inflammatory genes, including *iNOS*, *IL-1*, and *TNF-α*, may enhance neuroinflammation and Aβ accumulation. Age-related methylation changes in *APP* and *BRCA1* further exacerbate disease pathology. Intronic hypomethylation of **TREM2** has been associated with increased expression and heightened microglial activation, suggesting its potential as a biomarker (25) Epigenetic clock studies also indicate accelerated biological aging in individuals with AD (26).
- **Histone modifications:** Disruptions in histone acetylation—a modification that facilitates gene expression—are commonly observed in AD. HDAC inhibitors, such as valproic acid and phenylbutyrate, have been shown to reduce Aβ levels, decrease tau pathology, and improve cognitive function in animal models (27). Dysregulated acetylation of histones H2B, H3, and H4 affects key genes like **BDNF** and **Tau**, contributing to inflammation and neuronal dysfunction. Although histone methylation changes are also evident in AD, their specific effects on gene expression remain complex and context-dependent.
- **Non-coding RNAs (ncRNAs):** MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are significantly dysregulated in AD, impacting Aβ production, tau phosphorylation, and neuronal integrity. For instance, **miR-346** promotes Aβ accumulation by targeting **APP**, whereas **miR-107** reduces Aβ by downregulating **BACE1** (28). LncRNAs like **BACE1-AS** enhance Aβ production and may serve as accessible biomarkers, given their presence in blood and cerebrospinal fluid (CSF) (29).

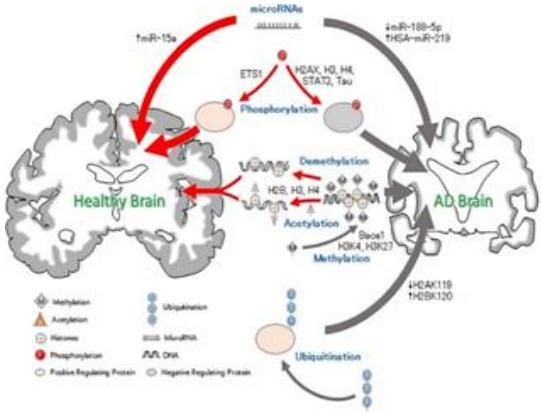

Figure 9

Parkinson's Disease (PD): The Shaking Truth

Parkinson's disease (PD) is a progressive neurodegenerative disorder predominantly affecting motor function. Its primary symptoms include resting tremor, muscle rigidity, and bradykinesia (slowness of movement), which stem from the degeneration of dopaminergic neurons in the substantia nigra pars compacta (30). While approximately 10–15% of PD cases have a clear familial link to mutations in genes such as *SNCA* (alpha-synuclein), *LRRK2*, and *PRKN*, the majority of PD cases are idiopathic, suggesting that environmental exposures in combination with genetic susceptibility play a critical role in disease development (31).

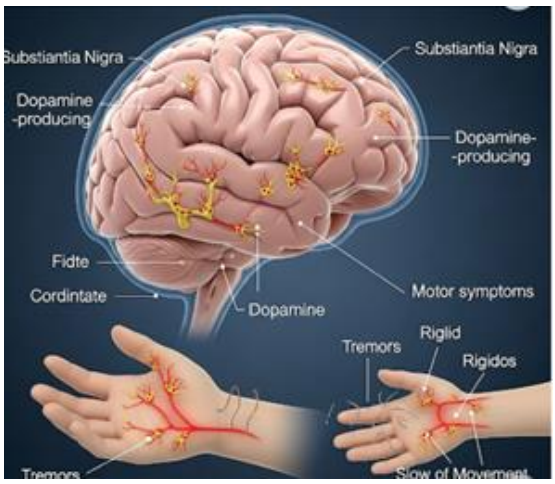

Figure 10

Epigenetic Mechanisms in Parkinson's Disease

1. DNA methylation: DNA methylation plays a central role in regulating gene expression in neurons. Altered methylation patterns have been observed in PD, particularly in genes involved in mitochondrial function, oxidative stress, and neuroinflammation. For instance, hypomethylation of the *SNCA* gene promoter is associated with increased alpha-synuclein expression, a key pathological hallmark in PD (32). Genome-wide methylation studies have revealed epigenetic dysregulation in multiple pathways relevant to PD pathogenesis, including immune signalling and synaptic transmission (33).

2. Histone Modifications: Histone acetylation and methylation patterns are also disrupted in PD. Reduced histone acetylation has been linked to transcriptional repression of neuroprotective genes. Pharmacological inhibition of histone deacetylases (HDACs) has shown neuroprotective effects in PD models, indicating a potential therapeutic target (34). Moreover, mutations in genes affecting chromatin structure, such as *LRRK2*, may directly influence histone modification patterns, further linking epigenetic regulation with disease risk

3. Non-coding RNAs (ncRNAs): MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression post-transcriptionally. Several miRNAs, including miR-34b/c and miR-7, are dysregulated in PD and modulate genes critical for mitochondrial function and oxidative stress response (35). Long non-coding RNAs (lncRNAs), such as *HOTAIR* and *NEAT1*, have

also been implicated in PD pathophysiology through the modulation of neuroinflammatory and apoptotic pathways (36).

4. Gene-Environment Interactions: Epigenetics serves as a key mediator between environmental exposures and genetic susceptibility in Parkinson's disease (PD). Exposure to pesticides, heavy metals, and air pollution has been linked to increased PD risk, potentially through oxidative stress and mitochondrial dysfunction—both of which can lead to epigenetic alterations (37). These environmental triggers may modify gene expression without altering the underlying DNA sequence, thereby influencing the onset and progression of disease.:

Huntington's Disease (HD): The Unravelling Code

Huntington's disease is a devastating inherited neurodegenerative disorder caused by an abnormal expansion of CAG trinucleotide repeats in the HTT gene. While the genetic cause is clear, the mutant huntingtin protein exerts its toxic effects through a variety of mechanisms, with epigenetic dysregulation being a central player.

Histone Deacetylation and Chromatin Remodelling:

The mutant huntingtin protein can directly interact with and sequester HATs, leading to a global reduction in histone acetylation. This widespread hypoacetylation results in a compacted, repressive chromatin state, leading to the silencing of critical genes important for neuronal survival, synaptic function, and overall brain health (e.g., *BDNF*, *CREB*) [38].

- DNA Methylation Changes: Altered DNA methylation patterns have been observed in HD, affecting the expression of various neuronal genes, further contributing to the complex pathology [39].
- **Non-coding RNAs:** Dysregulation of long non-coding RNAs (lncRNAs) has been extensively observed in HD brains, influencing transcriptional regulation and contributing to neurodegeneration [40]

Autism Spectrum Disorder (ASD): The Spectrum of Connection

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition marked by difficulties in social interaction and communication, along with restricted and repetitive behaviors. While genetics are important, high—but not absolute—concordance in identical twins and environmental influences suggest a strong role for epigenetics [41]

Key Epigenetic Mechanisms in ASD

1. DNA methylation: Altered methylation patterns affect genes vital to brain development, including UBE3A and ASH1L. Differences in methylation between ASD-discordant identical twins support an epigenetic contribution. While some studies show inconsistent results (e.g., ROR-α), others, like reduced methylation in PRRT1 in ASD brains, offer promising biomarkers.[42]

2. Histone Modifications: Abnormal histone acetylation affects genes linked to synaptic function and neuronal excitability. A shared histone acetylation pattern was found in 68% of

individuals across ASD subtypes. Mutations in chromatin-related genes (e.g., CHD8, ADNP) can disrupt gene expression during brain development.

3. MicroRNA (miRNA): Dysregulation in several miRNAs (e.g., miR-128, miR-146a, miR-132-5p) are disrupted in ASD. These regulate genes involved in neurogenesis, synaptic plasticity, and neuronal signalling—key processes often altered in ASD [43].

4. Gene-Environment Interactions: Environmental exposures can trigger or modify gene expression via epigenetic changes, influencing ASD risk and severity even in those with genetic predispositions [44].

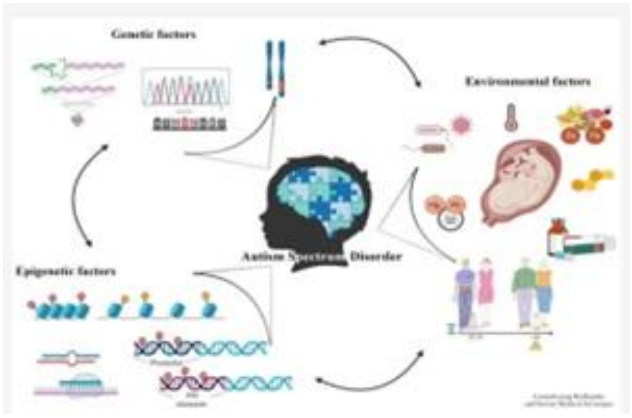

Figure 11

Schizophrenia: The Fragmented Reality

Schizophrenia, commonly referred to as "The Fragmented Reality," is a serious and long-lasting mental illness marked by significant disturbances in thinking, perception, feelings, and behaviour [45] Epigenetics plays a vital role in schizophrenia, connecting genetic and environmental factors in disease development. These heritable changes in gene expression are key to understanding the disorder's complex causes [46] Key epigenetic mechanisms implicated in schizophrenia include:

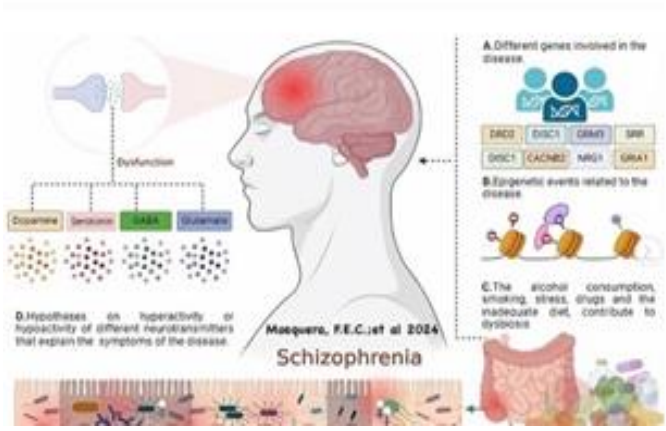

Figure 12

Key Epigenetic Mechanisms in Schizophrenia:

1. **DNA Methylation:** Schizophrenia involves abnormal DNA methylation at CpG sites in genes tied to neurodevelopment and neurotransmission. Genes like GAD1 and RELN show

hypermethylation, reducing expression and disrupting GABA function. Methionine may worsen symptoms by boosting methylation through increased SAM. Environmental factors like childhood trauma can also alter gene methylation. Reduced reelin in neurons is linked to increased promoter methylation and decreased histone acetylation. DNMT and HDAC inhibitors can restore GAD67 and RELN expression in models.

2. **Histone Modifications**: Schizophrenia-related genes are strongly affected by epigenetic changes. Altered histone acetylation and methylation impact neuronal and myelination genes. Increased HDAC1 expression correlates with reduced GAD67 and decreased H3K4 methylation at its promoter, especially in females. Although HDAC1 knockdown impairs learning and causes cell death, elevated HDAC1 is consistently seen in schizophrenia, suggesting a key role in the disease [47]
3. **MicroRNAs (miRNAs):** Dysregulated miRNAs are commonly found in the brains and fluids of people with schizophrenia. [48]. In the prefrontal cortex, miR-130b, miR-92a, miR-495, and miR-134 are linked to disrupted neurodevelopment, while elevated levels of miR-328, miR-17-5p, miR-134, miR-652, miR-382, and miR-107 appear in post-mortem brain tissue of patients. Figure 8-12 illustrates Alzheimer's, disease, Parkinson's disease autism and schizophrenia.

Innovative Biomarkers & Therapies:

- Early Detection: Epigenetic changes can signal neurological disorders like Alzheimer's and Parkinson's early for timely treatment.
- Subtyping & Prognosis: Unique epigenetic patterns help classify and predict disease progression.
- Non-invasive Testing: Biomarkers in blood or CSF enable easy diagnosis and monitoring.
- Treatment Monitoring: Epigenetic markers track therapy response for personalized care.

Therapeutic Strategies:

- Targeting enzymes (DNMTs, HDACs) shows promise in reducing disease pathology.
- Modulating ncRNAs (e.g., miR-107) can correct harmful gene regulation.
- Reversing epigenetic changes restores beneficial gene expression.

Addressing gene-environment effects supports targeted lifestyle or therapeutic interventions.

Conclusion:

Epigenetics serves as a crucial, yet often unseen, force in shaping both healthy and disordered brain function. By influencing gene activity without changing the underlying DNA, epigenetic processes offer a valuable understanding of how neurological disorders begin, evolve, and differ between individuals. Growing knowledge in this field opens the door to earlier diagnoses, more accurate classification of diseases, and personalized treatment strategies. As scientists continue to explore the intricate relationships between genes, the environment, and

epigenetic changes, new diagnostic tools and therapies are emerging that could significantly change the landscape of neurological care. Unlocking the potential of epigenetics may lead to groundbreaking advances in neuroscience and bring new hope to those living with neurological conditions.

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BRIDGING THE FISH SEED GAP IN ASSAM: A PATHWAY TO FOOD SECURITY AND SUSTAINABLE AQUACULTURE

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Abstract:

Fisheries and aquaculture play pivotal roles in global food production, contributing significantly to agricultural exports, food security, and the livelihoods of millions worldwide. Among these, freshwater fish farming stands out as one of the fastest-growing sectors. In India, the state of Assam has emerged as a critical hub for fish seed production, supported by a network of hatcheries. In 2022–23, Assam produced approximately 4.43 lakh tonnes of fish, underscoring its vital role in meeting local consumption needs. Despite a high per capita fish consumption rate of 13.06 kg annually, Assam continues to face challenges in fulfilling its fish demand domestically, leading to substantial daily imports from other states. This study aims to assess the existing gap between fish seed demand and supply in Assam. An analysis of recent data indicates a positive trend in fish seed production, driven by increasing aquaculture activities. However, projections from 2023 to 2030 reveal a growing disparity between fish supply and demand. These findings highlight the urgency of adopting sustainable aquaculture practices and implementing strategic interventions to bridge the production-consumption gap. Ensuring long-term food security and economic resilience in Assam's fisheries sector will require coordinated efforts in planning, investment, and policy support. This study offers a comprehensive evaluation of current trends and future projections, serving as a foundation for evidence-based decision-making in the sector.

Keywords: Fish, Assam, Supply, Demand, Gap

Introduction:

Fisheries and aquaculture are integral components of global food production, contributing significantly to agricultural exports, ensuring food security, and supporting the livelihoods of millions worldwide. In recent years, freshwater fish farming has emerged as one of the fastest-growing sectors in global food production. The industry directly and indirectly employs millions, underscoring its substantial role in the global economy. Currently, aquaculture accounts for over 52% of the fish consumed by humans globally (Naylor *et al.*, 2021), reflecting its increasing importance in meeting the world's nutritional demands.

In India, fisheries play a crucial role, employing over 14 million people (Department of Fisheries, 2020). India is the 3rd largest fish producing and 2nd largest aquaculture producing nation in the world (PIB,2023). The fisheries sector is prominent in India's development

programmes due to its vital contribution to employment, food security and foreign exchange earnings. Indian fisheries contribute INR 1.75 lakh billion (1.03%) in gross value added to the country's economy every year (Rajeev and Bhandarkar 2022). In 2019, the estimated per capita fish consumption was about 7.8 kg (FAO,2024). Export earnings from fish and fishery products were about USD 7.9 billion in 2022 (MPEDA,2024). India has important freshwater resources that can be used for fish production. India's fresh water resources consist of 195 210 km of rivers and canals, 2.9 million hectares of minor and major reservoirs, 2.4 million hectares of ponds and lakes, and about 0.8 million hectares of flood plain wetlands and water bodies. The country's major and minor rivers along with their tributaries, minor streams, creeks and all other related systems have an estimated combined length of 45 000 km. These along with the numerous man-made canals have a combined length of 0.17 million km (Economic Survey, 2023-24). Fish production in India has increased from 24.42 lakh tonnes in the year 1980-81 to 175.45 lakh tonnes in 2022-23. The average annual fish production growth rate is 7.98 % (Statistical Handbook Assam, 2023-24).

Assam, situated in India's northeast, stands out as a leader in regional fish production, contributing approximately 77 percent of the total fish output in the northeastern region. With its favorable climate and abundant water resources, Assam has also become a significant hub for fish seed production, bolstered by the establishment of numerous hatcheries. This paper explores the socio-economic impact of fisheries in Assam, focusing on its role in regional development, resource management practices, and the expansion of fish seed production through hatchery operations. Assam produces around 85 percent of all fishery production in NE states, around 4.43 lakh tonnes in 2022-23.

The Fishery sector has been emerging as one of the most potential sectors in the state with immense natural resources in the form of ponds, derelict water bodies and beels/ flood plain wetlands etc. covering 2.59 lakh hectares. Besides this, the two major river systems i.e. the Brahmaputra; Barak and its tributaries which spread up to 11304 KM as a riverine fishery. Thus, the entire water spread areas have immense opportunities to boost up the fish production and provide ample employment opportunities as well as livelihood to the society and nutrition to the individual’s health.

The state has achieved fish production level of 4.43 lakh MT in 2022-23 with an increase of per capita consumption to 13.06 Kg from 12.18 Kg in the last year 2021-22. As more than 90% of the state population is fish consumers, there is a huge demand for fish throughout the year and there is an ever-increasing scope of harnessing the fish production potential to the optimum level in a sustainable manner.

Fish is a staple in the diet of many residents of Assam, with the average person consuming about 13 kilograms of fish per year. Despite this high consumption rate, the state faces significant challenges in meeting its fish production needs internally. In the fiscal year

2020-21, Assam produced 3.93 lakh metric tons (MT) of fish. This figure increased to approximately 4.32 lakh MT in 2021-22, indicating a positive trend in local fish production. However, this increase has not been sufficient to meet the state's demand. As a result, Assam imports between 12 to 15 MT of fish daily from other states to satisfy its daily fish consumption requirements. This reliance on imports highlights a critical gap between local production and demand. The year 2022-23 saw a continuation of this trend, with a small rise in production. However, the demand for fish also grew correspondingly, maintaining the pressure on the state's fish supply system. To bridge this gap and achieve self-sufficiency by 2026, a comprehensive plan and significant investment are required. During the Chintan Shivir held in Kaziranga in 2022, the state's fishery department estimated that ₹3,997 crore would be needed to attain this goal. This investment would cover various aspects of the fish production supply chain, from infrastructure to operational costs. Currently, Assam has 431 hatcheries. To meet the state's fish production needs, an additional 169 hatcheries are required, bringing the total to 600. These hatcheries are essential for producing fish seeds, which are critical for increasing fish production. The current fish seed production in Assam is 4,000 MT. However, to achieve self-sufficiency, the state needs to produce 2.5 lakh MT of fish seeds.

The current study aims to analyze the gap between the demand and supply of fish seeds within the state. Its objective is i. Estimate the projected production of fish seed in Assam; ii. Estimate the demand supply projection and gap analysis of fish and fish seed in Assam

Methodology:

Secondary data was obtained from a variety of published sources regarding the fishery production and related variables in Assam. Data from the 68th round of the National Sample Survey (NSS) was assembled to provide information on consumer spending on fish among both rural and urban consumers. Population data for Assam from the 2001 and 2011 census were obtained, and projections were estimated up to the year 2030

The expenditure elasticity for rural and urban areas was calculated separately, and then final expenditure elasticity was calculated by proportionate addition of urban and rural elasticity. The elasticity obtained was used for demand projections.

$$Q_i = ae_{it}^{b}$$

$$\ln Q_i = \ln a + b \ln e_{it}$$

Q_i = quantity of commodity consumed by i^{th} household

e_{it} = total consumption expenditure of the i^{th} household at time period t

a = intercept, and b = coefficient

The total per capita projected demand up to the year 2030 has been obtained by using base period consumption and expenditure elasticity and growth rate of per capita NSDP. The aggregate demand of the state was estimated by multiplying projected demand by the projected

adult population of Assam up to 2030. The total production of fish was projected up to the year 2030, which would also serve as the projected supply of the same.

Population and supply projection was made by using the following formula

$P_t = P_0 (1+ r)^t$

P_t = Population/ supply to be estimated

P_0 = Population /supply of the base year

r = Population growth rate/supply growth rate, t = Time period

Estimation of Demand

The future demand for fish was estimated by the following formula,

$Q_{it} = P_t\{q_{io}(1+g)^{ne}{}_{it}\}$

Q_{it}= Quantity demanded of the commodity

P = Projected adult population

Q_{io}= Base period consumption of the commodity

g= Growth rate of per capita NSDP

n= number of years

e_{it}= expenditure elasticity

Autoregressive integrated moving average (ARIMA) model

The model was first introduced by Box and Jenkins (1970) [15] for the purpose of analyzing and forecasting of univariate time series data. The ARIMA model is characterized by the notation ARIMA (p, d, q) where p, d and q denote the orders of auto-regression, integration (differentiation) and moving average, respectively. The auto-regressive process of the order (p) is computed as:

$$Y_t = c + \varphi_1 Y_{(t-1)} + \varphi_2 Y_{(t-2)} + \ldots\ldots\ldots\ldots\ldots \varphi_p Y_{(t-p)} + \varepsilon_t$$

Moving average process of order (q) is computed as:

$$Y_t = \mu - \theta_1 \varepsilon_{(t-1)} + \theta_2 \varepsilon_{(t-2)} + \ldots\ldots\ldots\ldots\ldots \theta_q \varepsilon_{(t-q)} + \varepsilon_t$$

And the general form of ARIMA model of order (p, d, q) is:

$$Y_t = \mu + \varphi_1 Y_{(t-1)} + \varphi_2 Y_{(t-2)} + \ldots \Phi_p Y_{(t-p)} + \varepsilon_t - \theta_1 \varepsilon_{(t-1)} - \theta_2 \varepsilon_{(t-2)} - \ldots \theta_q \varepsilon_{(t-q)} + \varepsilon_t$$

Where, Y_t = The value of the time series at time t; c = constant; $\varphi_1, \varphi_2, \ldots \varphi_p$ = Parameters of component; the Autoregressive; ε_{t-1}, ε_{t-2}... ε_{t-q} = Lagged forecast errors of the Moving Average component; $\theta_1, \theta_2, \ldots \theta q$ = Parameters of the Moving Average component; ε_t = White noise error term at time t. The whole methodology of Box-Jenkins process which involves parameter estimation, diagnostic checking and forecasting was done in R-studio v4.4.3 software using required plug ins.

Analysis of ACF & PACF plots

Autocorrelation (ACF) and Partial Autocorrelation (PACF) plots are the graphical tools which are used in time series analysis. ACF shows the correlations between a time series and its

lags while PACF displays partial correlations for intermediate lags in the time series. Significant values at certain lags indicates patterns and dependencies in the dataset.

Stationarity test

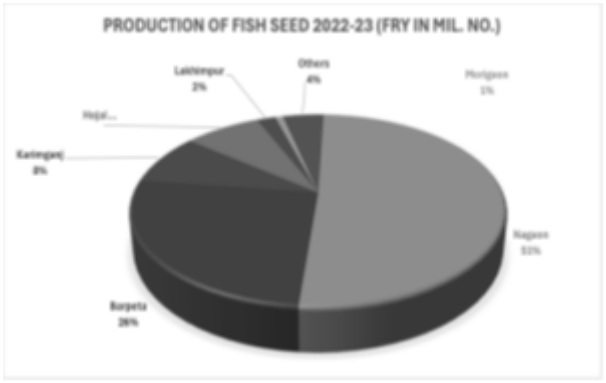
The statistical test known as Augmented Dickey-Fuller (ADF) test is commonly used for determining stationarity in a time series dataset. The null hypothesis of the ADF test is that the time series possesses a unit root and is non-stationary. If the test statistic is significantly negative and falls below critical values from a table, the null hypothesis is rejected, suggesting that the time series is stationary. On the other hand, failure to reject the null hypothesis indicates that the time series is non-stationary.

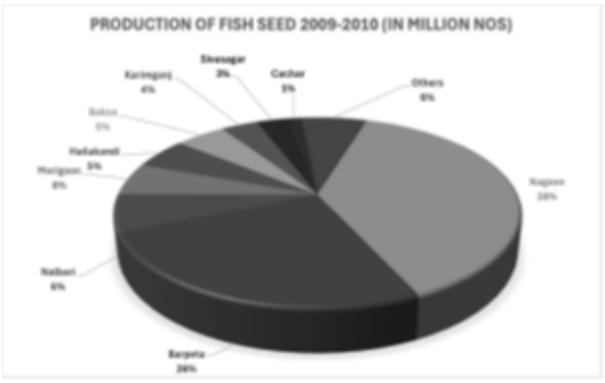
Ljung-Box test

It is a common test used in time series and forecasting for identifying significant autocorrelation in the residual of a fitted ARIMA model. The test helps ensure that the model adequately captures the autocorrelation structure in the data, and if there are any remaining systematic patterns in the residuals that need to be addressed. The test is designed to check the null hypothesis that there is no autocorrelation in the time series up to a specified lag. In our present lag order of 5 was considered for validating the best fitted ARIMA model.

Result and Discussion:

The yearly production of fish seeds in millions from 1986–1987 to 2022–2023, shows both a noticeable increase tendency and considerable volatility over the years. Production levels have varied greatly in the past, which may have been caused by shifting environmental factors and changing aquaculture techniques.





Production has been steadily increasing since the mid-2000s, and this trend continued until the most recent reported years, 2021–2022 and 2022–2023, when production peaked at 18,219 million and 20,843 million, respectively. This spike points to increased market demand for fish stocks, better management techniques, and technological developments in aquaculture. In order to satisfy future demands while maintaining environmental and economic sustainability, sustainable practices and strategic planning are essential. The data highlights the sector's resilience and growth potential.

When the output of fish seed is compared between 2009–2010 and 2022–2023, Assam's districts exhibit notable variations. Nagaon took the lead in 2009–2010 with 38%, followed by Barpeta with 26%. With 'Others' contributing 8% and Cachar contributing 1%, Nalbari, Morigaon, Baksa, Hailakandi, Karimganj, and Sivasagar contributed lesser portions. Nagaon's stake rose to 51% by 2022–2023 while Barpeta's stayed at 26%. Karimganj's share increased to 8%, while Hojai—who was not previously identified—showed up with 6%. Lakhimpur secured a 2% percentage of the vote. The notable 2009–2010 contributions from Nalbari, Morigaon, and Baksa are no longer mentioned separately, suggesting a potential reduction or consolidation into the 'Others' category, which now stands at 4%. Morigaon's percentage dropped sharply from 5% to 1%. These modifications imply that output is concentrated in a smaller number of areas, with Nagaon and Barpeta as key hubs.

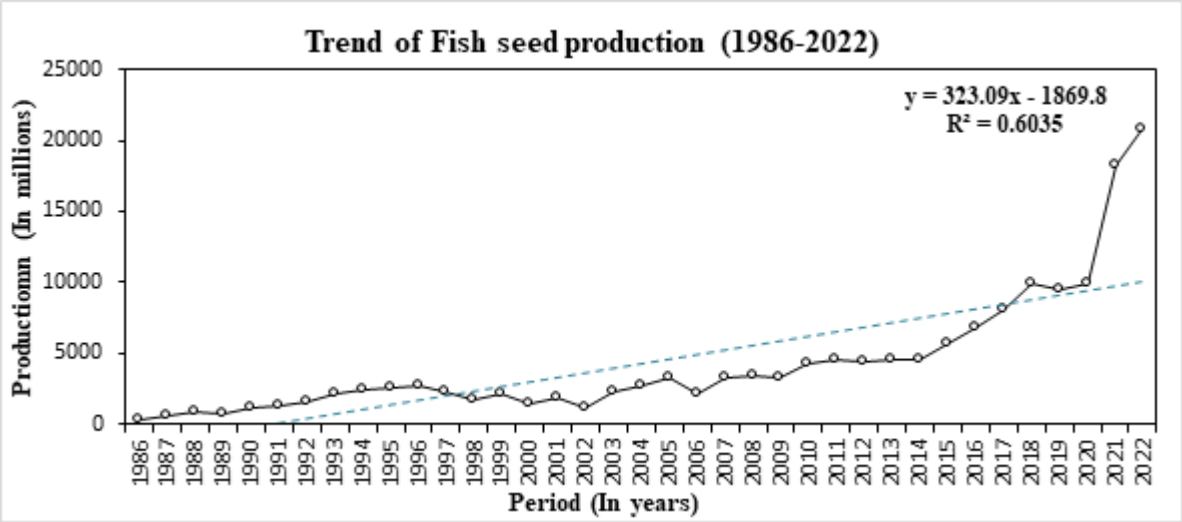


Table 1: Supply-demand projection and gap analysis of fish in Assam

Year	Supply (Lakh Tonnes)	Demand (Lakh Tonnes)	Gap (Lakh Tonnes)
2023	4.83	10.24	-5.41
2024	5.09	11.03	-5.94
2025	5.37	11.87	-6.50
2026	5.66	12.77	-7.11
2027	5.97	13.75	-7.78
2028	6.29	14.79	-8.5
2029	6.63	15.91	-9.28
2030	6.98	17.09	-10.11

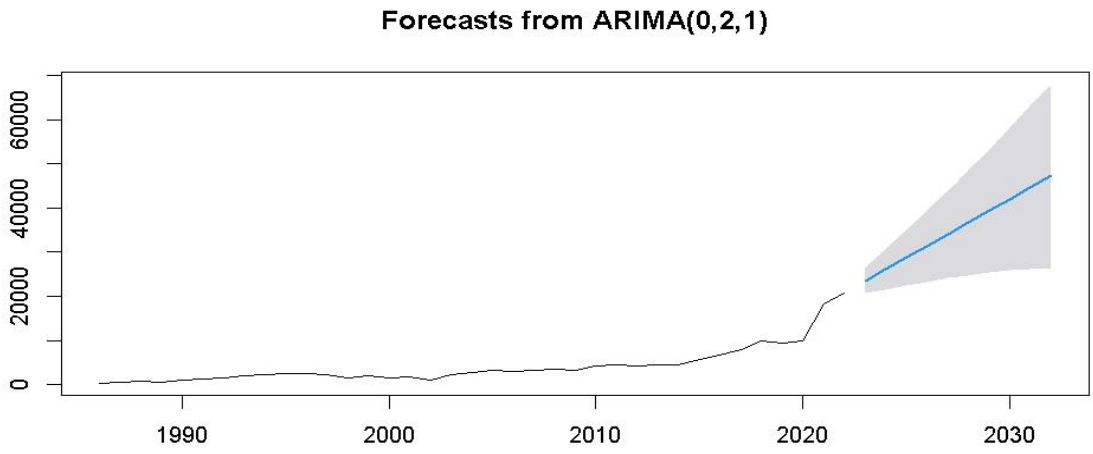
The table 1 presents the projected supply and demand trends in lakh tons for the years 2023 to 2030. The supply figures represent the anticipated availability of the product, while the demand figures denote the expected consumption. Each year, the gap between supply and

demand widens, indicating a growing deficit over time. By 2030, the supply is predicted to be 6.98 lakh tons, while the demand is forecasted to reach 17.09 lakh tons, resulting in a deficit of 10.11 lakh tons. These projections highlight the critical need for strategic planning to address the increasing supply-demand imbalance and ensure adequate product availability in the coming years.

Table 2: Forecasted Fish seed production for next 7 years (2023 to 2030)

Year	Fish seed production (Nos. in million)	Lower limit of forecasted production	Higher limit of forecasted production
2023-24	23486.47	20663.64	26309.29
2024-25	26129.93	21576.63	30683.23
2025-26	28773.40	22495.10	35051.70
2026-27	31416.86	23345.52	39488.20
2027-28	34060.33	24108.16	44012.49
2028-29	36703.79	24777.40	48630.19
2029-30	39347.26	25352.59	53341.93
2030-31	41990.72	25835.00	58146.41

The table forecasts fish seed production from 2023-24 to 2030-31, showing estimates in millions for each year. It includes a range from lower to upper limits, considering factors like environment, technology, and economics. In 2023-24, production is expected around 23,486.47 million fish seeds, possibly ranging from 20,663.64 million to 26,309.29 million. In 2024-25, production is forecasted to increase to 26,129.93 million fish seeds, with a potential range of 21,576.63 million to 30,683.23 million. This upward trend continues each year, aiming to meet growing demands in aquaculture. These insights help policymakers and industry leaders plan for sustainable growth and secure a steady fish seed supply.



Production Forecasting using ARIMA (0,2,1) model

At the rate at which the fish seed production is increasing it is estimated to reduce the gap and create a sureplus for fish seed in the state. This may be attributed to the fact that there have been a rise in hatcheries in the state and other intiatives such as

Supporting Fish Farmers and Conservation: The Fishery Department of Assam has been actively supporting fish farmers through various initiatives. One of the major efforts is the State Owned Priority Development (SOPD) schemes, focusing on enhancing fish and fish seed production. This includes expanding fish culture areas, providing essential inputs, and improving infrastructure to boost productivity. A notable project under SOPD is the Gene Bank Scientific Conservation Programme for Indigenous Fish (SCoPIF), which aims to safeguard the diversity of local fish species and develop new propagation technologies.

Infrastructure Development and Financial Support: Through the Rural Infrastructure Development Fund (RIDF), the department has implemented crucial projects such as refrigerated fish carrying vehicles and mini fish feed plants. These initiatives aim to improve transportation and feed availability, crucial for maintaining fish quality and supporting rural livelihoods.

Central Government Initiatives: Under the Pradhan Mantri Matsya Sampada Yojana (PMMSY), there's a strong focus on modernizing fishery infrastructure and providing comprehensive support to fish farmers. This includes establishing hatcheries, constructing new ponds, and offering livelihood support programs. Additionally, the Group Accident Insurance Scheme (GAIS) ensures that fishermen and fish farmers are covered for accidents, providing a safety net for those involved in risky occupations.

Community Engagement and Future Plans: The Fishery Mission Society under CMSGUY is dedicated to doubling fish farmers' income through sustainable practices and creating new water bodies. Projects like APART further enhance fish productivity through innovative farming techniques and support systems. Looking ahead to 2023-24, the department plans to expand infrastructure, continue conservation efforts, and introduce new technologies to further improve fish farming across the region.

Conclusion:

The study reveals a growing disparity in fish production within the state of Assam. This gap highlights the need for strategic interventions to bridge the shortfall between fish supply and demand. A key factor in addressing this challenge lies in the continuous increase of fish seed production. By scaling up fish seed production, supported by government initiatives, there is a potential to mitigate the widening gap in fish production. Government efforts aimed at enhancing fish seed production are crucial in this endeavour. These initiatives encompass various schemes and policies designed to boost the availability of high-quality fish seeds, which are essential for sustaining and expanding fish farming operations across Assam. As fish seed production rises, it

is anticipated to play a pivotal role in narrowing the gap between fish production levels and the increasing demand within the state.

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BRIDGING THE BIODIVERSITY GAP: ADDRESSING DATA DISCREPANCIES IN MODERN ZOOLOGICAL SURVEYS

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Abstract:

Modern biodiversity research increasingly relies on digital observation records, often sourced from citizen science platforms and field surveys. While this trend has democratized data collection, recent studies reveal significant gaps and biases in these datasets, threatening the integrity of zoological research. This chapter discusses recent findings highlighting the discrepancies between observational and specimen-based biodiversity data. The analysis emphasizes the implications for conservation science and suggests integrated approaches to bridge the data gap and improve the reliability of zoological surveys globally.

Keywords: Biodiversity, Zoological Surveys, Observational Data, Specimen Records, Citizen Science, Data Bias

Introduction:

The twenty-first century has witnessed an unprecedented transformation in the way biodiversity data is collected and shared. With the proliferation of digital technologies, mobile applications, and global citizen science platforms, biodiversity monitoring has become more accessible than ever before. These advances have empowered thousands of amateur naturalists, bird watchers, photographers, and environmental enthusiasts to contribute valuable observational records to public databases. Platforms such as iNaturalist, eBird, and GBIF (Global Biodiversity Information Facility) have dramatically increased the volume and geographic spread of biodiversity data (Bonnet *et al.*, 2020). This surge in participation is widely celebrated as a step toward democratizing science and enriching zoological knowledge.

However, alongside this digital revolution, several critical concerns have emerged regarding the quality, representativeness, and scientific utility of such data. Observational data—especially that generated through citizen science—is often opportunistic rather than systematic. Contributors tend to document easily accessible areas or aesthetically appealing and familiar species, leading to notable geographical, temporal, and taxonomic biases (Troudet *et al.*, 2017). This results in significant discrepancies in the recorded presence, distribution, and abundance of species, particularly in less-populated regions or among cryptic, nocturnal, or understudied taxa.

Moreover, a parallel decline in traditional specimen-based data—obtained through structured fieldwork and preserved in natural history museums—is creating a gap in the foundational elements of zoological science. These specimens are vital not just for taxonomic verification, but also for downstream research in ecology, climate impact studies, evolutionary biology, and genetics (Suarez & Tsutsui, 2004). Unlike digital observations, preserved specimens can be re-examined years or even centuries later, enabling longitudinal studies that observational records alone cannot support.

Recent analyses, such as the one led by Stanford University researchers (2023), have brought these issues into sharp focus. Their review of over 1.9 billion biodiversity records from multiple global databases revealed a striking imbalance—while observational data volume has skyrocketed, it tends to be highly clustered around populated regions and megafauna, leaving critical ecological zones and smaller taxa significantly underrepresented.

Materials and Methods:

This chapter uses a literature review approach. Primary data was collected through published research chapters, online articles, and official databases. Sources include peer-reviewed journals such as Nature Ecology & Evolution, Cambridge Core, and the Wiley Online Library, as well as institutional websites from Stanford University and the Global Biodiversity Information Facility. The review compared studies focused on both observational datasets and specimen-based records, examining geographic coverage, taxonomic biases, and scientific usage to understand discrepancies and their implications for zoological research.

Results and Discussion:

The literature reveals a sharp rise in digital observation records but highlights their regional and taxonomic biases. Observational data from platforms such as iNaturalist and eBird are heavily concentrated in the Global North and tend to favor large, charismatic species. In contrast, physical specimens, though fewer in number, show more even global distribution and represent a broader range of taxa. This discrepancy affects ecological modeling and conservation planning, potentially leading to misinformed policies. A Stanford University study (2023) confirmed that physical specimen records offer more comprehensive coverage, emphasizing the need to integrate these with digital data to ensure a holistic understanding of biodiversity (Nature Ecology & Evolution, 2023).

Conclusion:

The surge in biodiversity data through citizen science has transformed zoological research, yet the associated biases demand urgent attention. Bridging the gap between observational and specimen-based data is critical for producing accurate ecological insights and conservation strategies. Future biodiversity assessments should emphasize data integration,

technological innovation, and broader participation across geographic and taxonomic boundaries to build a more reliable picture of global biodiversity.

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Multidimensional Research Perspectives in Life Science

ISBN: 978-93-48620-76-7

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ISBN 978-93-48620-76-7

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