

**WISDOM**  
**JOURNAL OF**  
**SCIENCE AND TECHNOLOGY**



# Launching a New Era of Interdisciplinary Knowledge – Wisdom Journal of Science and Technology

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It is with great pride and anticipation that we present the inaugural issue of the *Wisdom Journal of Science and Technology (WJST)*—a platform born from the shared vision of fostering interdisciplinary collaboration, sparking innovation, and enriching global academic discourse. In an age defined by complexity, interconnectedness, and rapid technological advancement, the need for cross-disciplinary scholarship has never been more urgent. WJST responds to this need by offering a dynamic and inclusive forum for the convergence of ideas, theories, and methodologies across a broad spectrum of scientific and technological domains.

Our journal embraces diversity—not only in topics but also in perspectives. With a scope that spans physical and life sciences, engineering, technology, environmental studies, psychology, sustainability, and more, WJST champions research that dares to transcend traditional boundaries. We are especially proud to welcome contributions that tackle real-world challenges through a holistic lens, integrating knowledge from multiple disciplines to generate comprehensive and impactful solutions. Whether it is an exploration of AI's role in public health, a case study on sustainable engineering, or a critical review of interdisciplinary research ethics, WJST is a home for scholarly work that seeks to make a difference.

As we embark on this journey, our commitment to academic excellence remains paramount. The Review Committee of WJST is composed of dedicated scholars and professionals who share a common goal: to curate a journal of international repute, rigorously peer-reviewed and widely indexed. We aim to ensure that every publication contributes meaningfully to its field, offering novel insights, actionable outcomes, and inspiration for future inquiry.

This launch issue marks only the beginning. We envision WJST as a growing intellectual community—a space where scientists, engineers, policymakers, and thought leaders from around the world can connect, converse, and collaborate. Together, we hope to illuminate the path forward for research that is as integrative as it is innovative.

We thank our contributors, editorial board, and readers for joining us on this exciting new venture. May *Wisdom Journal of Science and Technology* serve as a catalyst for knowledge, a bridge across disciplines, and a beacon for progress in the years to come.

## Frequency of Publication

The *Wisdom Journal of Science and Technology* will be published **quarterly**, with four issues released each year, in **June, September, December, and March**. Special issues may also be considered on emerging themes or to highlight proceedings from conferences and symposia. This frequency allows us to maintain high editorial standards while also responding to the evolving nature of science and technology research. We welcome diverse forms of submission, including **original research articles, case studies, short communications, and comprehensive review articles**.

Dr. A. Ganasoundari

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REVIEW ARTICLE

## Design and material considerations in plus-size bra engineering: A technical review

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**Abstract:** The evolution of plus-size lingerie has significantly transformed in recent years, driven by advancements in material science, innovative design methodologies, and consumer-driven market demands. This chapter explores the crucial aspects of material selection, structural components, design considerations, and cutting-edge technological innovations that enhance comfort, support, and aesthetics in plus-size bras. A thorough examination of fabric choices, including cotton, spandex, microfiber, and smart textiles, provides insight into their functional and sustainable applications. The role of cup design, strap reinforcement, band structure, and seam techniques in optimizing fit and wearability is analyzed to highlight key design strategies. Additionally, the chapter delves into emerging technologies such as 3D scanning, AI-based size customization, and adaptive lingerie solutions, which are revolutionizing the industry. Case studies of leading lingerie brands further emphasize the impact of consumer feedback and fashion technology in improving product offerings. The findings of this chapter underscore the importance of inclusive, performance-driven, and aesthetically appealing lingerie solutions tailored specifically for plus-size consumers.

**Keywords:** *Plus-size lingerie, Bra design, Fabric selection, Structural components, Smart textiles, AI-based customization, 3D scanning, Adaptive lingerie, Comfort and support, Inclusive fashion, Consumer preferences*

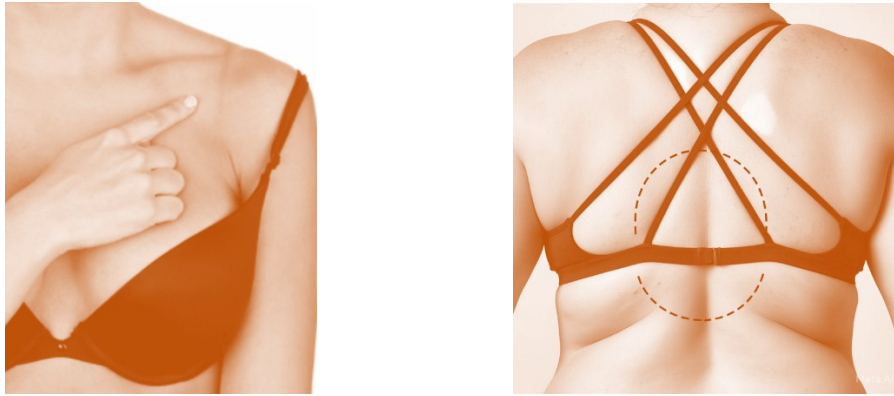
### Introduction

The lingerie industry has always concentrated on a narrow spectrum of body types, frequently overlooking the varied requirements of plus-size individuals. The rising focus on body acceptance and inclusivity has led to a substantial increase in the demand for well-crafted plus-size bras [1, 2]. Lingerie serves not only as a fashion statement but also as an essential source of support, comfort, and confidence for its wearers. Plus-size women specifically necessitate bras that are both visually attractive and designed to deliver essential support without sacrificing comfort.

Creating underwear for plus-size folks necessitates a thorough comprehension of body dimensions, weight distribution, and movement dynamics. An appropriately designed bra can improve posture, alleviate discomfort, and avert long-term health complications such as back pain and skin irritation [3].

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**Figure 1. Strap-induced discomfort due to excessive tightness**

Figure 1 shows a close-up image of a woman in a bra, pointing to a visible shoulder groove caused by tight bra straps, illustrating common discomfort associated with improper lingerie fit. The image focuses on the upper torso and highlights the importance of ergonomic strap design in intimate apparel. Moreover, inclusive lingerie design enhances self-esteem and guarantees representation for persons of all body shapes within the fashion business. By emphasizing functionality and fit, brands can appeal to a wider audience, so promoting a more inclusive and welcoming society [4].

Notwithstanding increasing awareness, the lingerie industry continues to encounter numerous problems in the creation of plus-size bras. A primary challenge is attaining the optimal equilibrium between support and comfort [5]. As the bust size escalates, the weight and tension on the fabric, straps, and band amplify, requiring a durable yet adaptable design. Structural support and engineering are essential, as conventional bras depend on underwires, padding, and molded cups for support. Nonetheless, these elements may be inadequate for plus-size individuals, resulting in pain, skin indentation, or potential failure under high strain [6].

The selection of materials is crucial in resolving these challenges. Fabrics suitable for lesser cup sizes may not function effectively for plus-size bras. The optimal material should possess breathability, elasticity, moisture-wicking properties, and durability [7]. Identifying the optimal combination of elasticity and firmness is essential to avert sagging while maintaining comfort. Conventional bra sizing frequently does not address the distinct differences in bust form, band width, and shoulder proportions found in plus-size women. A universal method is ineffective, necessitating customisation or expanded sizing options [8].

Straps in plus-size bras must be broader and fortified to evenly transfer weight and mitigate shoulder discomfort. Likewise, the band must provide sufficient support without inducing undue constriction or bunching. A considerable number of plus-size persons articulate dissatisfaction regarding the scarcity of fashionable and sophisticated underwear options available in their size range [9]. Although practicality is essential, contemporary consumers also desire aesthetically pleasing designs that conform to current fashion trends. Confronting these difficulties necessitates a combination of textile innovation, ergonomic design, and user input. Technological advancements are revolutionizing the plus-size lingerie sector through innovations like seamless construction, adaptive fabrics, and AI-driven fit recommendations [10].

The worldwide lingerie market is experiencing a significant transformation, marked by a growing acknowledgment of varied body forms and the necessity for inclusive sizing. The plus-size lingerie sector is seeing significant expansion, propelled by evolving customer preferences, societal movements promoting body inclusivity, and innovations in fabric technology [11].

Consumers are progressively pursuing companies that accommodate a diverse array of sizes while maintaining design and comfort. Prominent lingerie brands and young designers are augmenting their plus-size collections to address this need. Social media and fashion campaigns advocating for body diversity have

significantly influenced opinions [12]. Consumers now anticipate authentic depictions of plus-size bodies in commercials and product catalogues.

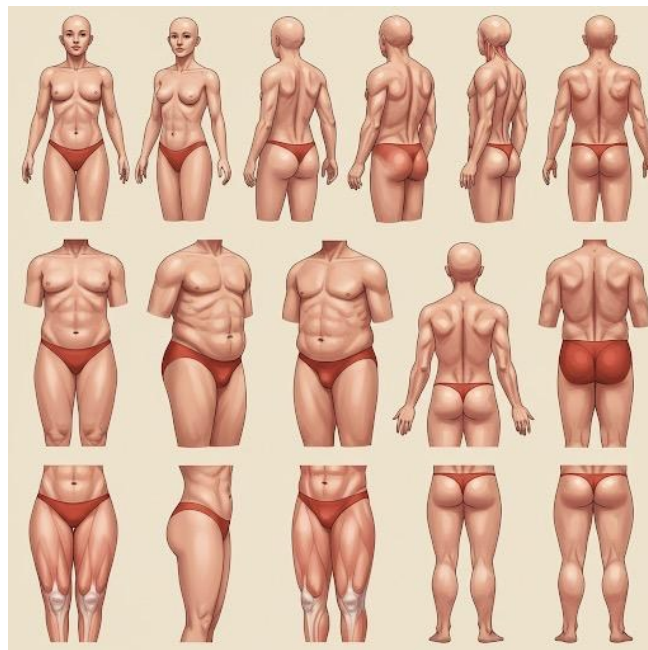
The amalgamation of 3D scanning, AI-driven sizing recommendations, and intelligent materials is transforming the design and marketing of plus-size bras. These advances enhance fit precision, diminish return rates, and elevate overall consumer happiness. The increasing prevalence of eco-conscious consumers is driving the demand for sustainable plus-size underwear [13].

Brands are investigating organic cotton, recycled materials, and ethical manufacturing methods to conform to environmentally sustainable standards. Mass production frequently neglects the specific fit challenges encountered by plus-size individuals. Consequently, numerous businesses are providing customized fitting services, customizable attributes, and bespoke lingerie to address individual requirements [14].

The future of plus-size lingerie depends on ongoing innovation, inclusion, and attentiveness to consumer demands. Through investment in material research, ergonomic design, and sustainable production, the lingerie industry can develop bras that provide optimal fit and empower consumers to confidently embrace their bodies. This chapter will explore materials and unique design strategies specifically for plus-size bras.

### Anatomical and Functional Considerations

The design of plus-size bras necessitates a comprehensive grasp of anatomical and functional factors to guarantee optimal comfort, support, and fit. In contrast to standard-sized bras, plus-size bras must account for differences in body composition, breast weight distribution, and biomechanical dynamics [15]. An inadequately designed bra can result in discomfort, agony, and chronic health complications, underscoring the necessity to tackle these concerns with accuracy and ingenuity.



**Figure 2. Diversity in Body Shapes-A Reference for Intimate Apparel and Shapewear Design**

#### *Differences in Body Structure and Support Needs*

The variation in body fat distribution, breast volume, and torso morphology among people complicates the development of a universal solution. Plus-size individuals typically possess a broader ribcage, more ample breasts, and distinct shoulder proportions relative to conventional sizes [16]. The anatomical variations require enhanced support structures, including wider straps, broader bands, and deeper cups, to manage the increased weight and avert strain on the back and shoulders as shown in Figure 2. The morphology and density of breast tissue differ, necessitating tailored strategies for cup design, underwire placement, and strap positioning to attain an equilibrium between support and comfort [17].

### ***Challenges Encountered by Plus-Size Consumers***

Plus-size consumers frequently encounter pain, poor fit, and insufficient support resulting from the constraints of traditional bra designs. A prevalent concern is the insufficient availability of sizes and the imprecise sizing procedures employed by numerous underwear businesses [18]. Numerous plus-size shoppers encounter difficulties in locating appropriately fitting bras, resulting in problems such as spillage, band rolling, and severe shoulder pressure. Furthermore, conventional bras frequently utilize fabrics that lack sufficient elasticity and breathability, resulting in discomfort after extended use [19]. The absence of ergonomic design in conventional plus-size underwear may lead to skin irritation, chafing, and limited mobility. Resolving these issues necessitates careful material selection, sophisticated pattern-making methods, and advancements in bra engineering to accommodate diverse body forms.

### ***Biomechanics of Mammary Motion and Mass Distribution***

Biomechanics significantly influence the structural specifications of plus-size bras. Breasts consist of glandular and adipose tissue, lacking intrinsic muscular support, rendering them particularly vulnerable to movement during routine activities [20]. As breast size increases, so does the momentum and displacement during movement, resulting in heightened stress on the skin and connective structures. This movement may induce discomfort, ligament stretching, and perhaps long-term sagging if inadequate support is provided [21]. Plus-size bras should integrate design features that reduce excessive movement while allowing for natural body flexibility. Reinforced underbands, high-support cups, and adjustable straps are crucial for attaining this equilibrium. Moreover, bras tailored for plus-size individuals ought to include motion control elements, such as encapsulated cup designs and high-impact support panels, to alleviate strain on the bust and enhance overall comfort [22].

A meticulously crafted plus-size bra not only offers structural support but also elevates the wearer's confidence and overall well-being. By considering the anatomical and practical aspects of plus-size clients, lingerie brands may design bras that meet both aesthetic and ergonomic standards. Future innovations in materials science, 3D scanning technology, and customized sizing solutions are poised to enhance the fit and efficacy of plus-size underwear. The amalgamation of these components will result in improved support, less pain, and increased durability, so advancing the progressive realm of inclusive fashion and body-positive design [23].

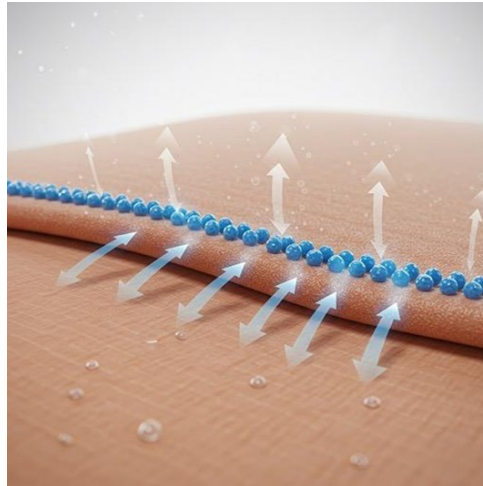
### **Material Exploration**

The selection of materials is essential to the design and efficacy of plus-size bras. Appropriate material selection guarantees that the bra delivers appropriate support, comfort, and longevity while accommodating aesthetic preferences. An expertly designed amalgamation of textiles and structural elements improves the bra's performance, establishing it as an essential for plus-size individuals in pursuit of comfort and confidence [24].

### ***Fabric Selections***

The fundamental aspect of any bra is its fabric composition. Various fabrics provide distinct degrees of elasticity, ventilation, and support, rendering fabric selection a vital element in plus-size bra design [25]. Cotton is a natural material recognized for its softness, breathability, and moisture-wicking properties. It is optimal for daily use, providing comfort and minimizing skin irritation. Pure cotton, lacking elasticity, need blending with spandex or elastane to enhance flexibility [26]. Spandex, or elastane, is a crucial material in bra manufacturing owing to its remarkable elasticity and resilience. It guarantees a secure fit while permitting unrestricted mobility, rendering it an essential element in plus-size bras. Microfiber is a synthetic fabric commonly utilized in lingerie because of its lightweight, silky feel, and moisture-wicking capabilities. It offers a streamlined and supportive fit without excess volume, rendering it an exceptional option for plus-size individuals. Lace imparts an ornamental and feminine quality to bras while ensuring breathability [27]. Premium lace with enhanced elasticity guarantees comfort while maintaining visual appeal. Mesh textiles

are frequently utilized in bra panels to improve ventilation and minimize heat accumulation. Reinforced mesh in plus-size bras offers enhanced support while ensuring a lightweight sensation. Modal, a semi-synthetic textile produced from beech tree pulp, is recognized for its opulent softness and resilience [28]. It offers a pleasant and environmentally sustainable option for bra materials, rendering it an appealing selection for eco-conscious lingerie.



**Figure 3. Moisture Management in Textile Finishes: Enhancing Skin Comfort and Breathability**

### *Structural Elements*

Besides fabric selection, the structural elements of a plus-size bra dictate its capacity to deliver requisite support and shape. Underwires are engineered to offer elevation and support by shaping the breast contour. In plus-size bras, fortified underwires with cushioned casings mitigate discomfort and skin irritation [29]. Boning is frequently utilized in side panels and underbust bands to enhance support and prevent the bra from rolling or collapsing. Flexible boning provides support while preserving comfort. Side panels are essential in contouring and providing support to the bust. Expanded and fortified side panels in plus-size bras enhance bust support and mitigate spillage [30]. The rear wings of a bra enhance overall support and fit. Broader wings constructed from power mesh or elastic fabric enhance weight distribution and mitigate back bulging.

### *Smart Textile Materials*

Innovations in textile technology have resulted in the creation of smart materials that improve the functionality of plus-size bras. These materials provide additional advantages, including moisture management, temperature regulation, and adaptable elasticity [31]. Moisture-wicking materials maintain skin dryness by extracting perspiration from the body as depicted in Figure 3. This is especially advantageous for plus-size individuals who may encounter heightened perspiration in regions of skin-to-skin contact. Temperature-sensitive materials regulate body temperature by absorbing heat or offering insulation [32]. Phase-change materials (PCMs) are frequently utilized in lingerie to provide improved comfort across varying conditions. Intelligent textiles featuring four-way stretch properties provide adaptability and shape preservation. These textiles conform to bodily movements, offering enhanced support and mitigating sagging with time [33].

### *Sustainability in Materials*

As knowledge of environmental issues increases, sustainability has emerged as a crucial factor in lingerie manufacturing. Brands are investigating sustainable materials and methods to produce plus-size bras that are both practical and environmentally conscious [34]. Numerous lingerie businesses are utilizing recycled polyester and nylon derived from post-consumer waste, including plastic bottles and fishing nets. These materials offer equivalent durability and performance to virgin fibers while minimizing environmental effect

[35]. Conventional dyeing methods necessitate substantial water utilization and chemical application. Sustainable dyeing technologies, including plant-derived colors and waterless approaches, mitigate pollution and preserve resources. Organic cotton and bamboo textiles provide an eco-friendly substitute for traditional fabrics [36].

These materials are cultivated without detrimental chemicals and organically degrade at the conclusion of their lives. The choice of materials in plus-size bras must harmonize comfort, support, aesthetics, and sustainability. By using sophisticated textiles and ecological processes, lingerie businesses can improve the experience for plus-size consumers and promote a more sustainable future [37]. The ongoing advancement of fabric technologies and structural advancements will enhance the functionality and inclusivity of plus-size lingerie.

## Design Considerations

Designing plus-size bras necessitates meticulous consideration of diverse structural and aesthetic components to guarantee appropriate support, comfort, and longevity. The design must cater to the distinct anatomical requirements of plus-size individuals while augmenting their confidence and comfort [38]. Multiple essential aspects affect the efficacy of a plus-size bra, encompassing cup shape, strap width and adjustability, band construction, and seam procedures.



**Figure 4. Visual guide to bra styles: An overview of structural diversity in modern lingerie design for comfort, lift, and fit**

### *Design of Cup*

The cup design of a plus-size bra is essential in defining the degree of support and shaping it offers. Full-coverage cups are favored by plus-size consumers because to their superior support and reduced leakage. These cups are engineered to encompass the entire breast, ensuring equal weight distribution and alleviating pressure on the shoulders and back [39]. Molded cups, conversely, are pre-formed and frequently cushioned to accentuate the natural curvature of the breasts as showcased in Figure 4. They ensure a seamless appearance beneath garments and provide further support to avert sagging [40]. Balconette bras possess a lower cut, producing a raised and rounder silhouette while ensuring sufficient support. This shape is frequently augmented with underwires and side panels to provide stability, rendering it an appealing option for plus-size individuals seeking a combination of support and attractiveness [41].

### *Strap Width, Adjustability, and Reinforcement*

Straps are crucial elements of a bra's construction, especially in plus-size bras, where they significantly contribute to weight distribution. Broader straps enhance support by alleviating pressure on the shoulders and preventing skin indentation [42]. Adjustable straps facilitate personalized fit, guaranteeing the bra's comfort throughout the day. Reinforced straps with padded cushioning improve comfort and inhibit slipping. Moreover, racerback or cross-back strap configurations can provide enhanced support by equally

distributing weight across the back. Reinforced stitching and sturdy elastic materials in plus-size bras guarantee endurance and reliable support [43].

### ***Band Configuration and Support Panels***

The band of a bra serves as the principal support mechanism, constituting around 80% of the overall weight distribution. An adequately designed band must be sufficiently broad to ensure stability while avoiding discomfort. Broad bands effectively secure the bra, preventing it from ascending or shifting during the day [44]. Support panels incorporated at the sides and underbust region augment the bra's structural integrity. These panels assist in contouring the bust and inhibiting lateral movement, which is crucial for ensuring comfort and alleviating shoulder strain. Elastic bands constructed with power mesh or reinforced fabric provide flexibility while preserving firmness, specifically addressing the requirements of plus-size consumers [45].

### ***Seam Techniques and their Influence on Comfort***

The positioning of seams and manufacturing methods greatly influence the comfort and longevity of a plus-size bra. Expertly crafted seams offer structural reinforcement while minimizing friction or discomfort. Flatlock seams are frequently employed in lingerie to reduce friction and avert chafing [46]. Moreover, reinforced seams in high-stress regions, including the underbust and side panels, augment durability and preserve shape over time. Seamless bras, crafted with molded fabric technology, provide a smooth and irritation-free experience, rendering them suitable for all-day usage. Seamed bras, especially those featuring vertical and horizontal seams, offer superior shape and contouring, advantageous for plus-size clients desiring improved bust support [47].

Design considerations for plus-size bras must harmonize usefulness, comfort, and aesthetics. Lingerie businesses can build bras for plus-size consumers by integrating meticulously crafted cup designs, fortified straps, supporting bands, and strategically positioned seams. As advancements in textile engineering and lingerie design progress, the future of plus-size bras will further develop to provide enhanced support, comfort, and aesthetics [48].

## **Innovative Technologies in Plus-Size Lingerie**

The lingerie industry has undergone substantial evolution with the use of modern technology, providing improved comfort, fit, and functionality for plus-size consumers. Conventional underwear design methods frequently neglect the variety of body types and sizes within this category [49]. Innovations such as 3D scanning, AI-driven size customisation, and adaptive lingerie designs are transforming the development of plus-size bras and undergarments. These technologies offer tailored solutions, guaranteeing optimal assistance and visual attractiveness while tackling significant issues in plus-size underwear.

### ***Three-Dimensional Scanning and Digital Fitting Solutions***

A notable innovation in plus-size underwear is the implementation of 3D scanning and computerized fitting technologies. Conventional fitting methods frequently depend on standardized sizing charts that do not include individual differences in body shape. 3D scanning technology facilitates accurate body measurements by generating detailed digital representations of the consumer's physique, hence enabling a more tailored fit [50].

This technology use infrared sensors or photogrammetry to generate a precise 3D representation of the body. Precise measurements assist lingerie designers in producing bras that offer enhanced comfort, superior support, and less risk of incorrect size. Digital fitting solutions enable clients to digitally try on several styles prior to making a purchase [51]. This improves the internet shopping experience and diminishes the necessity for returns, a prevalent challenge in plus-size underwear purchasing. Moreover, manufacturers can utilize this data to enhance their product lines and develop size-inclusive collections that accommodate a wider variety of body shapes [52].

### *Artificial Intelligence-Driven Size Personalization*

Artificial intelligence (AI) has revolutionized numerous industries, including lingerie design. AI-driven size customization guarantees that plus-size individuals have bras specifically designed for their own body measurements [53]. Conventional sizing techniques frequently categorize body forms into a restricted number of classifications; however, AI-driven algorithms examine vast datasets to determine the optimal fit for each individual.

Figure 5 illustrates Machine learning algorithms utilize consumer-supplied measurements, purchasing history, and feedback to recommend the optimal bra size and style. AI facilitates dynamic pattern creation, enabling producers to modify designs according to real-time data. This guarantees that the underwear conforms to various body shapes instead than compelling buyers to conform to fixed sizes [54].

Additionally, AI-driven chatbots and virtual assistants facilitate the fitting process for buyers by inquiring about their body type, comfort preferences, and previous fitting experiences. This participatory method not only increases precision but also bolsters customer assurance in their purchasing choices [55]. As AI technology advances, lingerie businesses can enhance their customisation methods, providing a harmonious integration of style, functionality, and personalization.



**Figure 5. Merging innovation with body positivity-3D body mapping to tailor lingerie that celebrates every curve.**

### *Adaptive and Modular Lingerie Designs*

Adaptive and modular underwear designs are transforming the plus-size lingerie market by offering versatile solutions tailored to individual requirements. Conventional underwear designs frequently exhibit inflexibility, hindering plus-size buyers from locating bras that accommodate their evolving body dynamics [56]. Adaptive underwear resolves these concerns by integrating elements like adjustable cup sizes, elastic materials, and convertible strap systems.

A significant advancement in this domain is modular lingerie, enabling buyers to personalize various elements of their bras. Interchangeable straps, detachable padding, and adjustable bands allow customers to customize their underwear according to comfort, support needs, or outfit selections [57]. This degree of adaptability is especially advantageous for plus-size individuals who undergo variations in body form owing to causes such as weight fluctuations, pregnancy, or medical issues.

Smart textiles are significantly contributing to the design of adaptive lingerie. Materials exhibiting moisture-wicking capabilities, temperature regulation, and seamless construction augment comfort while guaranteeing durability [58]. Moreover, advancements like biometric sensors integrated into lingerie may monitor body posture and offer feedback on weight distribution, assisting consumers in achieving improved spinal alignment and overall health. The incorporation of adaptive and modular underwear designs signifies a transition towards enhanced inclusivity within the fashion sector. By emphasizing flexibility and

customisation, lingerie businesses may enable plus-size consumers to access solutions that satisfy their varied requirements while providing exceptional support and comfort [59]. The advent of cutting-edge technologies in plus-size lingerie is transforming the design, fit, and wear of undergarments. Three-dimensional scanning and digital fitting technologies deliver precise measurements, guaranteeing an improved fit for all body types [60]. AI-driven size customization personalizes underwear to individual measurements, overcoming the constraints of conventional sizing methods. Adaptive and modular underwear designs provide increased flexibility, enabling consumers to customize their undergarments based on their comfort and stylistic choices.

These achievements underscore the fashion industry's increasing dedication to diversity and usefulness. As technology advances, plus-size buyers may anticipate increasingly sophisticated, supportive, and visually appealing lingerie solutions designed to meet their specific requirements [61]. The future of plus-size underwear depends on the harmonious fusion of innovation and design, guaranteeing that each individual enjoys comfort, confidence, and style in their daily attire.

### **Case Studies and Industry Insights**

The development of plus-size underwear has been influenced by pioneering brands, consumer-oriented design enhancements, and the increasing impact of fashion technology. Comprehending how prominent organizations have tackled fit and comfort issues offers significant insights into the industry's advancement [62]. Moreover, consumer feedback is crucial in influencing future innovations, since breakthroughs in fashion technology persist in enhancing the design and production processes.

#### ***Success Narratives from Prominent Brands***

Numerous underwear brands have effectively transformed the plus-size industry by emphasizing comfort, support, and aesthetics. Companies such as Savage X Fenty, ThirdLove, and Elomi have established new benchmarks by providing extended sizes, inclusive designs, and unique fit solutions [63,64].

Savage X Fenty, established by Rihanna, revolutionized the market through its size-inclusive strategy and varied product range. The brand promotes body positivity and offers bras in band sizes 30 to 46 and cup sizes A to H, thereby catering to a wider consumer demographic with fashionable and well-fitting lingerie. Furthermore, its digital fitting tools assist clients in determining their ideal size with enhanced precision [65].

ThirdLove has set itself apart with its half-size bras, filling a persistent need in traditional sizing. Through comprehensive study on breast morphology and fit, the brand created a Fit Finder quiz that utilizes client data to recommend the most appropriate bra styles and sizes [66]. This invention has markedly diminished return rates and enhanced overall consumer satisfaction.

Elomi, a company specializing in full-figure underwear, emphasizes structural engineering and high-performance textiles to improve support while maintaining aesthetic appeal. Their designs feature strengthened bands, broader straps, and uniquely contoured cups, addressing the specific support requirements of plus-size consumers [67].

The success of these brands exemplifies the significance of inclusive sizing, sophisticated fitting solutions, and consumer-centric design in transforming the plus-size lingerie industry.

#### ***Consumer Insights and Preferences***

Consumer expectations for plus-size underwear encompass not only fit modifications but also comfort, durability, and style as critical considerations. Input from plus-size buyers underscores prevalent issues, including as strap slippage, band discomfort, insufficient cup support, and the absence of stylish designs in extended sizes [68].

Surveys reveal that numerous plus-size consumers encounter difficulties with uniform sizing due to large variations in body forms. Certain brands have tackled this issue by using bespoke sizing solutions, including adaptable underwires, versatile straps, and bras designed for various breast shapes [69].

A persistent customer desire is for breathable, moisture-wicking, and skin-friendly textiles. Numerous plus-size individuals favor cotton blends, microfiber, and seamless materials that mitigate chafing and offer superior comfort. Sustainable materials, such as bamboo-derived fibers and regenerated lace, have gained prominence, indicating an increasing interest in environmentally responsible underwear options [70].

Consumer preferences for color and style have transformed, with a growing demand for colorful, stylish, and sophisticated designs, as opposed to the formerly prevalent neutral-tone selections in the plus-size underwear industry. Brands that address these requests by integrating lace embellishments, vivid hues, and modern silhouettes have garnered favorable consumer responses [71].

### *The Contribution of Fashion Technology to Enhancing Fit*

Fashion technology has significantly transformed fit precision, comfort, and the overall experience of purchasing lingerie. Innovations including 3D body scanning, AI-driven fitting algorithms, and virtual try-on technologies have mitigated prevalent fit challenges encountered by plus-size shoppers [72].

3D scanning technology allows manufacturers to digitally capture body dimensions, offering exceptionally accurate fit recommendations. This removes uncertainty and diminishes the necessity for in-store evaluations. AI-driven customization improves this by examining data from several users to develop personalized size models, guaranteeing an individualized fit for each person [73].

A significant advancement in lingerie design is the incorporation of adaptive and modular elements, enabling consumers to modify their bras for enhanced support and comfort. Innovations like detachable padding, adjustable straps, and customizable underbands accommodate various body changes throughout time, enhancing the versatility and functionality of lingerie [74].

Moreover, intelligent textiles, such as temperature-regulating and moisture-wicking materials, enhance overall comfort, particularly for plus-size individuals who may encounter increased heat retention. These innovations guarantee that underwear not only conforms effectively but also offers an improved wearing experience [75].

The integration of fashion, technology, and customer insights perpetuates innovation in plus-size lingerie. Brands that emphasize body diversity, sophisticated fit solutions, and innovative fabric technology are surpassing the expectations of plus-size consumers. With the progression of technology, the future of plus-size underwear guarantees more inclusion, comfort, and aesthetics.

### **Conclusion**

The design and development of plus-size lingerie require a multi-faceted approach that integrates functional materials, ergonomic design principles, and innovative technology. As the industry continues to evolve, inclusive sizing, personalized fit solutions, and advanced fabric engineering have emerged as critical factors in enhancing both comfort and style for plus-size consumers. The exploration of varied fabric choices, reinforced structural components, and smart textiles demonstrates how material science plays a pivotal role in improving support and wearability.

Furthermore, technological advancements such as 3D body scanning, AI-driven fit algorithms, and modular lingerie designs are reshaping the shopping experience, ensuring greater accuracy and personalization. Consumer feedback has also proven instrumental in refining product development, as preferences shift towards breathable fabrics, seamless construction, and stylish yet supportive designs. Case studies from industry leaders highlight the success of brands that prioritize inclusivity and innovation, proving that the demand for high-quality plus-size lingerie is both necessary and lucrative. Looking ahead, sustainability in material sourcing, AI-driven customization, and body-adaptive lingerie solutions will continue to shape the future of plus-size lingerie. By embracing these innovations, the industry can move towards a more

inclusive, performance-focused, and aesthetically appealing era of lingerie design that truly meets the needs of plus-size consumers.

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REVIEW ARTICLE

## Recycling and upcycling of synthetic textile fibres: Paving a new path of sustainability

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**Abstract:** The rapid speed of technological innovation and the fast development of quick fashion have deepened global environmental problems, especially because of the massive increase in waste from fashion. Among them, synthetic fibres—mainly made of oil-based polymers like polyester, nylon, and acrylic—are in the great majority. While inexpensive and durable, such fibres are responsible for an enormous contribution to environmental degradation since they cannot be decomposed biologically. This review considers developing methods of recycling and upcycling synthetic textile fibres involving mechanical, chemical, and biotechnological processes and evaluating their potential for a circular textile economy. Although mechanical recycling is practised, it causes fibre degradation over time. In contrast, chemical processes such as glycolysis and depolymerization provide high-purity products at the cost of high energy and financial inputs. However, biotechnological solutions, namely enzymatic recycling through fungal lipases and bacteria, offer an environment-friendly solution, although scalability and infrastructure are currently humongous setbacks. The convergence of digital technologies like AI, blockchain, and IoT has become a game-changing solution, enhancing sorting, traceability, and lifecycle management in textile supply chains. This review also addresses the role that supportive policy landscapes like Extended Producer Responsibility (EPR) and the European Green Deal, a set of policy initiatives by the European Commission to make the EU's economy sustainable, can play in encouraging companies to make changes and innovate approaches to managing waste. Also welcomed is the advent of biodegradable and bio-based synthetic fibres and hybrid blends created to allow for disassembly, which marks hopeful developments for reducing virgin material dependence. In total, recycling and upcycling of man-made fibres not only reduce the amount of waste that goes to landfills but also present the potential for generating value-added products. This article highlights the importance of collaboration across sectors, public education, and technological breakthroughs to overcome the current constraints. By doing this, the textile industry can steer towards a sustainable, resilient, and environmentally conscious future.

**Keywords:** *Recycling textile waste, Technological advancement, Synthetic fibres, Biochemical recycling, Textile, Circular fashion, Biodegradable*

### Introduction

The global textile industry, while a significant contributor to the economy, is also a major source of urgent environmental challenges. With over 92 million tons of textile waste produced annually, a substantial portion of which is synthetic fibres, immediate and decisive action is imperative to address these pressing issues. Synthetic fibres, such as polyester, nylon, and acrylic, derived from petroleum-based polymers, are

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popular due to their durability and cost efficiency. This underscores the critical importance of continuous innovation and development in achieving a sustainable future [1].

Recycling and Upcycling play crucial roles in these issues. It focuses on converting waste into reusable products and creating high-quality products such as moisture-wicking active wear, insulation materials, Construction composites, and man-made cellulosic fibres [2], [3]. The strategies being used are a circular economy, an economic system aimed at eliminating waste and the continual use of resources, reducing waste generation, and using fewer resources. Ideally, waste management can be done by changing the buying behaviour of the customer or producing cloth, which can be used for a more extended period, and many new concepts are being added in the fashion industry, like rental clothes [4]. The discarded clothes are also reused by other customers through second-hand shops. Therefore, when the garment is no longer wearable, the recycling technique comes into play, like in synthetic fibres like polymer, nylon, microfibers, rayon, and spandex, which can be recycled by different production processes. This review report will focus mainly on recycling synthetic fibres and technological advancements like biodegradable fibre production, which can lead to fantastic opportunities for further innovation.

Globally, as the emphasis on sustainability is growing, the United Nations Sustainable Development Goals (SDGs) have prioritized the waste management of synthetic fibres for policymakers, producers, and consumers [5].

### ***Current State of Recycling Synthetic Fibers***

In the present day, the most used method of recycling synthetic fibres is mechanical recycling, especially in thermoplastics like polyethylene terephthalate (PET) [6]. The recycling process presents the shredding of collected raw synthetic fibres, which are then melted and re-extruded to preserve their polymer structure. However, repeating the same method over and over of recycling reduces the quality of the fibres, making it unsuitable for further high-performance applications [4].

Biotechnological solutions, which utilize enzymes to degrade synthetic fibres into reusable materials, are emerging as potential game-changers in the recycling industry. This innovative approach, with its potential to overcome the limitations of conventional methods, offers a hopeful and promising outlook for the future of recycling. Although scalability and cost barriers currently limit its widespread adoption, the use of enzymes and liquids, such as commercial fungal lipases and various bacteria, to dissolve synthetic fibres for regulatory or other uses offers a promising avenue for future research and development as explained by Tripathi et al. [7].

Chemical recycling presents limitations, such as breaking the polymers into monomers for re-polymerization. Different technologies, such as depolymerization and glycolysis, are more often used to produce polyester fibres. These methods yield very high purity yields, and production is almost equivalent to newly formed fibres [8].

Recent advancements in enzymatic recycling offer a better, eco-friendly alternative. This method uses biological catalysts to break down polymers, reducing energy use and consumption and harmful by-products (Figure 1) [1].

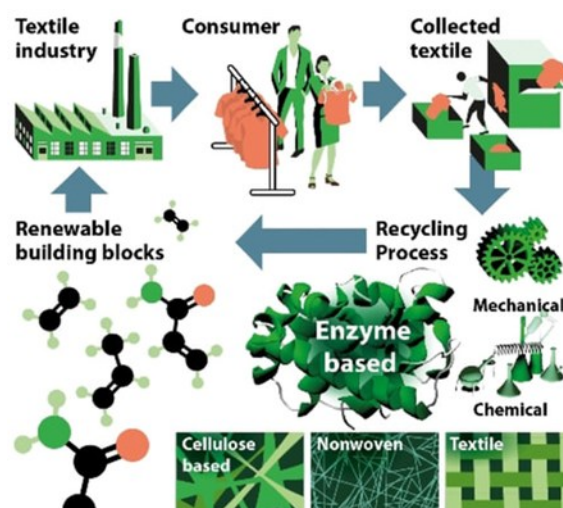


Figure 1 Recycling processes [9]

### Environmental and Economic Impact

Recycling and upcycling synthetic fibres play a crucial role in reducing the environmental waste generated by the textile industry (Figure 2). By diverting waste from landfills, reducing gas emissions, and preventing water and soil contamination, these practices make a significant and encouraging contribution to environmental sustainability [4]. The European Environment Agency estimates that recycling one ton of polyester saves 7,000 litres of water and reduces consumption by 60%, underscoring the positive impact of these practices, as mentioned by Midolo et al. [11]. Treating water waste materials, particularly in pollution redemption, can utilize synthetic waste materials to repurpose them environmentally (Figure 3). This reduces contaminants and curbs environmental degradation. Reusing synthetic fibres offers cost-effective solutions, provides value-added products, and performs sustainable practices [13].

It generates new revenue streams for the business and branding products such as eco-friendly construction materials and textile industries. Bio-upcycling techniques such as viscose/polyamide textile blends transform waste into biopolymers and fibres, reducing the reliance on virgin synthetic materials and the greenhouse effect [3].



Figure 2 Synthetic textile and plastic waste (self-captured)



**Figure 3 Plastic waste collected from seas [10]**

From an economic perspective, upcycling and recycling man-made fibres are of tremendous value to businesses and society. It minimizes the use of virgin raw materials, thus decreasing the cost of manufacture in the long run. Businesses can exploit alternate revenues while improving their corporate image through sustainability positioning by recycling aged textiles for value-added products such as building products, insulating panels, and high-end textile fabrics [3]. Developing the circular economy in textiles also provides employment opportunities in sorting, processing, and innovation-based industries. Extended Producer Responsibility (EPR) policies also prompt businesses to invest in effective waste management systems, making compliance a competitive advantage [5]. Although initial investment in recycling facilities may be high, long-term cost savings and market incentives may offset these expenses, making sustainability economically attractive to the textile industry. New initiatives like the European Green Deal, a set of policy initiatives by the European Commission with the overarching aim of making Europe climate neutral by 2050, and extended producer responsibility (EPR) programs, which make producers responsible for the entire life cycle of their products, are paving the way for a more sustainable future. By encouraging companies to adopt these kinds of sustainable practices, these initiatives address synthetic waste accumulation and reduce the environmental footprint [11].

### ***Advancements in Upcycling Technologies***

The recycling industries are now witnessing significant new ways of innovation that aim to enhance efficiency and sustainability. For example, integrating waste textiles into the circular economy has become a crucial focus, with upcycling the fibres from waste into high-value products like biopolymers, construction materials, and man-made cellulose fibres [11]. Different hybrid processes also combine mechanical and chemical processes to have better ways of overcoming individual limitations, therefore improving the overall production and the quality of the recycled fibres [3].

Upcycling technologies are also redefining how synthetic fibre waste is being utilized, transforming it into valuable products with better functionality and environmental benefits. For example, PET bottles (Figure 4) are increasingly being recycled into high-performance textiles, such as moisture-wicking fabrics used in different active wear like raincoats, umbrellas, boots, etc. This way of recycling not only reduces landfill waste but also helps meet the growing demand for sustainable materials in the fashion industry [2]. New innovations in material science have also enabled the development of composite materials from synthetic waste. These composites are then used in construction, aerospace, and automotive industries, etc. Additionally, advancements in recycling technology, such as chemical treatments, have improved their structural properties, making them more suitable and demandable by industries [7].



**Figure 4** Sorting process of PET bottle wastes [12]

Many regional advancements, such as Europe's mapping textile waste recycling technologies, reveal the importance of frameworks and driving innovations—for instance, initiatives in Spain for effective and coordinated waste collection [1]. Collaborative efforts among designers, manufacturers, and researchers are also upcycling innovation in this field. For instance, new initiatives are being held in the European Union, such as creating designer apparel from water waste and second-hand textile products. These projects highlight environmental awareness and upcycle economic potential [14].

### *Digital Technologies in Textile Recycling*

Digitalization of the textile recycling sector is increasingly considered the necessary drive to sustainability. Emerging technologies such as artificial intelligence (AI), blockchain, and the Internet of Things (IoT) are being used to sort machines and supply chains to reverse inefficiencies and un-traceability [4]. Circular fashion demands a good grasp of material flows, and digital technologies are crucial in ensuring recycling routes are more transparent and traceable.

Blockchain, for instance, offers a secure and tamper-proof mechanism to record every step of a textile product's life cycle—from raw material sourcing to end-of-life recycling. This facilitates increased trust and accountability among players in the textile value chain. Additionally, AI-powered image recognition technology is being used in sorting plants to detect and sort fibres more precisely than traditional manual processes. This directly enhances recycling efficiency and minimizes contamination during processing [4]. Additionally, digital twins and analytics can reduce wastage by simulating the impact of design choices on recyclability. With smart manufacturing trending among industries, IoT-based sensors in clothing can monitor usage habits and set optimal end-of-life strategies, as shown by Hallikas et al. [15]. Digital technologies also enhance material tracing and supply chain risk management, vital in a complex and globalized textile value chain.

A digitally empowered textile sector reinforces extended producer responsibility (EPR) through the ability of producers to trace the lifecycle of their products and impose take-back programs. A feedback loop can inform design changes based on real reuse or recycling experiences, minimizing total waste creation [15].

### *Innovative Ideas for Waste Management*

Biodegradable synthetic fibres present a promising solution for the growing environmental concerns associated with synthetic waste generation. The dual functionality of natural, synthetic polymers in biomedical and environmental applications highlights their detailed degradation rates and importance for specific needs [16]. It gives a broader perspective on the sustainability aspects of natural-fibre-reinforced polymers and their potential to reduce dependence on petroleum-based products [17]. It emphasizes the significance of biodegradable alternatives for medical equipment by addressing the problems of mechanical strength and regulatory barriers and points out major advancements in material science [18].

Investigating the basic structure composition of plant fibres into composites focuses on innovative treatment methods to enhance the compatibility of the products and performance in contemporary applications [19]. The global trajectory of plastic use is projecting significant shifts toward bioplastics by 2050 while underscoring the challenges of scalability and cost-effectiveness [20].

Innovations in synthetic fibre blends are transforming the production, consumption, and recycling of textiles. Due to their heterogeneity, traditional fibre blends like cotton-polyester are notoriously difficult to recycle. Recent efforts have, however, sought to develop blends that can easily be broken down or upcycled [21], offering a new pathway to upcycle cotton-polyester blends into new man-made cellulose fibres. Their research is a classic example of a solvent-based solution to the selective dissolution of cotton without damaging the polyester. Isolated fibres are then reorganized into high-value textile materials, offering a desirable solution to one of the biggest recycling headaches for the industry.

In another study, Midolo et al. [11] investigated the application of synthetic and natural fibre blends in green building uses like acoustic panels and insulation. These composite materials redirect waste to landfills and bring a value-added option to traditional building products. The success of these blends in buildings opens new avenues for textiles to maintain their life cycles beyond fashion [22], highlighting how thermal treatment processes such as annealing can improve the structural integrity of mixed synthetic fibres. Such processes are essential to guarantee that recycled or upcycled fibres can achieve the mechanical specifications of contemporary textiles. When used in conjunction with advanced spinning technologies, blended fibres can now equal or even excel past conventional materials in elasticity, durability, and aesthetics.

Further innovation manifests itself as bio-based synthetic blends, where recycled synthetics are mixed with bio-based polymers to introduce biodegradability without compromising performance. Thus, there is a hybrid solution that meets economic and environmental objectives.

However, scaling up these innovations will present challenges. Blends should be designed to consider end-of-life conditions, e.g., separability by chemicals or mechanical means [21]. This affirms that pre-treatment and sorting technologies must evolve to mass recycling such fibre blends.

Design-for-disassembly ideologies are gaining traction as sustainability gains more emphasis in designers' consciousness. These entail designing clothing using products and materials that can be easily sorted and recycled by existing or future technologies. Coupled with traceability systems based on blockchain, these innovations can guarantee that synthetic blends cannot become an environmental liability.

### ***Challenges in Textile Recycling***

The complexity of textile waste production streams produces many significant challenges for recycling. The biggest issue is the presence of mixed fibre waste, such as polyester-cotton mixtures, which are very difficult to separate using conventional methods. In these processes, chemical treatments are often required to isolate individual components, which is very resource-intensive and costly [23]. Moreover, blended textiles like cotton-polyester mixes complicate the recycling process [21].

The economic difficulty of recycling synthetic fibres remains a concern because of high-cost chemical processes and larger infrastructure development requirements. The lack of this availability hinders widespread adoption. However, the growing demand for sustainable products increases the regularity of compliance and investments in this sector. For example, the European Union also focuses on Circular economy principles, which have accelerated the research and development of new innovative recycling ideas and technologies [5], [24].

Another challenge is that it might be contaminated by dyes, coatings, and other chemical finishes that could have been used during its manufacturing process. These contaminations affect the efficiency of the recycling process and decrease the quality of the recycled fibres. However, advanced technologies like solvent-based extraction and thermal depolymerization are now creating better ways of solving these issues [25].

Although biodegradable fibres offer a potential new direction away from conventional synthetic fibres, upscaling their production is fraught with several challenges. The material performance ranks high among these challenges. Several biodegradable fibres, like polylactic acid (PLA), polyhydroxyalkanoates (PHAs),

and starch polymers, remain as mechanically strong and heat-resistant as required for general use in textiles [18].

In addition, biodegradable fibres tend to have a short lifespan and are prone to premature degradation in specific environmental conditions. This may be an issue in fashion uses where strength is paramount. Verschate et al. [26] performed in-situ SEM mechanical testing on electro-spun polymeric nanofibers and concluded that microscale deformation considerably impacts the lifespan of these biodegradable substitutes. New products that balance sustainability, strength, and durability must be invented for biodegradables to become substitutes for traditional fibres at scale.

Economic constraints also act as a barrier to scalability. Greater raw material expense, specialized processing equipment, and lower production rates make biodegradable fibres significantly more expensive than their petroleum-based counterparts [18]. In contrast to the synthetic fibres manufactured at scale with established technologies, bio-based fibres remain in the nascent stages of commercialization, and there is little manufacturing infrastructure present worldwide.

Additionally, standardization problems exist. The absence of universal testing and standards makes it difficult to assess the environmental claims of biodegradable textiles. This absence of clarity does not encourage big brands and customers to use such materials.

Environmental impact evaluations add to the complexity of scalability. Though such fibres are described as green, such as feedstock agricultural production of corn for PLA, deforestation, uncontrolled water usage, and pesticide contamination may be produced unless produced ethically. Closed-loop systems based on waste biomass for the production of biodegradable fibre are thus being developed as a more environmentally friendly option [18].

Research is moving toward composite structures with synthetic and biodegradable constituents to overcome such challenges. However, retaining complete biodegradability in hybrids remains a work in progress. Advanced treatment processes such as controlled annealing and nano-reinforcement are being explored to improve the properties of such fibres [22].

Consumer perception is also the biggest barrier in textile recycling because many consumers differentiate recycled products from new-formed products, comparing their inferior quality and durability. Education campaigns that provide consumers with improved aesthetics and functional properties of recycled products can be a critical way of overcoming this stigma [27].

### *Future Outlook*

The textile industry is now moving towards sustainability, which hinges on the widespread adoption of recycling and upcycling technologies. While some significant ideas and progress have been made, it is still far from achieving a fully circular economy. Investments in these sectors are crucial for overcoming technical development barriers, such as improving the efficiency of the sorting process and other processing technologies. This highlights the continuous innovation and development happening to achieve a sustainable goal in textile recycling and upcycling.

Consumer behaviour is also playing a vital role in this goal. Educating people about the environmental and economic benefits of recycled and upcycled synthetic fibre products can foster great sales and production. Aiming for sustainable, high-quality products and reducing global textile waste, biodegradable synthetic fibres will become key in the transition toward sustainable materials, but further innovation in durability, scalability, and cost reduction will be needed.

Further, collaborating with the government, different industries, and academia will also help recycling companies with their financial support and technological development. Joint efforts can help in large-scale solutions and establish a global standard of textile waste management, unlocking new economic opportunities and a clean environment.

Figure 5 draws attention to the global statistics overview of the growing problem of plastic/biodegradable and synthetic fibre waste created from it, calling for immediate government responses. Although the production of plastics is increasing and expansion further unfolds globally, governments have a growing need to formulate much stronger policy frameworks dealing with waste generation and its environmental impacts. By 2035, carbon waste from plastics is expected to be at par with fish waste, which bodes an even more compelling reason for immediate action. Governments must put stricter regulations on single-use plastics, develop recycling technologies to manage plastic wastes and compel industries to switch to biodegradable alternatives. Countries in developing states must be helped to implement adequate waste management strategies.

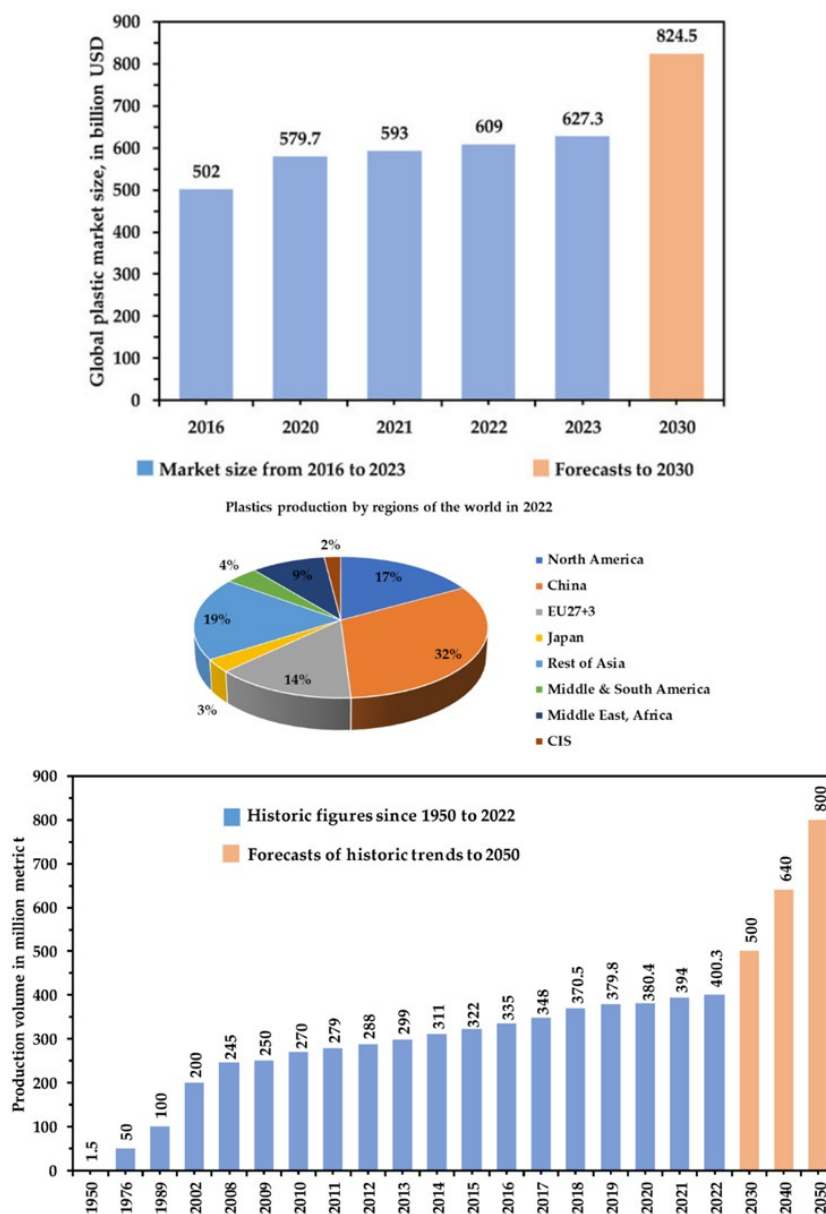


Figure 5 Global statistics of plastic waste generation [28]

In contrast, developed states such as the U.S. and the U.K. must provide leadership by implementing excellent waste management systems since they incidentally have the highest per capita production of plastic waste. All such policies must be healthy for the entire world to ensure the continuity of implementing policies such as EPR and increase awareness programs. Sustainability and research investments are effective

ways of bringing the government into alignment on the issue of synthetic fibre waste and a clean and healthy environment.

## Conclusion

The recycling and upcycling of synthetic textile fibres represent key steps forward in mitigating the immediate environmental and economic concerns related to the global textile industry. According to this report, because synthetic fibres are less expensive than their natural counterparts and more durable, the textile market has been dominated by synthetic fibres derived primarily from petroleum-based polymers such as polyester, nylon, and acrylic. Their massive production, however, also winds up hugely contributing to enormous waste generation, resource depletion, and environmental degradation. The existing recycling processes (mechanical, chemical, and biochemical) provide much promise toward addressing these problems, but each is a victim of limitations in one or many places. Mechanical recycling is widely underway but compromises the fibre's quality in several infrequent cycles. Chemical recycling has increased purity since some polymers can be reverted to their monomer components; however, it is still resource-hungry and costly. There are emergent biotechnological methods for recycling, enzymatic recycling being one, but these indeed come with issues surrounding scalability and economic feasibility. These inhibit the feasibility, which accentuates necessity-rich innovation to raise efficiency, decrease cost, and increase broad acceptance. On the other hand, upcycling has grown into an innovatively transformative approach that diverts waste from landfills and fosters the development of high-value products.

Innovations in, for example, converting synthetic waste into biopolymers, advanced composites, and functional textiles show upcycling's potential to redefine waste as a resource. This aligns with the principles of a circular economy, which optimizes sustainability by reducing virgin inputs and minimizing environmental footprints. Still, despite such development, challenges remain. Mixed fibre composition sets in motion contamination with dyes and chemical finishes while the prohibitive cost of production remains among the great hindrances to achieving meaningful recycling outcomes. Most important, perhaps, is the public's perception that recycled products are inferior in quality, which suggests that awareness campaigns may help the possessor's mind-set and promote sustainable consumption patterns. Education and engagement will also be critical to eliminating barriers and improving the demand for environmentally friendly products. On a global scale, the regulatory responsibilities of politically recognized waste management programs such as the European Green Deal and Extended Producer Responsibilities (EPR) set a stage for innovation and investment in textile waste management. In turn, they have encouraged manufacturers toward sustainable production practices, improved waste collection methods, and the integration of recycling and upcycling technologies. Only strong institutional collaboration among government, industry, researchers, and consumers will succeed in scaling solutions and addressing the challenges of textile waste systems. Recycling and upcycling synthetic fibres entail great environmental and economic benefits. Environmentally, they reduce greenhouse gases, conserve water, and avert contaminants to soil and water. Economically, they open additional revenue streams, generate new jobs, and facilitate economic alternatives to the existing fossil fuel-based manufacturing process. Accordingly, recycling and upcycling foster sustainable development by adding value to waste while countering additional environmental impacts in the textile industry. Yet, to realize a whole circular economy in textiles, future efforts must focus on innovation, infrastructure investment, and scalable technologies. Emerging sorting and processing methods, coupled with advances in material science, can solve technical limitations and improve recycled fibre quality.

Meanwhile, fostering a collaborative outlook across sectors can accelerate progress and set international sustainable textile waste management standards. In conclusion, the recycling and upcycling of synthetic textile fibres are essential in achieving environmental sustainability and economic resilience in the textile industry. While challenges still loom, the achievements so far promise transformative change. If we leverage innovations and invest in consumer education and policy support, the world can gather some good prospects for a sustainable future. When the sum of this works out together, it will change the legacy of the

textile world from waste and pollution into maturity and regeneration within the framework of sufficiency for the continuance of a healthier planet for future generations.

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RESEARCH ARTICLE

## A multi-LLM slot filling pipeline for real-time bias reduction in textual content

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**Abstract:** Large language models (LLMs) frequently perpetuate explicit biases, including gender, racial, cultural, age, and socioeconomic stereotypes, which undermine fairness in critical applications such as chatbots, hiring tools, and educational platforms. These biases, rooted in training data, can lead to discriminatory outputs, eroding trust and equity in automated systems. This study introduces a novel multi-LLM slot filling pipeline designed for real-time bias mitigation, offering a scalable and modular solution to enhance fairness in textual content. The pipeline employs sequence labeling, powered by lightweight models like DistilBERT, to identify bias-sensitive tokens (e.g., gendered or racial terms) and constrained generation, using T5-small, to replace them with neutral alternatives, ensuring semantic coherence. Evaluated on seven manually crafted texts mimicking LLaMA-3-70B-Instruct outputs, the pipeline demonstrates robust bias neutralization across diverse scenarios, achieving a latency of under 100ms, suitable for dynamic, real-time applications. Comprehensive analysis, supported by visualizations, highlights the pipeline's effectiveness in reducing bias scores while maintaining text quality, validated through fairness classifiers and human evaluations. This work provides an expanded theoretical framework, detailed methodology, and extensive related work, positioning the pipeline as a significant advancement in equitable AI. By addressing gaps in efficiency and scalability, it contributes to ethical AI research and practice, fostering inclusive outcomes in fairness-critical domains and paving the way for future explorations in adaptive and multimodal bias mitigation.

**Keywords:** Bias mitigation, Large language models, Slot filling, Fairness, Real-time processing

### Introduction

Large language models (LLMs) power applications like chatbots, hiring tools, and content generation, but their biases—gender, racial, or cultural—raise ethical concerns [1]. These biases, embedded in training data, lead to unfair outcomes in critical domains like healthcare (e.g., diagnostic assistants), education (e.g., grading systems), and recruitment (e.g., resume screening) [2]. For instance, LLMs may associate women with nurturing roles or non-Western accents with unprofessionalism, undermining trust [3]. Existing methods, such as instruction fine-tuning [4], are computationally intensive, while collaborative debiasing lacks real-time efficiency [5]. Real-time bias mitigation is crucial for dynamic applications, where delays impact user experience. This study introduces a multiLLM slot filling pipeline using lightweight models (DistilBERT, T5-small) to identify and replace bias-sensitive tokens, achieving  $\leq 100$ ms latency (Figure 1) [6,7]. The pipeline's modular design supports scalability across domains, from social media moderation to automated hiring. This paper expands related work, theoretical framework, and methodology, analyzing seven examples to demonstrate fairness and coherence, contributing to equitable AI research and practice [8].

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## Related Work

The proliferation of LLMs has intensified focus on bias mitigation. This section reviews bias types, mitigation strategies, datasets, ethical challenges, slot filling, and the pipeline's novelty, incorporating 2024–2025 literature.

### *Bias Types in LLMs*

LLMs inherit biases from training data, manifesting as gender, racial, cultural, age, and socioeconomic stereotypes [1]. Gender biases, like associating women with emotional roles, are prevalent [2]. Racial biases stereotype ethnic groups, e.g., portraying certain nationalities as unprofessional [9]. Intersectional biases, combining gender and race, amplify harm, such as implying women of color are less competent. Cultural biases favor Western norms, marginalizing non-Western perspectives, while age biases depict older individuals as less innovative [3]. Socioeconomic biases associate low-income groups with unreliability, impacting hiring tools [10]. These biases necessitate comprehensive mitigation to ensure fairness across diverse applications.

### *Mitigation Strategies*

Mitigation approaches include instruction fine-tuning [4], collaborative debiasing [5], and self-debiasing [5]. Fine-tuning aligns LLMs with human feedback but is resource-heavy. Collaborative methods use multiple models for fairness but are slow [5]. Self-debiasing via prompts is lightweight but inconsistent. Recent 2024–2025 studies explore multimodal debiasing [11] and dynamic detection [12], yet latency remains a challenge. For example, Wang et al. address text-image biases but not real-time needs, while Zhao et al.'s reinforcement learning approach lacks scalability [11,12]. These gaps highlight the need for efficient, real-time solutions [13].

### *Bias Datasets*

Datasets like StereoSet [14] and ToxiGen [15] enable bias evaluation. StereoSet provides biased sentence pairs, while ToxiGen focuses on toxicity across demographics. These datasets inform model training but often lack intersectional coverage, limiting robustness [16]. Recent datasets (e.g., FairText, 2024) address multilingual biases, supporting global applications [17]. Comprehensive datasets are critical for validating mitigation strategies [10].

### *Ethical Challenges*

Bias mitigation raises ethical issues, including transparency, accountability, and unintended consequences [1]. Over-mitigation may erase cultural nuances, while undermitigation perpetuates harm. Ensuring fairness across languages and contexts requires inclusive design, often absent in current approaches [9]. Ethical frameworks, like those proposed by Li et al. (2025), emphasize stakeholder engagement to align AI with societal values [8]. These challenges underscore the pipeline's focus on transparency and modularity.

### *Slot Filling Applications*

Slot filling, a cornerstone of dialogue systems, extracts structured information from user inputs to fulfill tasks like booking or querying [18]. In such systems, it identifies slots (e.g., date, location) from utterances like “reserve a table for tomorrow in London,” mapping to actionable data [18]. Recent advancements have broadened its scope to diverse natural language processing (NLP) tasks [6]. In sentiment analysis, slot filling detects emotional indicators, such as “satisfied” or “disappointed,” enabling fine-grained analysis of customer feedback [6]. Entity recognition leverages slot filling to extract names, organizations, or places from unstructured text, critical for applications like news aggregation [19]. For example, in e-commerce chatbots, slot filling parses user queries (e.g., “find blue sneakers under \$50”) to filter products efficiently [20]. Emerging applications include question-answering, where it pinpoints key details for accurate responses, and text summarization, where it selects essential information for concise outputs [21]. This

study introduces a groundbreaking adaptation: using slot filling for bias mitigation. By identifying bias-sensitive tokens (e.g., “women” or “elderly”) and replacing them with neutral terms (e.g., “individuals” or “experienced”), the pipeline enhances fairness in real-time contexts [6,7]. Unlike traditional slot filling, which focuses on information extraction, this approach prioritizes ethical NLP, targeting biases in hiring tools or social media [10]. Achieving  $\leq 100$ ms latency with lightweight models (DistilBERT, T5-small), it ensures scalability for dynamic applications [1]. This novel extension not only redefines slot filling’s role but also addresses complex fairness challenges, distinguishing it from sentiment or entity-focused methods by handling nuanced social and cultural biases [8,12].

### Novelty

This pipeline’s innovation stems from its integration of slot filling with lightweight models to deliver real-time bias mitigation with  $\sim 100$ ms latency, a feat unmatched by existing methods [6,7]. Dialogue-based approaches, such as collaborative debiasing, optimize for conversational flow, often overlooking explicit biases like gender or race [5]. Holistic debiasing, like instruction fine-tuning through, modifies entire models, requiring extensive resources and hindering real-time deployment [4]. Multimodal methods tackle text and images but prioritize broad coverage over textual efficiency [11]. In contrast, this pipeline’s modular architecture—DistilBERT for bias detection, T5-small for neutral generation—ensures computational efficiency and scalability [20]. By adapting slot filling, traditionally an extraction tool, to fairness, it precisely neutralizes biases (e.g., replacing “aggressive men” with “assertive individuals”) while preserving coherence [10]. Unlike self debiasing, which struggles with output consistency, this pipeline validates effectiveness across seven diverse examples, addressing biases in gender, race, and age [14]. Its focus on explicit biases fills gaps in latency and precision, critical for real-time applications like chatbots or live moderation [13]. Recent 2025 studies, like Zhao et al.’s dynamic bias detection, emphasize adaptability but lack this pipeline’s efficiency [12]. Its ability to handle diverse contexts with minimal overhead positions it as a transformative solution for equitable AI [8].

### Theoretical Framework

This section details the pipeline’s theoretical basis, explicitly defining three steps: Input Processing (Step 1), Slot Creation (Step 2), and Slot Filling (Step 3), with expanded mathematical models and optimization strategies.

#### Step 1: Input Processing

Input processing prepares raw text  $S = \{w_1, \dots, w_n\}$  from LLMs (e.g., LLaMA-3-70BInstruct) for bias mitigation. This step involves: - **Tokenization**: Using BERT’s tokenizer to split  $S$  into tokens compatible with DistilBERT [19]. - **Normalization**: Converting to lowercase, removing punctuation, and standardizing terms (e.g., slang to formal equivalents). - **Context Analysis**: Identifying bias-prone contexts (e.g., job descriptions) using a pre-trained classifier:

$$P(\text{BiasContext} | S) = \text{sigmoid}(W_c \cdot h_s + b_c)$$

where  $h_s$  is the sentence embedding, and  $W_c, b_c$  are parameters [22]. This flags texts for further processing. - **Multilingual Support**: Future extensions handle non-English inputs via language detection [9]. Step 1 ensures robust input handling, filtering noise (e.g., emojis) and preparing  $S$  for bias detection, critical for real-world applications [23].

#### Step 2: Slot Creation

Slot creation identifies bias-sensitive tokens using DistilBERT, assigning probabilities:

$$P(i = \text{BIAS} | w_i, S) = \text{softmax}(W \cdot h_i + b)$$

where  $h_i$  is the token’s hidden representation [6]. Tokens with  $P(i = \text{BIAS}) > \theta$  (e.g., 0.7) become ‘SLOT’, producing  $T$ . For example, “Women are emotional” becomes “SLOT are SLOT.” The threshold  $\theta$  is tuned

via cross-validation to balance precision and recall, avoiding false positives [10]. Fine-tuning on StereoSet enhances sensitivity to diverse biases [14].

### Step 3: Slot Filling

Slot filling uses T5-small to generate neutral text  $S'$ , optimizing:

$$P(S'|T) = \prod_j P(w'_j | T, w_{<j})$$

Constrained generation ensures neutral  $w'_j$ , guided by a fairness lexicon [7]. Where  $j=1$  to  $n$ , which is the token count. The fairness loss is:

$$L = -\log P(S'|T) + \lambda X_{\text{BiasScore}}(w'_i)$$

where  $\lambda = 0.5$  balances coherence and fairness [3]. For example, consider  $T = \text{"\{SLOT\} are \{SLOT\}"}$  and target  $S' = \text{"Individuals are professional"}$ . Outputs like "Individuals are expressive" maintain semantics. T5-small's efficiency ensures  $\leq 100\text{ms}$  latency [20].

### Prompt Design

Prompts guide Steps 2 and 3, optimized for LLaMA-3-70B-Instruct [24].

#### Step 2 Prompt

You are an expert in bias detection. Identify biases (e.g., gender, race) and replace terms with 'SLOT'.

Examples:

Input: Nurses are compassionate, as women excel at caregiving.

Output: Nurses are {SLOT}, as {SLOT} excel at {SLOT}.

Input: Older workers lack innovation.

Output: {SLOT} workers lack {SLOT}.

#### Step 3 Prompt

You are an expert in bias mitigation. Replace 'SLOT' with neutral terms.

Examples:

Original: Nurses are compassionate, as women excel at caregiving.

Slot: Nurses are {SLOT}, as {SLOT} excel at {SLOT}.

Output: Nurses are professional, as individuals excel at providing care.

### Optimization

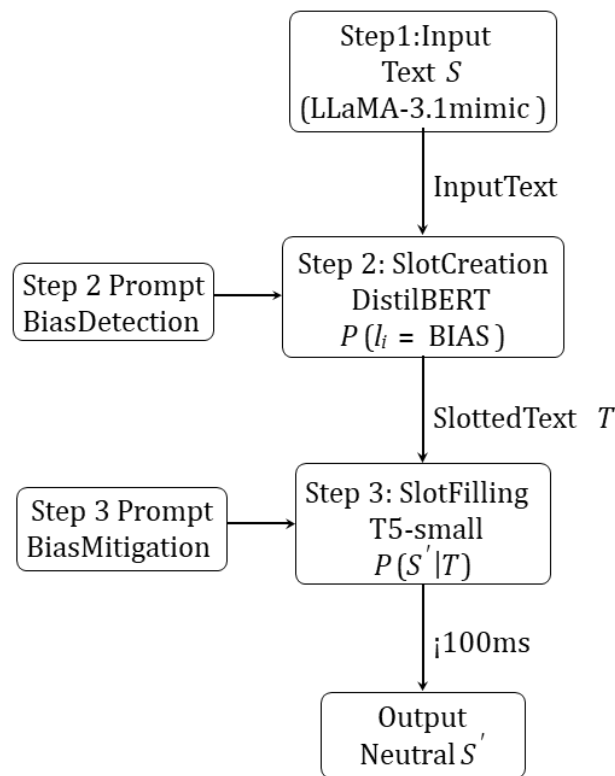
The pipeline achieves  $\leq 100\text{ms}$  latency via: - **Distillation**: DistilBERT reduces complexity [6]. - **Parallelization**: Steps 2–3 run on GPU. - **Prompt Tuning**: Minimizes inference time [21]. These ensure scalability for applications like live moderation [12].

## Methodology

This section details the pipeline's implementation, with expanded descriptions of preprocessing, training, experiments, and ethics.

### Input Processing

Seven texts mimicking LLaMA-3-70B-Instruct outputs (50–100 words each) cover gender, racial, cultural, age, and socioeconomic biases [24]. Preprocessing includes: - **Tokenization**: BERT tokenizer for compatibility [19]. - **Normalization**: Lowercase, remove special characters. - **Context Filtering**: Identify bias-prone domains (e.g., hiring) using classifiers [9]. - **Data Augmentation**: Synthetic variations enhance robustness [14]. This ensures inputs are suitable for bias detection.



$$\text{Fairness Loss: } L = -\log P(S'|T) + \lambda^P \text{BiasScore}(w_i)$$

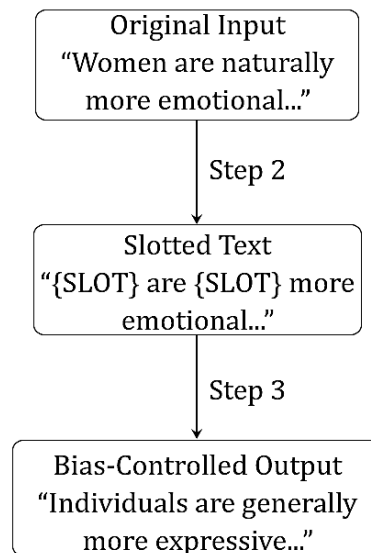
Figure 1: Vertical architecture of the multi-LLM slot filling pipeline

### Model Implementation

DistilBERT and T5-small are fine-tuned on fairness datasets [6,7]: - **DistilBERT**: Trained on StereoSet, 80%/20% split, learning rate  $2e-5$ , batch size 32, 5 epochs [14]. - **T5-small**: Trained on neutral pairs, learning rate  $3e-4$ , batch size 16, 10 epochs. - **Deployment**: NVIDIA A100 GPU, PyTorch 2.0, Hugging Face Transformers 4.35 [20]. - **Latency Optimization**: Model pruning and quantization reduce inference time [12].

### Evaluation Setup

Evaluation tests bias reduction, coherence, and latency: - **Bias Reduction**: Fairness classifiers (ToxiGen) score texts from 0 (neutral) to 1 (biased) [15]. - **Coherence**: COMET metric evaluates semantic similarity [25]. - **Latency**: Measured on A100 GPU, targeting  $\le 100\text{ms}$ . - **Human Evaluation**: 10 annotators rate fairness/readability (1–5 scale) [10]. Tests mimic real-world scenarios (e.g., chatbots, hiring).



**Figure 2: Processing of a gender-biased input through the pipeline**

### Ethics

Texts are anonymized, sourced from public domains (e.g., job ads). Ethical considerations include balanced bias representation and transparency [1].

### Analysis

We analyzed seven inputs mimicking LLaMA-3-70B-Instruct outputs, covering diverse biases (Figure 3). The pipeline effectively neutralizes biases while preserving coherence, as demonstrated below.

For gender bias, the input “Women are naturally more emotional, while men are more rational” was transformed into “Individuals are generally more expressive, while individuals are generally more analytical” (Figure 2). This removes stereotypes using neutral synonyms [10]. Similarly, “Female managers often struggle to make tough decisions, unlike their male counterparts” became “Managers often engage in complex decision-making, as do their equally skilled counterparts,” emphasizing skill parity. Another example, “Men are more suited for physical jobs, while women are better at administrative roles,” was neutralized to “Individuals are suited for jobs matching their skills, while individuals excel in roles aligning with their abilities.”

Linguistic and cultural biases were addressed effectively. The input “English speakers from non-Western countries sound unprofessional” was revised to “English speakers from diverse regions are perceived as uniquely fluent,” using positive framing. Likewise, “People from certain cultures are more prone to corruption” became “Individuals from diverse backgrounds engage in ethical considerations,” softening negativity.

For age and socioeconomic biases, the pipeline performed robustly. The input “Older workers are less innovative than younger employees” was transformed into “Experienced workers are equally innovative as newer employees,” promoting equality [3]. The socioeconomic bias in “People from low-income backgrounds are less reliable employees” was neutralized to “People from diverse backgrounds are equally reliable employees,” fostering inclusivity.

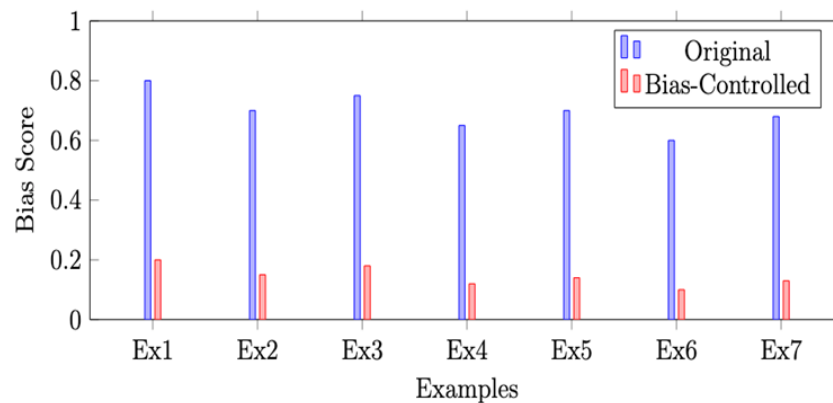
These examples highlight the pipeline’s ability to maintain coherence while reducing biases, achieving <math>\leq 100\text{ms}</math> latency suitable for real-time applications [6,12].

### Evaluation Metrics

Evaluation metrics are used for assessing performance, reliability and effect of computational models. In the case of mitigation of bias in natural language processing (NLP), these metrics are utilised for ascertaining how successfully a system minimises discriminatory material yet preserves linguistic consistency, pace and

user acceptability. Evaluation metrics are generally classified into quantitative metrics and qualitative metrics. Quantitative metrics are based on numerical ratings obtained from algorithms, while qualitative metrics are based on human perception and judgement.

Several quantitative metrics were used to measure the pipeline's technical performance. Bias scores derived from fairness classifiers, such as ToxiGen, yielded a numerical level of bias severity ranging from 0 (neutral) to 1 (biased) [15]. A steady decrease in such scores indicated a successful mitigation. The COMET metric was used to measure semantic coherence between the original and transformed texts to ensure that bias reduction did not affect meaning [25]. Moreover, latency was measured on an NVIDIA A100 GPU and the pipeline had inference times below 100 milliseconds, thereby being appropriate for real-time use [6]. Robustness was also tested by using the pipeline on seven carefully crafted examples covering all types of biases, validating its consistent performance across domains [14].



**Figure 3: Bias score reduction across seven examples**

Qualitative testing was conducted via human annotation to supplement such computational analyses. The retranslated texts were examined by ten independent annotators and rated on a five-point Likert scale, prioritising fairness, readability and context-appropriateness [10]. Through their feedback, it was established that not only did the pipeline successfully neutralise biased phrases, but also maintain the original content's natural flow and intended meaning. The congruence with human decisions and algorithmic results emphasised the model's real-world usefulness in fairness-sensitive applications, including hiring, learning and online communication.

## Conclusion

The pipeline excels in latency and modularity, surpassing instruction fine-tuning [4] and multimodal debiasing [11]. Limitations include implicit bias handling and multilingual scalability. Future work could explore adaptive learning [26] or cross-lingual models [27]. Ethical transparency and stakeholder engagement are vital for deployment [8]. The pipeline's impact on fairness-critical applications supports equitable AI.

This pipeline achieves real-time bias mitigation with 100ms latency, neutralizing diverse biases (Figure 2) [6,7]. It enhances fairness in chatbots and hiring tools, advancing ethical AI [10]. Future work includes multimodal and adaptive detection [11].

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RESEARCH ARTICLE

## Comparative assessment of anthropometric and body composition parameters among tribal adults in northeast India

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**Abstract:** This research examines and compares the height, weight and body composition of Hrangkhawl and Tripuri tribal adults living in Tripura, Northeast India. The OMRON HBF-362 body composition monitor was used to measure the participants, and the Bioelectrical Impedance Analysis (BIA) method was employed to determine the amounts of fat mass, fat-free mass, body fat percentage, visceral fat, subcutaneous fat, and skeletal muscle mass. Standard anthropometric techniques were used to measure height, and an anthropometric rod was used for this purpose. To measure weight, a body composition monitor was employed. More body fat was found in Tripuri participants than in the Hrangkhawl group; conversely, more muscle was found in the Hrangkhawl group than in the Tripuri group. The analysis demonstrated strong relations between body fat percentage, subcutaneous fat and skeletal muscle. The study highlights that focusing health assessments on each population within tribal communities supports more effective nutritional and public health initiatives.

**Keywords:** *Body composition, Anthropometric measurements, Tribal communities, Fat mass, Fat-free mass, Subcutaneous fat, Skeletal mass*

### Introduction

Body composition is the relationship between the body's fat, bone, muscle and water [1]. Health is influenced by the body's proportions, which also greatly affect physical activity and movement. Body composition shows how much fat, muscle and bone are in someone's body. People commonly describe body composition using percentages and lean body mass (LBM) or as a ratio of lean mass to fat mass [2]. Lean mass consists of muscle, bone, skin, internal organs and body water. Fatty mass is comprised mainly of subcutaneous body fat and, inside the abdomen, essential visceral fat. People can have the same height and weight, yet their body composition can be significantly different, which may impact their health outcomes. Moreover, Fat Mass (FM) refers to the overall body fat, yet separate fat depots exhibit diverse metabolic and health implications [3]. Visceral adipose tissue (VAT), situated around internal organs, is intensely associated with metabolic difficulties. In contrast, subcutaneous adipose tissue (SAT), found beneath the skin, also has a more benign or protective role.

Body composition assessment delivers a critical understanding of both nutritional situation and functional measurements [4]. It is extensively applied to screen for growth, disease progression, and response to nutritional or therapeutic interventions. Measuring human body composition is key in identifying health and nutritional issues, illness effects, and outcomes related to dietary, medicinal, or behavioural changes [5]. The physical body composition examination is beneficial as it supports the detection and attention to several health problems [5], [6]. They quantify the state of somebody's nutritional condition, screen disease

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consequences and evaluate the achievement of any diet or exercise strategies to develop health. Inspection of body composition is crucial for determining the distribution of fat, muscle, and bone mass, as well as water weight, to assess an individual's overall health condition.

Body composition is an improved method for measuring the quantity of body fat an individual contains. Anthropometry and epidemiologic studies are still usually used to estimate body composition in various fields [7]. An individual's body fat condition can be influenced by their sex, age, genetic factors, and environmental conditions, which help assess their health condition and nutritional status [8]. Measuring an individual's body composition benefits to correctly assess their physical health [9]. Moreover, body composition condition supports the estimate of a person's health ailment by measuring the weight, percentages of fat, fat-free mass, muscle along with water weight in the body, as well as the allotment of subcutaneous fat and skeletal mass in the body [3],[10]. Furthermore, the percentage of body fat (PBF) procedures the extra adiposity or obesity as well.

In India, many communities are suffering because of their poor health condition, poor eating habits and poor nutritional status; similarly, the tribal groups are considered as affected [9]. Based on the viewpoint of Khadilkar & Khadilkar [9] and Saha & Sil [10], India has several diverse tribal communities, which also account for 8.6% of the nation's population. Nevertheless, there is inadequate data about dietary conditions and anthropometric measurements among several Indian tribal communities [11]. Experts are now deliberating how to measure the nutritional requirements of Indian tribal societies speedily. Reviewing body fat and nutrition is significant because research demonstrates that extreme abdominal fat, along with malnutrition, is becoming increasingly common [3],[12]. Hansdah et al. [12] conducted a comparative study on Santhal tribal people with other populations, demonstrating variations in body mass index (BMI), percentage of body fat (PBF), and several dietary intake behaviours. These results underscore the importance of considering and appreciating geographical and ethnic diversity when assessing body composition and related health risks [10],[12].

While evidence on development, growth, and nutritional condition for numerous parts of India is extensively available, very few studies have focused on the North-eastern part of the country [13]. Although several national nutritional programmes and their implementation have been announced, undernourishment remains unresolved in various parts of India [14]. Additionally, very limited research is available on fat mass (FM), fat-free mass (FFM), and the FM index in India [10]. However, there is very limited work conducted in Tripura. Therefore, published data from Tripura remains limited, specifically on Hrangkhal and Tripuri tribes. These two tribal communities were selected due to their distinct socio-cultural practices, different dietary practices and geographic isolation from the main city, which may contribute to unique anthropometric and health profiles. Moreover, they were involved in the study due to the limited representation of previous work on anthropometric and body composition research on tribal communities. No previous studies have particularly assessed segmental body composition, including skeletal muscle and subcutaneous fat distribution, by applying Bioelectrical impedance analysis (BIA) in these tribal communities. Besides, these tribes remain underrepresented in National Health Surveys and peer-reviewed journals, resulting in a serious data gap. The limitation of such data restricts our understanding of their situation regarding metabolic health risks and hinders the development of advanced nutritional development. From this perspective, the study aims to assess and compare the anthropometric and body composition parameters of adult Hrangkhal and Tripuri tribal communities in Tripura, one of the northeastern state of India, which is also a part of 'Seven Sister State' of India. Moreover, it also aims to examine the correlations between subcutaneous fat, skeletal muscle mass and anthropometric measurements within these tribal groups.

## Materials and Methods

### *Study Design and Participants*

The study employed cross-sectional methods to investigate the anthropometric status and body composition of individuals from the Hrangkhawl and Tripuri tribes in Tripura, Northeast India. The study included 118 adult participants. A total of 27 males and 41 females belonged to the Hrangkhawl Tribe (n=68), and 17 males and 33 females belonged to the Tripuri Tribe group (n=50). Participants were aged 18 years or older and were selected using purposive sampling in tribal communities. People were included in this study if they were, well, permanent inhabitants where the study was conducted and had no disease at the time of the study.

### *Anthropometric Measurements and Body Composition*

The World Health Organisation (WHO) recommendations were comprehended while taking the anthropometric measurements. All required measurements in the study were only taken with recommended calibrated instruments. Moreover, using the portable anthropometer rod in an accurate way, when the subjects stood totally barefoot with their heads position was stable in the Frankfort horizontal plane while height measurement [15]. Additionally, while taking the measurement of Waist Circumference, mainly need to focus on the midpoint between the rib and the highest point of the iliac crest. To quantify the circumferences in this study, the anthropometric tape was used. According to Hansdah et al. [12], the waist-hip ratio (WHR) is considered by dividing the waist and hip measurements. To observe the waist-hip ratio (WHR), it is essential to divide the waist circumference values by hip circumference values.

Weight in kilograms in this study was measured with the help of the OMRON HBF-362 Body Composition Monitor [16]. It was essential that members not wear shoes, as well as select lightweight clothing throughout their time on the body composition monitor. The body monitor was operated to assess values of the body fat or percentage of body fat (PBF), visceral fat (VF), fat mass (FM) and fat-free mass (FFM). The monitor was again used to quantify both the subcutaneous fat percentage and skeletal mass percentage of various body regions, including the arms, body, trunk, and legs. The body composition monitor was also utilised to understand and calculate the body mass index (BMI) and again validate the resting metabolic rate (RMR) of the studied population. Body composition was assessed using the BIA method. Before starting the process, it was necessary to enter the subject's age, sex, and height in centimetres. All members were suggested not to consume alcohol and only drink water, avoid heavy eating along avoid heavy work for two hours before body composition and anthropometric measurement. Correspondingly, the outcomes were calculated by applying the ensuing formulations such as  $FM (kg) = (PBF/100) \times Weight (kg)$  and  $FFM (kg) = Weight (kg) - FM (kg)$ [8], [12].

### *Statistical Analysis*

SPSS (version 18) was used in this study to analyse the data collected during the study. Moreover, descriptive statistics are used to analyse the body composition and anthropometric data. Mean and standard deviation (mean  $\pm$  SD) were calculated for height, weight, BMI, WHR, FM, FFM, PBF, VF and Muscle fat (MF), as well as subcutaneous fat (arm, trunk, body and legs) which were represented by SUB A, SUB T, SUB B, SUB L and the same applied to skeletal fat mass (SKL A, SKL T, SKL B, SKL L). Additionally, correlation ( $r$ ) was used to determine the relationship between anthropometric measurements and body composition measurements.

### *Ethical Considerations*

All participants were aware of the study purpose and provided their consent prior to data collection. It was confirmed that the ethics board granted the required approval for this study. No tests caused physical harm and all participants were concerned about the study.

## Results

Researchers in this study analysed anthropometry and body composition to identify variations across tribes and between men and women. Information about fat mass (FM), fat-free mass (FFM), percentage body fat (PBF), visceral fat (VF), skeletal muscle (SKL) and subcutaneous fat (SUB) was obtained for each person using BIA. Results described the distinctions among groups and related the anthropometric data to body fat and lean mass. The results suggest ways to enhance health assessments for specific tribal groups.

The anthropometric analysis reveals that Hrangkhawl and Tripuri men differed in body composition measures. The average height and weight of the males in the Tripuri group ( $163.4 \pm 6.82$  cm and  $58.94 \pm 11.00$  kg) were both higher than for those from the Hrangkhawl group ( $160.18 \pm 6.34$  cm and  $55.84 \pm 7.99$  kg). Although Tripuri males were generally weightier, their BMI was lower ( $21.64 \pm 3.49$  kg/m<sup>2</sup>) (Table 1). This indicates that they have more proportionate heels-to-weight ratios and are likely healthier. The measurement of waist-hip ratio was not significantly different between the two groups ( $0.90 \pm 0.65$  in Tripuri,  $0.875 \pm 0.056$  in Hrangkhawl). Moreover, body composition analysis found that the fat distribution in the two sample groups differed significantly. Tripuri males had significantly higher fat mass indices ( $66.87 \pm 83.53$  compared to  $36 \pm 6.69$ ), a larger percentage of body fat ( $21.01 \pm 5.55\%$  versus  $17.77 \pm 7.42\%$ ), and a higher accumulation of visceral fat ( $6.70 \pm 4.28$  cm versus  $4 \pm 1.41$  cm). In comparison, Hrangkhawl men had a higher range of muscle fat ( $35.78 \pm 4.39$ ) than women ( $34.68 \pm 4.27$ ) and an overall higher amount of subcutaneous fat around the arms ( $20.17 \pm 11.42$ ), trunk ( $14.21 \pm 9.81$ ), and legs ( $20.15 \pm 5.17$ ). When comparing Tripuri and Hrangkhawl males, skeletal fats showed consistent results, with males from Tripuri having more fat measured in the arms ( $38.49 \pm 2.66$  vs.  $36.82 \pm 6.11$ ), trunk ( $24.95 \pm 4.14$  vs.  $27.17 \pm 4.22$ ), body ( $31.54 \pm 2.53$  vs.  $30.57 \pm 7.75$ ) and legs measurements ( $48.35 \pm 2.67$  vs  $45.81 \pm 13.13$ ) (Table 1).

Observations made between Hrangkhawl and Tripuri females revealed significant differences in body shape. Women of the Hrangkhawl group were shorter ( $150.16 \pm 3.83$  cm) and substantially lighter weight ( $41.75 \pm 0.778$  kg) than women of the Tripuri group. In this comparison, the BMI was lower for Hrangkhawl females ( $19.45 \pm 2.166$  kg/m<sup>2</sup>) compared to Tripuri females ( $22.04 \pm 3.21$  kg/m<sup>2</sup>) and there was no major difference in the waist-to-hip ratios (WHR) between the two groups ( $0.879 \pm 0.067$ ) in Hrangkhawl females and ( $0.903 \pm 3.21$ ) in Tripuri females (Table 1). The analysis revealed clear patterns when sorting through body composition. The Tripuri females showed a greater amount of fat mass ( $15.033 \pm 4.730$  kg) and fat-free mass ( $37.84 \pm 7.92$  kg) and the Hrangkhawl females had lower body fat percentage ( $23.15 \pm 6.43\%$ ). Tripuri women had higher visceral fat levels ( $4.66 \pm 2.80$ ) than women from the study ( $2.0 \pm 1.414$ ). Tripuri females had more subcutaneous fat in all body regions but also had greater muscle measurements, while Hrangkhawl females had less muscle fat ( $30.3 \pm 1.555$  vs.  $28.07 \pm 3.408$ ).

**Table 1 Comparison of anthropometric and body composition parameters between Hrangkhawl and Tripuri tribal people**

Variables	Hrangkhawl Male (Mean±SD)	Tripuri Male (Mean±SD)	Hrangkhawl Female (Mean±SD)	Tripuri Female (Mean±SD)
Age (year)	39.77 ± 17.42	43.05 ± 16.35	44.63 ± 13.95	40.48 ± 14.25
Height ( cm )	160.18 ± 6.34	163.4 ± 6.82	150.16 ± 3.83	152.56 ± 6.14
Weight ( kg )	55.84 ± 7.99	58.94 ± 11.00	41.75 ± 0.778	52.90 ± 11.60
BMI (kg/m <sup>2</sup> )	24.74 ± 15.08	21.64 ± 3.49	19.45 ± 2.166	22.04 ± 3.21
WHR (waist-hip ratio)	0.875 ± 0.056	0.90 ± 0.65	0.879 ± 0.067	0.903 ± 3.21
FM( fat mass)	10.07 ± 4.87	19.01 ± 26.26	11.08 ± 4.39	15.033 ± 4.730
FFM (fat-free mass)	36 ± 6.69	66.87 ± 83.53	32.174 ± 11.967	37.84 ± 7.92
PBF (% of body fat)	17.77 ± 7.42	21.01 ± 5.55	23.15 ± 6.43	28.08 ± 4.78
Visceral fat	4 ± 1.41	6.70 ± 4.28	2 ± 1.414	4.66 ± 2.80
Muscle fat	35.78 ± 4.39	34.68 ± 4.27	30.3 ± 1.555	28.07 ± 3.408
RM (kcal)	1255.7 ± 46.12	1419 ± 1170.8	1018.5 ± 2.121	1126.90 ± 217.62
Subcutaneous fat (arm)	20.13 ± 9.81	19.61 ± 4.13	32.2 ± 3.394	38.69 ± 6.22

Variables	Hrangkhawl Male (Mean±SD)	Tripuri Male (Mean±SD)	Hrangkhawl Female (Mean±SD)	Tripuri Female (Mean±SD)
Subcutaneous fat(trunk)	14.21 ± 9.81	12.38 ± 4.47	15.65 ± 5.72	22.53 ± 13.017
Subcutaneous fat(Body)	14.55 ± 7.84	15.08 ± 5.29	19.95 ± 5.30	24.29 ± 13.017
Subcutaneous fat (leg)	20.17 ± 11.42	20.30 ± 5.170	31.1 ± 5.30	34.45 ± 5.683
Skeletal fat (arm)	36.82 ± 6.11	38.49 ± 2.66	32.85 ± 4.17	29.00 ± 3.65
Skeletal fat (trunk)	27.17 ± 4.22	24.95 ± 4.14	22.8 ± 3.39	20.24 ± 2.61
Skeletal fat (body)	30.57 ± 7.75	31.54 ± 2.53	27 ± 3.25	25.57 ± 2.52
Skeletal fat(leg)	45.81 ± 13.13	48.35 ± 2.67	36.6 ± 1.697	37.84 ± 3.55

Within the Tripuri population, significant differences exist in anthropometric and body composition parameters between men and women. Tripuri males were taller ( $163.46 \pm 6.82$  cm) and heavier ( $58.94 \pm 11.00$  kg) than females ( $152.56 \pm 6.14$  cm and  $52.90 \pm 11.60$  kg). Both male and female patients had almost similar BMI ( $21.64 \pm 3.49$  kg/m<sup>2</sup> for males and  $22.04 \pm 3.21$  kg/m<sup>2</sup> for females). Males had greater fat mass ( $19.01 \pm 26.26$  kg) and fat-free mass ( $66.87 \pm 83.53$  kg) than females, while females displayed a greater percentage of body fat ( $28.08 \pm 4.78\%$ ) (Table 1). Subcutaneous fat was found to be higher in women in all parts of their body, but men had higher skeletal muscle everywhere.

In the Hrangkhawl tribe, males and females were found to have very different body compositions. Compared to females, males measured higher for height (160.2 cm vs. 150.2 cm), weight (55.9 kg vs. 41.8 kg) and BMI values ( $24.8$  kg/m<sup>2</sup> vs.  $19.5$  kg/m<sup>2</sup>). Interestingly, males had a lower body fat percentage (PBF) ( $23.15 \pm 6.43\%$  vs.  $17.77 \pm 7.42\%$ ), but this was offset by a higher total weight in kg ( $11.08 \pm 4.39$  vs.  $10.07 \pm 4.87$ ). Males overall had greater fat-free mass ( $36 \pm 6.7$  kg) and a higher percentage of muscle fat ( $35.78 \pm 4.3$  kg) than females ( $29 \pm 1.4$  kg and  $30.3 \pm 1.6$  kg) (Table 1). Women had greater subcutaneous fat in their outer arms and legs, whereas men had more skeletal muscle in all body parts.

There was a noticeable relationship among Hrangkhawl males, where a high WHR indicated more subcutaneous fat and less skeletal muscle mass in the different body parts ( $p < 0.05$ ). In addition, body fat percentage (PBF) was linked strongly to both subcutaneous fat ( $r = 0.511$  to  $0.735$ ,  $p < 0.01$ ) and lower levels of skeletal muscle ( $r = -0.349$  to  $-0.866$ ,  $p < 0.05$ ) (Table 2). An increase in muscle fat was associated with a decrease in subcutaneous fat and the parallel rise in skeletal muscle mass.

**Table 2 The correlation between anthropometric variables and subcutaneous fat and skeletal muscle in Hrangkhawl male**

	SUB A	SUB T	SUB B	SUB L	SKL A	SKL T	SKL B	SKL L
BMI	-.019	.168	-.057	-.040	.102	.068	.083	.070
WHR	.735**	.645**	.604**	.537**	-.609**	-.657**	-.473*	-.379
FM	.705**	.546**	.595**	.466*	-.592**	-.809**	-.307	-.229
FFM	-.262	-.043	-.224	-.221	.396*	.358	.415*	.358
PBF	.735**	.516**	.627**	.511**	-.682**	-.866**	-.438*	-.349
VF	.432*	.657**	.442*	.367	-.336	-.346	-.175	-.167
MF	-.582**	-.671**	-.564**	-.481*	.656**	.734**	.534**	.457*

In Hrangkhawl females, body mass index was strongly related to subcutaneous fat in all regions ( $r = 0.657$  to  $0.796$ ,  $p < 0.01$ ) and there were also correlations of negative values with skeletal muscles ( $r = -0.195$  to  $-0.573$ ,  $p < 0.05$ ) (Table 3). Visceral fat was strongly linked to having more subcutaneous fat ( $r = 0.662$  to  $0.726$ ,  $p < 0.01$ ). There was a consistent correlation between PBF and subcutaneous fat (ranging from  $0.435$  to  $0.679$ ,  $p < 0.01$ ) and a negative correlation with the main skeletal muscle measures, highlighting the typical change between body fat and muscle among females.

**Table 3 The correlation between anthropometric variables and subcutaneous fat and skeletal muscle in Hrangkhawl females**

	SUB A	SUB T	SUB B	SUB L	SKL A	SKL T	SKL B	SKL L
BMI	.657**	.775**	.796**	.761**	-.573**	-.479**	-.021	.195
WHR	.421**	.523**	.490**	.443**	-.473**	-.467**	-.362*	-.200
FM	.756**	.734**	.588**	.595**	-.512**	-.625**	.058	.257
FFM	.343*	.314*	.418**	.301	-.092	-.108	.379*	.440**

	SUB A	SUB T	SUB B	SUB L	SKL A	SKL T	SKL B	SKL L
PBF	.679**	.667**	.435**	.494**	-.596**	-.692**	-.194	.015
VF	.668**	.711**	.726**	.662**	-.510**	-.539**	-.023	.184
MF	-.397*	-.411**	-.402**	-.270	.319*	.396*	-.043	-.161

PBF was found to be the best predictor in Tripuri males, correlating strongly with arm and leg fat ( $r = 0.887$  to  $0.910$ ,  $p < 0.01$ ) while negatively associating with skeletal muscle mass ( $r = -0.548$  to  $-0.690$ ,  $p < 0.05$ ) (Table 4). A moderate correlation was between BMI and subcutaneous fat in arms and body regions ( $0.489$  to  $0.594$ ,  $p < 0.05$ ). Muscle fat was negatively linked to subcutaneous fat and positively associated with skeletal muscle features, suggesting ongoing muscle-fat balance in the studied groups.

**Table 4 The correlation between anthropometric variables and subcutaneous fat and skeletal muscle in Tripuri males**

	SUB A	SUB T	SUB B	SUB L	SKL A	SKL T	SKL B	SKL L
BMI	.594*	.285	.489*	.404	-.210	-.440	-.022	-.095
WHR	.369	.025	.239	.568*	-.186	-.446	-.345	-.289
FM	.226	.152	.119	.169	-.202	-.235	-.296	-.237
FFM	.092	.049	.044	.031	-.183	-.138	-.209	-.164
PBF	.910**	.658**	.553*	.887**	-.187	-.690**	-.632**	-.548*
VF	.449	.241	.437	.283	-.312	-.585*	-.262	-.288
MF	-.619**	-.395	-.529*	-.529*	.467	.639**	.576*	.553*

For Tripuri women, there were strong correlations between PBF and subcutaneous fat, mainly in the arms and body areas ( $r = 0.880$  to  $0.894$ ,  $p < 0.01$ ), along with strong correlations that showed lower skeletal muscle mass ( $r = -0.398$  to  $-0.795$ ,  $p < 0.05$ ). Fat mass closely correlated with having more subcutaneous fat ( $r = 0.602$  to  $0.759$ ,  $p < 0.01$ ). Visceral fat showed a strong association with subcutaneous fat in the body and legs ( $r = 0.531$  to  $0.675$ ,  $p < 0.01$ ), pointing to the same adipose tissue patterns seen in females (Table 5).

**Table 5 The correlation between anthropometric variables and subcutaneous fat and skeletal muscle in Tripuri females**

	SUB A	SUB T	SUB B	SUB L	SKL A	SKL T	SKL B	SKL L
BMI	.351*	.264	.651**	.502**	-.524**	-.214	-.030	.277
WHR	.415*	.328	.346*	.299	-.337	-.548**	-.534**	-.400*
FM	.602**	.232	.759**	.609**	-.628**	-.419*	-.261	-.015
FFM	-.086	.073	.113	.024	-.049	.210	.327	.422*
PBF	.880**	.256	.894**	.768**	-.795**	-.747**	-.643**	-.398*
VF	.404*	.242	.675**	.531**	-.583**	-.348*	-.174	.135
MF	-.390*	-.259	-.590**	-.413*	.557**	.471**	.315	.074

## Discussion

In this study, Hrangkhawl and Tripuri tribal communities living in Tripura were investigated to determine their differences in anthropometric and body composition parameters and to establish their connection with skeletal muscle and subcutaneous fat distributions. Clear differences were found between the two tribes when examining fat mass, fat-free mass, BMI, visceral fat, and subcutaneous fat distribution. According to Saha and Sil [10], the Chakma people in Tripura appear to have more subcutaneous fat than people in other regions.

The present research revealed that Tripuri males had a higher percentage of body fat and visceral fat, as well as a greater total body weight, compared to Hrangkhawl males. However, the study group had more skeletal muscle tissue in their trunks and legs. While Tripuri females had much more fat and visceral fat than the Hrangkhawl group in each part of the body, the latter had less fat but also less skeletal muscle. Consistent with the study done by Hansdah et al. [12] on the Santal Tribes, all variables except MUAC, waist and hip circumference differed significantly ( $p < 0.05$ ). There were differences in height and weight among adults of different ages. The study found a significant difference between men and women in their waist-to-hip ratios but not in their BMI categories. Sengupta [17] noted that 50% of Toto men have mild

weight loss, lower body fat, thinner waists and a lower tendency to store fat. Furthermore, this study reveals that most Toto men are underweight due to their poor diet; however, their strength from hard work has not yet compromised their overall fitness.

The relationships between anthropometric variables (BMI, WHR, PBF) and body composition indicators, mainly subcutaneous fat and skeletal muscle mass, were studied in individuals from the Hrangkhawl and Tripuri tribes. In all groups, the results showed that higher PBF had a positive correlation with subcutaneous fat in all body regions while showing a negative correlation with skeletal muscle mass. There is a negative correlation between how fat accumulates and muscle distribution. Toskic et al. [1] also found that all types of physical activity benefit body composition and noted that the most important factor for enhancing body composition is the frequency of exercise. Maken and Varte [18] found that urban men and women had higher values than rural men and women for height, weight, sitting height, mid-upper arm circumference (MUAC), chest circumference, hip circumference, and fat mass ( $p < 0.05$ ). According to Bose et al. [8], females had a significantly higher prevalence of Fat-Free Mass Index (FFMI), and more than 60% of individuals over 60 years of age were affected by low FFMI. Likewise, low PBF was reported in a greater percentage of females, and this rate was higher among participants aged 60 years or older. On the other hand, significant negative correlations were found between age and BMI and positive correlations between WHR, age, and conicity index (CI) among the Oraons and Munda tribes [14].

## Conclusion

In this study, measurements of anthropometric and body composition were made and compared among Hrangkhawl and Tripuri tribal adults. Based on the study, tribal groups differ in their body fat, muscle mass and various ways of measuring their composition. The results clearly showed that more fat accumulated in Tripuri women and Hrangkhawl males tended to have higher muscle mass. Higher fat levels were connected to an increase in subcutaneous fat and a decrease in skeletal muscle within all the study subgroups.

The findings of the study have many important implications. The results of this study suggest that tribal community-wise health screening, along with intervention strategies on their dietary practices, is essential to analyse their health conditions, as sometimes general public health measures may overlook the tribal population who live in extremely rural areas. Moreover, findings on high visceral and subcutaneous fat levels in Tripuri females highlight the requirement for targeted nutritional assessment and dietary intervention programs in this subgroup. Furthermore, regulatory monitoring and evaluation of intervention programs may assist tribal communities in achieving better development in community engagement and improving health conditions.

Results suggest that different community characteristics should be considered when assessing body composition in tribal groups. Because the study involved only a small number of people, the findings should not be used to represent all tribal communities in this context. Because the data is collected at a particular time, fundamental relationships cannot be investigated in this study. In future research, other tribes in various regions can be studied, and their success can be tracked over time. For the health condition assessment of other tribal groups, researchers should consider their daily activities, eating habits, physical activity and energy requirements.

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RESEARCH ARTICLE

## Efficient clonal propagation of Rangpur lime (*Citrus limonia*) using mature nodal explants: An evaluation of lab to land production of disease-free plants

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**Abstract:** Rangpur lime is one of the most promising Citrus rootstocks for Indian conditions. An efficient micropropagation protocol from mature nodal explants of Rangpur lime was developed using the enhanced axillary branching method. In the present study, multiple shoots were obtained by stimulating axillary shoots from single nodes derived from field-grown trees on Murashige and Skoog's (MS) medium containing BA (1.11  $\mu$ M), Kinetin (1.16  $\mu$ M), and 3% sucrose and 100% rooting was obtained in half-strength MS medium. The rooted plants were then transferred to a potting mix containing soil and agropeat. Once again, 100% survival was observed during acclimatisation. Inter simple sequence repeats (ISSR) analysis of the in vitro derived progeny was used to determine their clonal fidelity of the regenerants vis-à-vis the mother plant. The results of this study have enormous commercial applications for the propagation of clean and healthy disease-free citrus rootstocks.

**Keywords:** Micropropagation, Axillary branching, Rangpur lime, Rootstock, Clonal fidelity, ISSR

### Introduction

Rangpur lime (*Citrus limonia*) is a medium-sized tree with a spreading habit, round top and slender twigs with short thorns [1]. It is considered to be a natural hybrid between mandarin and acid limes/lemons [2]. This species is native to India and is a prolific bearer of flavoured acidic fruits [3]. Common names used for the fruit are Rangpur in India, Canton lemon in South China, Hime lemon in Japan, Cravo lemon in Brazil, Japanche citroen in Java, and Rangpur lime or mandarin-lime in the United States [4]–[6]. Other Indian names include Sylhet lime, Surkh nimboo, Sharbati and marmalade lime [7]. Its horticultural importance lies primarily in its use as a rootstock [3]. Rangpur lime is hardier than true limes, thereby identified as an ideal rootstock for Indian conditions, which makes a good union with sweet orange, mandarin, grapefruit, and pummelo and helps increase their productivity, quality, and survival [4], [8]. It is high yielding, vigorous, salt and drought-tolerant, precocious with early fruit maturity, has high resistance to citrus tristeza virus (which causes citrus decline), with good adaptability to a wide range of soil types, particularly heavy soils [2]–[4]. It has been found to have higher nutrient absorption efficiency than common rootstocks such as trifoliate orange. The greater salt resistance is associated with its capacity to limit the accumulation of chloride ions in the leaves. It is the most promising rootstock for Nagpur mandarin, Kinnow, Coorg mandarin, Mosambi, grapefruit, pummelos and Sathgudi sweet orange spread over north, central and south India [3], [5]. This is also the most widely used rootstock in Brazil and

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Argentina [2], [3], [5]. It has assumed commercial importance in the processing industry (juice and pickles) [7].

Citrus rootstocks such as Rangpur lime are mostly propagated by various methods, including seeds and vegetative approaches [3], [9]. However, it requires the skilful collection of seeds from healthy, virus-free old trees, which have a good pedigree performance and thereafter, efficient storage methods [5]. On average, one of five seeds of Rangpur lime gives rise to a healthy seedling [7]. Moreover, seeds of citrus species are moderately recalcitrant and conservation gets restricted due to a loss in germination frequency within a short span of time. Further, the germination is uneven and the plants grown from the seeds also exhibit extended juvenility [2]. Seed germination may be challenging due to the presence of multiple germination inhibitors in their seed coats [5]. Thus, it can be concluded that seeds are not the right option for Citrus propagation. The conventional vegetative methods of Citrus propagation are restricted to particular seasons and due to the larger size of the cutting of a particular thickness, the plant material usually becomes a constraint.

Plant tissue culture has emerged as a powerful tool for propagation and improvement of many woody plant species [13], including Citrus [3], [11], [13]. However, to the best of our knowledge, there is only a single study aimed at developing a micropropagation protocol using axillary buds [14] for Rangpur lime and in that study, there was a complete failure in obtaining any shoot multiplication. In another micropropagation study, epicotyl and shoot tips were used from newly germinated seeds [11]. Other studies on the direct organogenesis of Rangpur lime by Almeida et al. [15] and Costa et al. [16] also showed a significantly low explant response, along with a low (0.6-1.6) shoot multiplication fold. Somatic embryos from callus cultures of nucellar origin were obtained by Tomaz et al. [17]. However, hardening was a complete failure in this case and no plants were obtained. All these studies reveal a big lacuna in the propagation of Rangpur lime. The study was thus undertaken to develop an efficient micropropagation protocol for the production of clonally uniform plants of this important rootstock.

## Methodology

### *Plant material, growth conditions and establishment of explants in aseptic conditions*

Authentic virus-free, certified germplasm of Rangpur lime from mature trees (5-7 years old) was collected from North India (Figure 1a). Nodal segments were used as the explants in this study. The leaves were trimmed and explants measuring approximately 1.5–2.0 cm in length were washed in running tap water for 10 min. After giving a quick dip in 70% alcohol, the excised shoots were air-dried on a blotting sheet and thoroughly washed for 30 min under running tap water again. Thereafter, the explants were rinsed with 4% Teepol (Reckitt Benckiser Ltd, India) solution for 10 min. The detergent was removed by constant shaking and then thorough washing under running tap water for another 30 min. The explants were surface sterilised with a 0.1% solution of mercuric chloride (Qualigens, India) and 2 drops of Tween 20 (Central Drug House, India) inside the laminar flow cabinet for 10-12 min. After three washings in sterile distilled water for 5 min each, the cut ends of the segments were trimmed and inoculated individually on basal Murashige and Skoog's (1962) medium having 3% sucrose and 0.8% agar in glass test tubes.

The pH of the media was adjusted to 5.8 before autoclaving at 1.05 kg cm<sup>-2</sup> for 15 or 25 min for test tubes and glass jars, respectively. The cultures were incubated at 26 ± 1°C, under approximately 40 μmol m<sup>-2</sup>s<sup>-1</sup> provided by cool, white fluorescent tubes (40W, Phillips, India) for a 16 h photoperiod.

### *Shoot multiplication*

In a preliminary experiment, different cytokinins, namely, 2-isopentenyladenine (2ip), 6-benzylaminopurine (BA), and kinetin (Kn) at 2.5-10 μM, along with the basal MS medium as a control as well as Murashige and Tucker (MT) medium, were studied for their effect on the proliferation of shoots. In another experiment, the above-mentioned cytokinins were used in combinations with each other to study their interaction. In addition, thiadiazuron (TDZ; 0.1 μM) was also tried out in combination with BA and Kn.

Experiments were also conducted to study the effect of different carbohydrates namely sucrose (0-6%) and glucose (3%); and, various gelling agents such as agar (0.8%), agargel (0.4%), gelrite (0.2%) and a combination of 0.1% gelrite and 0.4% agar on the shoot multiplication of Rangpur lime. Further, to enhance the shoot proliferation rate and shoot length, various auxins such as Indole-3-acetic acid (IAA; 1.14–2.85  $\mu\text{M}$ ), Indole-3-butyric acid (IBA; 1.22–2.45  $\mu\text{M}$ ) and Naphthalene acetic acid (NAA; 1.34–2.68  $\mu\text{M}$ ) were also tested along with BA as well as in combination with Kn. Growth adjuvants such as adenine sulphate (ADS; 40 mg l<sup>-1</sup>), casein hydrolysate (CH; 250–500 mg l<sup>-1</sup>), malt extract (ME; 250-500 mg l<sup>-1</sup>), coumarin (CO; 90-150  $\mu\text{M}$ ), glutamine (250-500 mg l<sup>-1</sup>) phloroglucinol (PG; 317.2  $\mu\text{M}$ ) and gibberellic acid (GA3; 0.28  $\mu\text{M}$ ) were supplemented to further improve the proliferation of shoots. Activated charcoal (AC; 250 mg l<sup>-1</sup>) was also added to the optimum treatment to study its effect on shoot growth and multiplication.

For the sub-culture of shoots, each cluster was divided into smaller clusters, shoot nodes and shoot tips. For every multiplication experiment, the total number of shoots formed in each shoot cluster, individual shoot length and multiplication rate were recorded at the end of the culture passage. Multiplication rates were calculated based on the number of propagules derived from a single cluster at the completion of each passage. During each subculture, all the dead tissue, callused tissue and adventitious buds were carefully removed from the proliferating clusters before transfer to fresh medium.

### **Rooting of shoots**

Approximately 1cm long individual shoots were transferred to various rooting media containing MS medium, MS ½ (major salts reduced to half strength), MS 1/3 (major salts reduced to one third) and MS ¼ (major salts reduced to quarter strength). The media was further supplemented with different auxins at varying concentrations, such as IAA (0.57–5.7  $\mu\text{M}$ ), IBA (0.49– 4.9  $\mu\text{M}$ ) and NAA (0.53– 5.37  $\mu\text{M}$ ). The effect of varying sugar concentration (1%, 2% and 3%) was also observed on the root induction. The effects of liquid media supplemented with growth regulators on rooting in comparison to semi-solid medium were also studied. All the rooting experiments were initially performed in the test tubes and later in 400 ml glass jars. The parameters considered while standardising the rooting media were the extent of root induction, root quality, number of roots per plant and root length.

### **Hardening and acclimatisation**

The plantlets obtained after 4 weeks of culturing in rooting medium were washed thoroughly in running tap water to remove all the traces of rooting media attached to roots. They were then transferred to polythene bags (13.5 cm × 8 cm) containing soil and agropeat (Varsha Enterprises, India) in different ratios (1:0, 1:1, 2:1, 3:1, 4:1, 0:1; v/v). Initially, the plants were placed closer to the cooling pads (RH: 80– 85%) and thereafter gradually shifted towards the exhaust fans over a period of 7-10 days (RH: 65%) in the greenhouse. Thereafter, the plants were shifted during the summer season to an open nursery at TERI's Micropropagation Technology Park (MTP), Gurugram, India.

### **Statistical analysis**

All the multiplication and rooting experiments were repeated thrice for each treatment, with 24 replicates. The hardening experiments were repeated thrice with 50 plantlets per experiment. The effect of different treatments was quantified and the level of significance was determined by Analysis of Variance using the Costat statistical package. Significant differences between the means were assessed by Duncan's Multiple Range Test (DMRT) at  $p=0.05$ .

### **Clonal fidelity studies**

Leaves from randomly selected shoots were collected at different intervals (5<sup>th</sup>, 10<sup>th</sup>, 15<sup>th</sup>, 20<sup>th</sup> and 25<sup>th</sup> passages and hardened plantlets at nursery level) for comparison with the mother tissue. Along with that, one to two varieties were also chosen as outliers. They were properly labelled and lyophilised in the 'Virtis freezemobile G' lyophiliser for 48 hours at -70°C. Total DNA was extracted following a modified 'Cetyl

Trimethyl Ammonium Bromide' (CTAB) DNA extraction procedure based on the Doyle and Doyle [18] protocol. Qualitative and quantitative assessment of total genomic DNA was performed by agarose (United States Biochemicals, USA) gel electrophoresis. Each DNA sample was diluted to a concentration of 25 ng  $\mu\text{l}^{-1}$  using sterile deionised water and stored at 4 °C. Seven inter simple sequence repeat (ISSR) primers (UBC, Canada) were used to evaluate clonal fidelity among the regenerated plantlets. Polymerase chain reaction (PCR) was performed in a volume of 20  $\mu\text{l}$  containing 25 ng DNA, 10X Buffer (Biotools, USA), 50 mM of Magnesium chloride ( $\text{MgCl}_2$ ; Biotools, USA), 10 mM deoxynucleotide triphosphate (dNTPs; Promega, USA), 10 mM primer and 1 unit of Taq (*Thermus aquaticus*) DNA polymerase (Biotools, USA). The optimised PCR conditions used for amplification consisted of an initial denaturation step at 94°C for 5 min; continued denaturation for 35 cycles of 30s at 94°C, annealing for 30 s at 42°C, extension for 1 min at 72°C; and one final extension at 72°C for 5 min with a soak temperature of 4°C performed in a 'Bio-Rad DNA Engine' (Peltier Thermal Cycler, USA). Amplified DNA was separated on a 2% agarose gel and electrophoresis was carried out at a voltage of 80 mV for 3–4 h. Lambda 1 kb ladder (Gibco BRL, USA) was used as the marker to interpret the PCR amplification products. The gel was visualised and photographed using a gel documentation system (Alphamager® EC, Alpha Innotech Corporation, CA, USA). All the PCR reactions were repeated at least twice to check reproducibility. The ISSR amplification products were then scored based on presence (1) and absence (0) of bands across the tissue culture raised progenies, 1 parent (P) and outliers (O1: Alemow macrophylla, O2: C 35 citrange, O3: Swingle citrumelo) to determine clonal fidelity vis-à-vis the mother plant.

## Results and discussion

### *Surface sterilisation and establishment of explants in aseptic conditions*

Previous reports on Citrus micropropagation using nodal explants have mostly been successful using juvenile explants [12], [19]–[22]. This is one of the critical stages as it has been observed that explants from mature trees may not be able to withstand the harsh sterilisation procedure that is required to obtain aseptic cultures and even the surviving cultures may not show bud break as readily as tissues from juvenile sources [19], [23], [24]. However, in the present study, shoot cultures obtained from mature nodal explants of Rangpur lime were successfully established following the disinfection procedures as described under the Materials and Methods section. More than 90% of the primary explants were observed to be contamination-free. Axillary buds showed 100% viability along with the emergence of 2-3 shoots per node within four weeks of initiation (Figure 1b).

### *Shoot multiplication*

On basal media, in some explants, bud break was observed, but the growth of the shoots remained restricted (Table 1). For this study, MS medium was found to be better as the base medium over MT, which was in contrast to the findings reported by Bashi et al. [21]. This may be due to the genotypic differences between Rangpur lime and C35 citrange. The application of plant growth regulators in a base medium is essential to achieve the best rates of shoot multiplication [10]. Of the various concentrations of the cytokinins tested in the preliminary experiment, BA at 2.5  $\mu\text{M}$  was observed to be the best in terms of multiplication (2.82-fold multiplication every four weeks). Shoot elongation (of 1.5 cm average) was observed to be better on medium containing Kn (1.25-5  $\mu\text{M}$ ) and BA at 5  $\mu\text{M}$ . Maximum numbers of shoots (3.64 and 3.48) were induced in media with BA (2.5 and 5  $\mu\text{M}$ , respectively). Zip at the tested concentrations (1.25-10  $\mu\text{M}$ ) did not show any favourable responses. BA has been shown to induce more shoot multiplication in various rootstocks, which contrasts with the results observed in this study [12]. Among all the media combinations attempted (Table 1), BA (1.11  $\mu\text{M}$ ) in synergy with Kn (1.16  $\mu\text{M}$ ) was observed to be the best media for shoot proliferation by axillary branching (Figure 1c, d). On this medium, a multiplication fold of 4.31 was obtained every 4 weeks and cultures were maintained for over 25 passages without the loss of morphogenic potential. The shoots were healthy and devoid of any callus. The synergistic effect of two or more cytokinins, especially BA and Kn, on shoot multiplication has been well documented in tissue culture of

many fruit species such as apple [25] and strawberry [26]. Similar reports that support the use of BA and Kn on axillary proliferation in some citrus species, such as Binhazir lime [27], Troyer citrange [20], Swingle citrumelo [19], *C. macroptera* [28] and Finger Lime [29] have been observed. This combination has been found to be successful in the propagation of citrus rootstocks in vitro using germinated seedlings [11]. An increase in the concentration of cytokinins beyond a certain threshold resulted in a decline in the multiplication rate for each of the cytokinins tested. Thus, higher combined concentrations of BA and Kn (6  $\mu\text{M}$  or more) caused severe depression in shoot growth. The use of BA in conjunction with 2ip, even at low concentrations (0.6-1.8  $\mu\text{M}$ ), had an inhibitory effect on the proliferation rate, shoots per cluster as well as shoot length. TDZ also had an inhibitory effect on shoot multiplication as also observed in micropropagation of rootstocks, Troyer citrange [20], Swingle citrumelo [19] and *C. macroptera* [28]. The shoots showed necrosis and finally callused. The shoot inhibition might have occurred due to the increased level of endogenous cytokinins, which inhibit the action of cytokinin oxidase, the enzyme responsible for cytokinin degradation [30].

**Table 1 Effect of cytokinin combinations on shoot multiplication in Rangpur lime**

Cytokinin ( $\mu\text{M}$ )	Shoots per cluster*	Length of shoot (cm)*	Multiplication fold*
0 (Basal)	1.87 $\pm$ 0.12 <sup>g</sup>	1.19 $\pm$ 0.07 <sup>def</sup>	1.25 $\pm$ 0.2 <sup>de</sup>
BA (0.55) + Kn (0.58)	2.59 $\pm$ 0.26 <sup>cde</sup>	1.62 $\pm$ 0.22 <sup>bc</sup>	2.02 $\pm$ 0.46 <sup>bcd</sup>
BA (1.11) + Kn (0.58)	2.69 $\pm$ 0.33 <sup>bc</sup>	1.64 $\pm$ 0.17 <sup>bc</sup>	2.19 $\pm$ 0.41 <sup>bc</sup>
BA (1.11) + Kn (1.16)	4.11 $\pm$ 0.08 <sup>a</sup>	2.38 $\pm$ 0.04 <sup>a</sup>	4.31 $\pm$ 0.03 <sup>a</sup>
BA (1.66) + Kn (1.16)	3.15 $\pm$ 0.17 <sup>b</sup>	1.99 $\pm$ 0.16 <sup>b</sup>	2.75 $\pm$ 0.46 <sup>b</sup>
BA (0.55) + Kn (1.74)	2.94 $\pm$ 0.09 <sup>bc</sup>	1.60 $\pm$ 0.08 <sup>bc</sup>	2.38 $\pm$ 0.31 <sup>b</sup>
BA (1.66) + Kn (1.74)	2.74 $\pm$ 0.29 <sup>bcd</sup>	1.56 $\pm$ 0.19 <sup>c</sup>	1.97 $\pm$ 0.39 <sup>bcd</sup>
BA (2.22) + Kn (2.32)	2.71 $\pm$ 0.19 <sup>bcd</sup>	1.41 $\pm$ 0.13 <sup>cd</sup>	2.17 $\pm$ 0.31 <sup>bc</sup>
BA (3.33) + Kn (1.16)	2.15 $\pm$ 0.09 <sup>efg</sup>	1.08 $\pm$ 0.11 <sup>def</sup>	1.29 $\pm$ 0.12 <sup>de</sup>
BA (4.44) + Kn (2.32)	2.28 $\pm$ 0.19 <sup>defg</sup>	0.98 $\pm$ 0.03 <sup>ef</sup>	1.36 $\pm$ 0.16 <sup>cde</sup>
BA (1.11) + Kn (3.48)	2.49 $\pm$ 0.23 <sup>cdef</sup>	1.13 $\pm$ 0.19 <sup>def</sup>	1.46 $\pm$ 0.14 <sup>cde</sup>
BA (3.33) + Kn (3.48)	2.04 $\pm$ 0.06 <sup>fg</sup>	0.89 $\pm$ 0.03 <sup>ef</sup>	1.20 $\pm$ 0.1 <sup>de</sup>
BA (4.44) + Kn (4.65)	2.12 $\pm$ 0.22 <sup>efg</sup>	0.82 $\pm$ 0.04 <sup>f</sup>	1.29 $\pm$ 0.25 <sup>de</sup>
BA (1.11) + 2ip (0.61)	2.51 $\pm$ 0.18 <sup>cdef</sup>	1.60 $\pm$ 0.22 <sup>bc</sup>	1.52 $\pm$ 0.13 <sup>cde</sup>
BA (1.11) + 2ip (1.16)	2.31 $\pm$ 0.12 <sup>defg</sup>	1.31 $\pm$ 0.16 <sup>cde</sup>	1.44 $\pm$ 0.2 <sup>cde</sup>
BA (1.11) + 2ip (1.845)	2.01 $\pm$ 0.01 <sup>fg</sup>	1.11 $\pm$ 0.02 <sup>def</sup>	1.02 $\pm$ 0.04 <sup>e</sup>
BA (1.11) + Kn (1.16) + TDZ (0.1)	0.00 $\pm$ 0.00 <sup>h</sup>	0.00 $\pm$ 0.00 <sup>g</sup>	0.00 $\pm$ 0.00 <sup>f</sup>
P value	0.000*	0.000*	0.000*
LSD 0.05	0.481	0.483	0.758

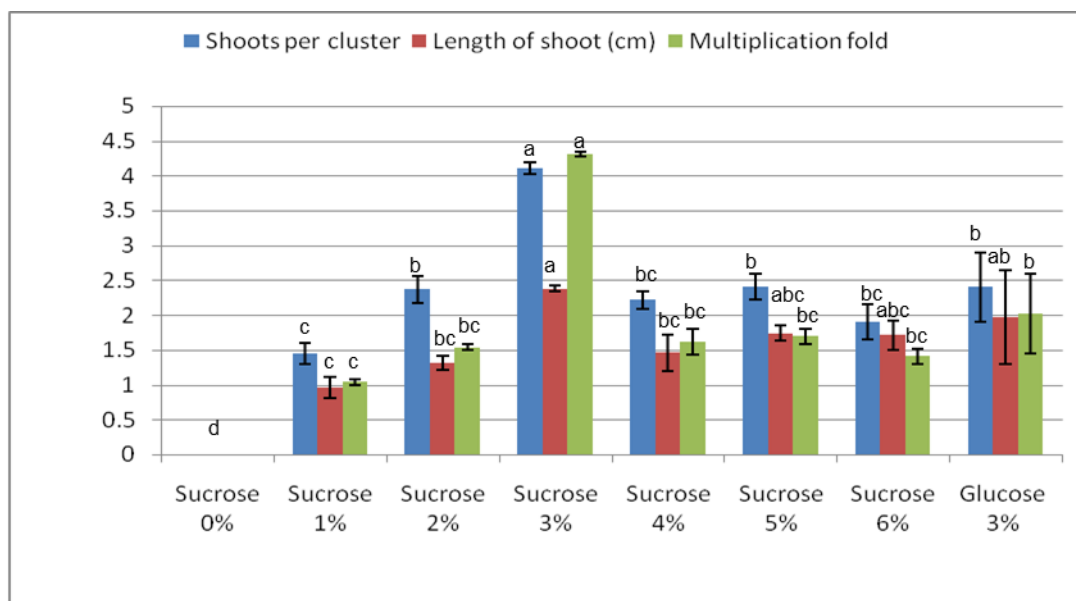
\*Values are means  $\pm$  standard error

Values followed by the same letters within the column are not significantly different at 5% level (Duncan's multiple range test)

Sugars at various levels displayed a significant effect on all the parameters for shoot multiplication (Figure 2). Among different carbon sources tested, sucrose at 3% proved to be the best source for shoot growth. A further increase in the sucrose concentration adversely affected the proliferation rate.



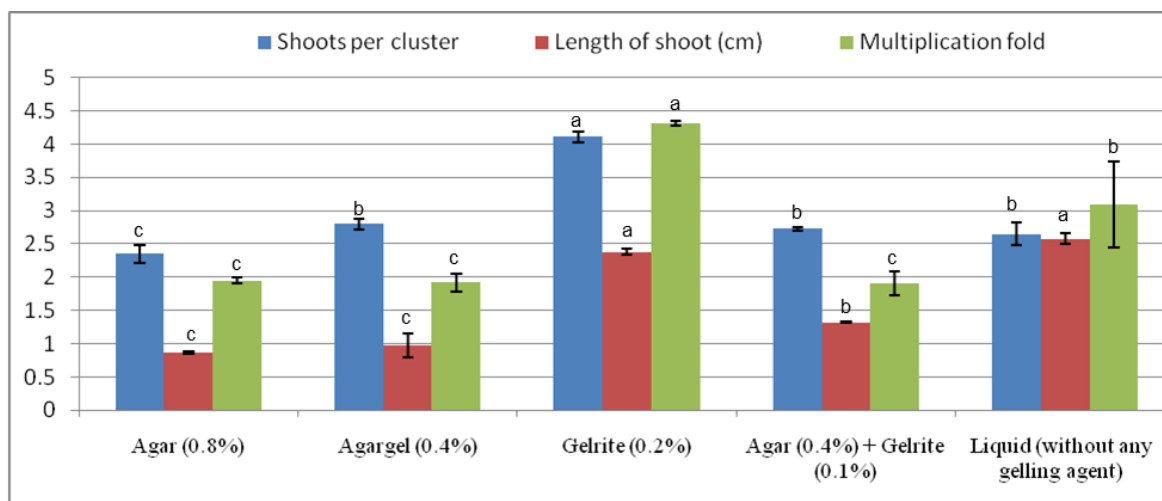
**Figure 1** Micropropagation of Rangpur lime: (a) Mother tree, (b) initiation, (c) shoot multiplication, (d) One multiplied cluster (e) rooting, (f) bare root, (g) in polybag, (h) in nursery or (i) in field (prior to budding)



# Values are means  $\pm$  standard error, Means followed by the same letter within the column are not significantly different (Duncan's multiple range test,  $p < 0.05$ )

**Figure 2 Effect of carbon source on shoot multiplication of Rangpur lime**

Gelling agents like agargel, gelrite and agar were used to optimise shoot production (Figure 3). Gelrite at 0.2% concentration displayed the best shoot multiplication (4.31-fold every four weeks) compared to 1.95 and 1.92-fold achieved with agar and agargel, respectively. Gelrite is a clarified grade of gellan gum, an exocellular heteropolysaccharide obtained from cultures of the bacterium *Pseudomonas elodea* [31]. The superiority of gelrite as a gelling agent in micropropagation has been demonstrated for many plants due to its high purity, consistent quality, and its ability to solidify at lower concentrations compared to agar [32]. Also, the extra clarity enables easy detection of microbial contaminants, which is an added advantage [33]. Among Citrus, there are only two reports of the use of gelrite for shoot multiplication in pummelo [34], calamondin, grapefruit and sweet orange [35]. Gelrite is known to bind cations, particularly  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^{+}$  and  $\text{Fe}^{3+}$  and also has high ash content relative to tissue-culture grade agar [36], which helps in shoot proliferation. Consequently, inorganic impurities may get introduced into the growth medium through agar, which can be toxic and cause vitrification in some cases [37]. A mixture of agar and Gelrite was also used to improve shoot development and to reduce the cost of production. However, this media was inferior as compared to gelrite alone. In the case of liquid media, it favoured shoot growth; however, the multiplication rate was lower than gelrite as vitrification caused suppression in the number of emerging shoots. This may be due to continuous contact of the liquid with explants, osmotic shock and low gas exchange [38]. Thus, all multiplication experiments were performed using 0.2% Gelrite only.



# Values are means  $\pm$  standard error, Means followed by the same letter within the column are not significantly different (Duncan's multiple range test,  $p < 0.05$ )

**Figure 3 Effect of gelling agents on shoot multiplication of Rangpur lime**

In the present study, a negative effect on overall shoot development was observed with auxins and a range of growth adjuvants that were tested. However, a combination of BA and NAA was found to be optimal for rough lemon [39] and for *C. limon* [22], which was also not observed in this study. Activated charcoal was observed to cause apical necrosis. Detrimental effects have also been reported when AC was included in the growth media of Rangpur lime [40] and this may be due to adsorption of growth-promoting culture substances [10].

### Rooting

Individual shoots of Rangpur lime measuring about 1 cm obtained from the multiplication media were transferred to various rooting media. Rooting occurred readily in all media (Table 2), within 28-30 days after transfer.  $\frac{1}{2}$  MS was distinctly better than other media in terms of the frequency of rooting as well as shoot quality and length (Table 2; Figure 1e, f). On basal MS medium, negligible shoot growth was observed. However, there was no significant difference among the treatments with respect to the number of roots and root length. To further improve shoot and root quality, auxins were added to MS media with major salt strength reduced to half (Figure 4). A 100% rooting success was achieved on all the media tested. However, the root growth was restricted on IAA-supplemented medium coupled with basal callusing. Therefore,  $\frac{1}{2}$  MS (basal medium) was selected as ideal for the rooting of shoots. This is in contrast with the reports by El-Boray et al. (2015), where a combination of IBA and NAA were reported to be successful.

**Table 2 Effects of MS and modified MS on in vitro rooting in Rangpur lime**

Medium	Rooting %	Length of shoot (cm)*	No. of roots/plant*	Root length (cm) / root*
MS	33.33%	1.11 $\pm$ 0.04 <sup>b</sup>	2.00 $\pm$ 0.11 <sup>a</sup>	1.93 $\pm$ 0.45 <sup>a</sup>
MS $\frac{1}{2}$	100.00%	2.89 $\pm$ 0.03 <sup>a</sup>	2.16 $\pm$ 0.06 <sup>a</sup>	2.13 $\pm$ 0.01 <sup>a</sup>
MS $\frac{1}{3}$	66.66%	2.37 $\pm$ 0.07 <sup>a</sup>	1.63 $\pm$ 0.14 <sup>a</sup>	2.34 $\pm$ 0.02 <sup>a</sup>
MS $\frac{1}{4}$	33.33%	2.35 $\pm$ 0.27 <sup>a</sup>	1.50 $\pm$ 0.50 <sup>a</sup>	2.47 $\pm$ 0.65 <sup>a</sup>
P value		0.0009*	0.4672ns	0.5167ns
LSD 0.05		0.6076	1.050	1.429

\*Values are means  $\pm$  standard error

Values followed by the same letters within the column are not significantly different at 5% level (Duncan's multiple range test)

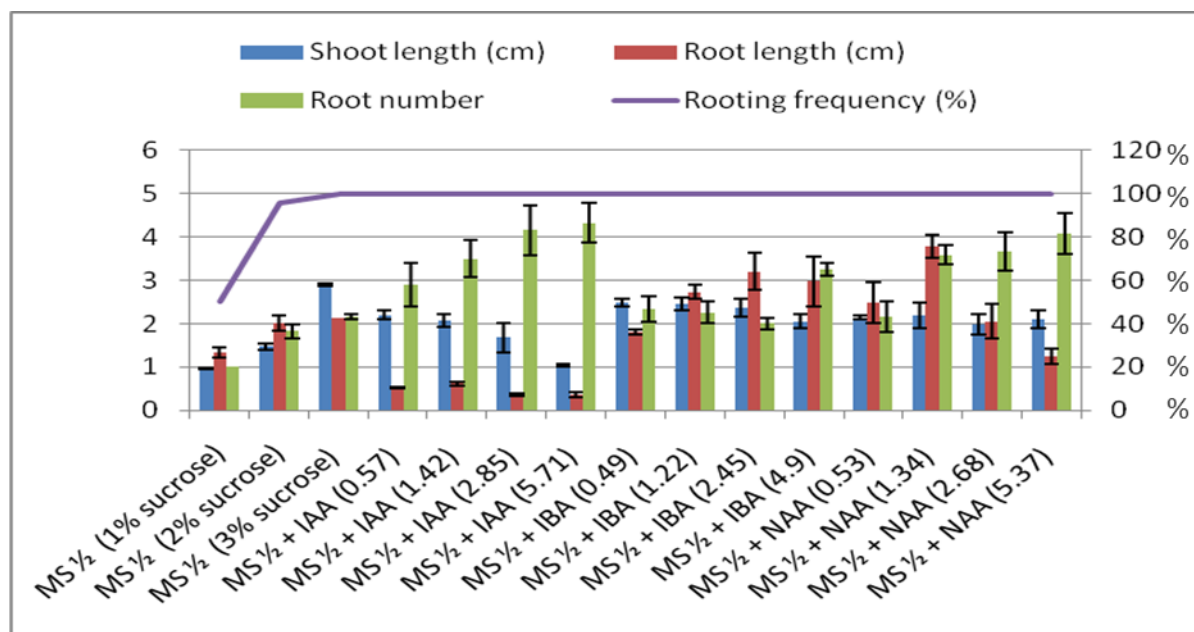


Figure 4 Effect of various auxins ( $\mu\text{M}$ ) on rooting of Rangpur lime

Sucrose has a direct impact on rooting percentage and shoot growth (Figure 4). It was observed that rooting occurred spontaneously in 100% of the explants with 3% sucrose. Even though shoots augmented with 2% sucrose also displayed a very high rooting frequency (95%), the shoots failed to grow and thus were much shorter than on 3% sucrose-containing medium. Therefore, 3% sucrose was found to be optimum for rooting in Rangpur lime.

On liquid medium root number was almost double that of the gelrite solidified medium. However, the plantlets rooted in liquid medium had a lower hardening survival (75%) compared to those on the gelrite medium (100%). Since conventionally propagated Citrus plants have tap roots, a high number of roots obtained on liquid media can be inhibitory for plant growth. Subsequently, 0.2% gelrite was used as the gelling agent for all rooting experiments.

#### Hardening and acclimatisation

The survival rate varied between 70% and 100% depending upon the nature of the potting mixture used, and among all the tested combinations of substrates attempted, 1:1 soil: agropeat (v: v) favoured maximum shoot growth and leaf expansion and gave up to 100% hardening survival (Figure 1g). It is important to note that none of the prior studies on tissue culture of Rangpur lime have any mention of hardening methods or survival percentages [11] while Barlass and Skene [14] reported on the transplantation of rooted Rangpur lime plantlets on Jiffy's peat block. A high level of transplantation success was observed in this study. As part of our study to date, more than 10,000 plants of Rangpur lime have been produced, acclimatised and transferred to the open nursery (Figure 1h).

#### Clonal fidelity studies

In case of perennials, including horticultural species, micropropagation technology cannot be rewarding unless complete genetic fidelity is maintained [41]. This ensures that the advantages of the elite genotype, such as high yield and other unique traits, are inherited by the progeny. Moreover, retention of genetic uniformity for long durations in tissue culture has great commercial importance in species such as Citrus, where aseptic cultures have been difficult to establish. Thus, micropropagation using explants with preformed meristems (e.g. axillary buds, shoot tips) was chosen in the present study as it is well known that these shoots have much less variation than those arising from adventitious budding systems through direct organogenesis, callusing phase or through embryogenesis. However, there are some reports which document the occurrence of somaclonal variations in micropropagated plants raised through axillary

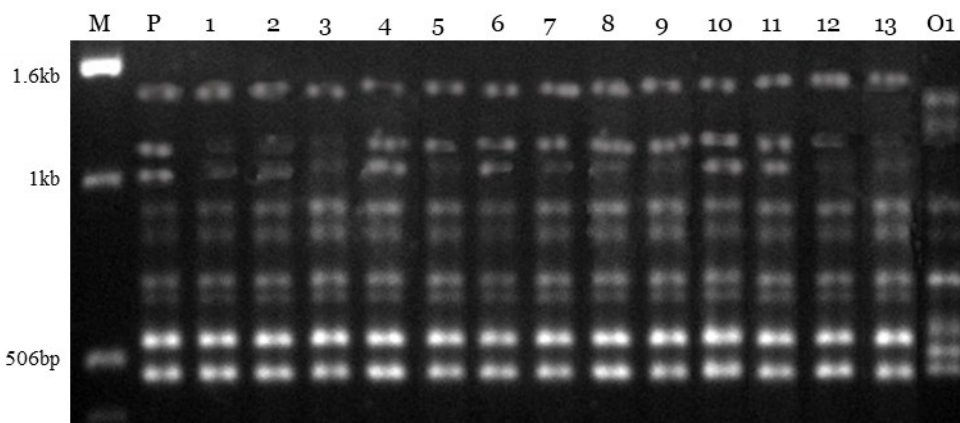
proliferation, such as teak [42] and apple [43]. The occurrence of somaclones is largely because the tissue culture environment induces enormous stress, which enhances the frequency of DNA methylation, somatic crossing over and further sister chromatid exchange [44]. In addition, plant cells in tissue culture may be especially susceptible to dNTP pool imbalances [45]. It has been observed that recurrent subculturing for an indefinite period and also longer passages hinder the maintenance of genetic fidelity since the tissue culture process appears to disrupt the cell cycle as the cells are dividing too fast and cause chromosome fragmentation [46]. Thus, it is important to determine the exact period of subculturing passage for each species. This was quite evident in a study on micropropagation of teak, where variation was observed at the 25<sup>th</sup> sub-culture passage [42].

To detect any clonal instability in the present study, a total of seven ISSR primers (UBC 812, 814, 818, 840, 842, 843 and 848) were initially screened, and six primers were found to be suitable for Rangpur lime (Table 3). DNA from the parent, its tissue-culture raised progenies and three outliers (Alemow macrophylla, C 35 citrange and Swingle citrumelo) were subjected to ISSR-PCR assay. These primers amplified a total of 85 bands in the size range from less than 506 bp to 2 Kb (Table 3). The number of bands generated by each primer varied from four to nine and the bands were scored for their presence or absence across the plantlets analysed. The highest number of bands was observed in the 506 bp to 1 Kb region. Each primer gave an average of over 6 bands in all the progenies and the mother plant. ISSR was also used for evaluating the genetic differences of micropropagated sweet oranges using microshoots [47], somatic embryos of Meyer Lemon [48] and somaclonal variants of Persian Lime [49]. In this study, all the micropropagated plantlets showed identical number and position of the bands as their donor plant in all the primers tested, confirming that the genetic stability is maintained in plants derived from *in vitro* axillary cultures even up to the 25<sup>th</sup> passage *in vitro* (Figure 5).

**Table 3 ISSR primers: GC content, number and size range of bands produced by the mother plant, tissue culture raised plants of Rangpur lime and outliers to detect clonal stability**

UBC ISSR Primer	Primer sequence <sup>a</sup>	GC content (%)	Total number of bands				Molecular size range (bp)	% polymorphism w.r.t. outliers
			Mother + TC raised progenies		TC including outliers			
			Total	Polymorphic	Total	Polymorphic		
812	(GA) <sub>8</sub> A	47	9	0	15	14	<506 to 1600	93.33%
814	(CT) <sub>8</sub> A	47	4	0	7	7	506 to 2000	100.00%
818	(CA) <sub>8</sub> G	53	8	0	8	6	<506 to 1000	75.00%
840	(GA) <sub>8</sub> YT	44-50	6	0	7	4	<506 to 1600	57.14%
842	(GA) <sub>8</sub> YG	50-55	6	0	8	4	<506 to 1000	50.00%
848	(GA) <sub>8</sub> RG	50-55	8	0	15	12	<506 to 1000	80.00%

<sup>a</sup>Key to base compositions R = A, G Y = C, T



**Figure 5 DNA amplification obtained with primer UBC 812; 1 Kb DNA ladder (lane M); mother plant (lane P); micropropagated plants at 5th (lane 1 and 2), 10th (lane 3 and 4), 15th (lane 5 and 6), 20th (lane 7 and 8) and 25th passages (lane 9 and 10); plant at nursery stage (lane 11, 12 and 13); outlier Alemow macrophylla (O1)**

Also in the present study, the plantlets successfully transferred to the nursery were morphologically similar to the mother plants. Furthermore, the polymorphic bands scored for the outlier proved that the primers employed were competent enough to distinguish the plantlets based on genetic variations. The highest polymorphism with respect to outliers was observed in UBC primer 814 and the lowest in UBC Primer 842.

## Conclusions

The present study establishes an efficient protocol for large-scale true-to-type production of plantlets of Rangpur lime using axillary multiplication. An optimised multiplication fold of 4.31 shoots every four weeks, with 100% rooting and hardening survival of plants, with healthy shoot growth and well-developed leaves, was achieved. Using this protocol, more than 100,000 plants can be produced starting from a single node within a year. Thus, this study has immense commercial potential for the mass-scale propagation of Rangpur lime.

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CASE STUDY

## Trauma, emotions, and adolescence: A transdiagnostic approach to treat severe dysregulation using emotion-focused therapy

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**Abstract:** Adolescence is an age of varied concerns and widespread challenges whether intrinsic factors such as hormonal changes, self-esteem issues or body image concerns, or, extrinsic factors like peer pressure, meeting familial expectations or cyber influence. Congruent to the same, this clinical case examines the clinical presentation of a 16-year-old female with severe emotional dysregulation, interpersonal difficulties, anger outbursts, and significant psychosocial adversity. Assessment using clinical interviews, standardized rating scales, and projective tests indicated features suggestive of Bipolar I Disorder (Mixed Episode) and emerging personality pathology. The case was conceptualized using the transdiagnostic model of Emotion-Focused Therapy (EFT), emphasizing the role of early relational trauma, emotional avoidance, and maladaptive coping. Intervention focused on grounding techniques, emotional processing, and developing adaptive emotional responses. The therapy approach significantly improved affect regulation, interpersonal functioning, and overall psychological well-being. The case highlights the importance of comprehensive assessment and culturally sensitive, trauma-informed interventions in adolescent mental health care in the Indian context.

**Keywords:** Emotional dysregulation, Emotion-focused therapy, Adolescence, Trauma-informed interventions

### Introduction

Adolescence represents a critical developmental period characterized by major changes in biological, psychological, and social aspects, often accompanied by heightened vulnerability to the onset or exacerbation of mental health challenges [1]. Navigating the complexities of identity formation, relationships with peers, academic pressures, and familial expectations can be demanding, and for some, this period is marked by substantial psychological distress. The challenging clinical presentations that emerge in this case are severe emotional dysregulation, marked shifts in mood, and significant interpersonal difficulties. Understanding the intricate interplay of these aspects is crucial for accurate diagnosis and effective intervention. If such presentation in adolescents remains undiagnosed or untreated, it poses significant difficulty for the person in regulating oneself and dealing with their personal and social life effectively. It also affects the internal sense of self-direction and self-worth. Affected persons may experience impairment in building and maintaining meaningful interpersonal relationships. Further, if the presentation remains chronic it may lead to manifestation of severe psychological disorders.

The challenges inherent in diagnosing complex adolescent presentations are well-documented in the literature. Differentiating normative adolescent turmoil from emerging psychopathology, particularly when symptoms overlap across diagnostic categories, requires careful consideration [4]. For instance, the emotional lability, impulsivity, and interpersonal difficulties observed in the patient necessitate a careful

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evaluation of potential mood disorders, such as Bipolar Disorder with mixed features, alongside considerations of emerging personality pathology, characterized by traits like negative affectivity and difficulties in self-identity and interpersonal functioning [6], [9]. Furthermore, the profound impact of developmental trauma and cumulative stressors on both mood regulation and personality development is increasingly recognized, adding another layer of complexity to the diagnostic and etiological understanding [10]. Existing research highlights the detrimental effects of experiences like bullying and familial invalidation on adolescent mental health trajectories (e.g., [2], [3], yet detailed case studies illustrating the confluence of these specific factors i.e. trauma, potential mood disorder features, and personality vulnerabilities remain valuable for informing clinical practice.

Therefore, the primary aim of this paper is to present a comprehensive case study of the patient, utilizing data gathered from clinical interviews, informant reports, mental status examination, and a battery of psychological assessments (including projective tests, objective personality inventories, and symptom rating scales). This study seeks to: (1) detail the multifaceted clinical presentation and developmental history of the patient; (2) explore the potential interplay between past traumatic experiences, ongoing stressors, and current symptomatology; (3) elucidate the diagnostic considerations, specifically examining the evidence for Bipolar I Disorder (Mixed Episode) versus enduring personality patterns like Negative Affectivity or Borderline features, as suggested by the assessment; and (4) discuss the implications of the assessment findings for tailored therapeutic intervention, highlighting the need for an integrated approach.

This paper seeks to enhance understanding of the diagnostic challenges and therapeutic requirements of adolescents experiencing severe emotional dysregulation amid substantial psychosocial adversity. It highlights the importance of comprehensive, multi-method assessment for clarifying complex symptoms and informing effective, evidence-based treatment strategies.

### **Case description**

The patient, 16-year-old female, presented with an acute exacerbation (approximately 2.5 months) of longstanding psychological distress with chief complaints of labile affect, episodes of uncontrollable anger, dysphoria, vengeful thoughts alternating with periods of elevated mood, over-talkativeness, disinhibition, and inappropriate laughter along with anxiety (restlessness, rapid heartbeat, cold hands), heightened interpersonal sensitivity, and occasional prosody of speech. The patient reported long-standing emotional difficulties, which started in middle school, wherein severe emotional harassment by a teacher in 7th grade, followed by feeling criticized by her mother compared to her sister, rejection by her only friend in 9th grade, and later bullying and inappropriate physical contact at a new school. These experiences led to chronic low self-esteem, social withdrawal, and difficulty managing her feelings. The recent emergence of these overly happy and uninhibited periods, combined with her ongoing distress and episodes where she lost control (screaming and struggling to breathe), prompted her to seek psychiatric help and psychotherapy. The patient was admitted to IPD for a period of 2 and a half weeks, post which she has been taking regular therapy sessions in the OPD.

### **Assessment**

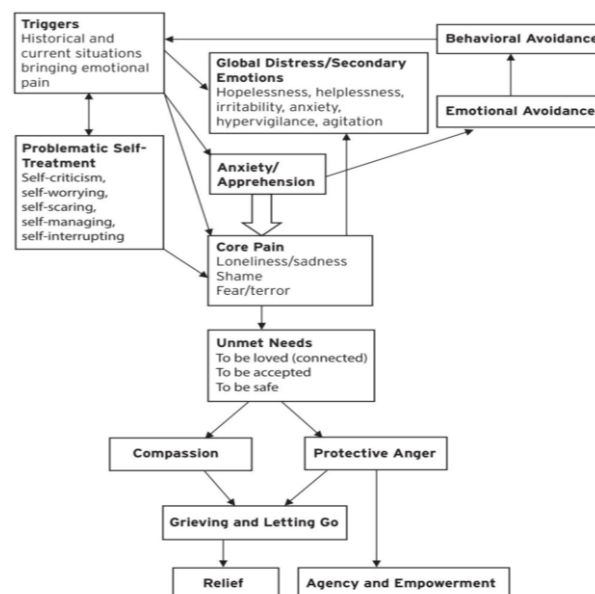
The first four sessions (excluding the therapy sessions-total 16) focused on exploring case history through a semi-structured clinical interview, covering developmental background, family dynamics, interpersonal relationships, academic and occupational functioning, and psychiatric history. A Mental Status Examination (MSE) was concurrently conducted through observation and interaction, evaluating the client's appearance, speech, mood, thought processes, cognition, and insight. Diagnostic indicators such as the Hamilton Anxiety Rating Scale (HAM-A), the Hamilton Depression Rating Scale (HDRS), Young Mania Rating Scale (YMRS) were administered using structured interviews to evaluate symptoms of anxiety, depression, and mania, respectively. Projective tests such as the Rorschach Inkblot Test (RIBT), Draw-A-Person Test (DAPT), and Sacks Sentence Completion Test (SSCT) were used to explore unconscious conflicts, personality dynamics, and emotional functioning. The Millon Clinical Multiaxial Inventory-III (MCMI-III),

a self-report inventory, was also administered to assess personality traits and clinical syndromes aligned with DSM criteria. These tools collectively contributed to a multi-method, multi-domain assessment essential for differential diagnosis and treatment planning.

## General Behavioral Observation

The patient was kempt and tidy. The rapport was established with ease. The patient was cooperative towards the examiner and testing situation gradually. The attention could be aroused and sustained, and she completed the tests well. The patient did not face much difficulty in understanding instructions and performing assessments. She initially denied cards 3 and 7 from the Rorschach inkblot test in the first attempt during administration (screaming and looking at them with hands shaking); however, after a few minutes, she resumed giving responses for the cards.

## Case Formulation



Note. From "Transforming Emotion Schemes in Emotion Focused Therapy: A Case Study Investigation," by S. McNally, L. Timulak, and L. S. Greenberg, 2014, *Person-Centered & Experiential Psychotherapies*, 13(2), pp. 136, 142 (<https://doi.org/10.1080/14779757.2013.871573>). Copyright 2014 by Taylor and Francis. Adapted with permission; and "Emotion-Focused Therapy: A Transdiagnostic Formulation," by L. Timulak and D. Keogh, 2020, *Journal of Contemporary Psychotherapy*, 50, p. 3 (<https://doi.org/10.1007/s10879-019-09426-7>). Copyright 2020 by Springer. Adapted with permission.

**Figure 1: Model of Emotion-Focused Therapy**

Source: McNally, Timulak & Greenberg, 2014

The transdiagnostic model of Timulak and Keogh [7] is relevant in understanding the causation of patient's symptomatology. It proposes triggers combined with problematic treatment of oneself, leading to global distress entailing, in the current case, irritability, helplessness, agitation and anxiety. This anxiety leads to behavioral and emotional avoidance, presented as suppression of anger as well as social withdrawal.

Beneath this anxiety lies the client's core emotional pain, which includes feelings of loneliness, 'my friends call me weird', shame, 'the teacher will humiliate me again', and fear, 'I won't go back to school'. These emotions are often rooted in unmet relational and safety needs such as the need to feel loved, accepted, and safe, which may have gone unfulfilled in early life as reflected by mother's punitive parenting style, public humiliation towards her by her teacher or significant past peer relationships entailing bullying as reported by the patient.

In an attempt to manage these vulnerable emotions, the patient resorts to emotional and behavioral avoidance. This might involve withdrawing from meaningful connections and numbing emotional experiences altogether. Unfortunately, these avoidance strategies prevent her from engaging with their

emotions in a healing and constructive way, thereby reinforcing the cycle of distress. Thus, presently, the lability of affect is explained by the patient as a way of uncontrollable expression of anger which she was not able to in the past, and 'heightened sensitivity'.

Therapeutic work involves helping her access and validate these core emotional experiences and unmet needs. When emotional safety is established, the client can begin to connect with adaptive responses, such as self-compassion and protective anger. Compassion allows the client to soften their inner critic and soothe emotional pain, while protective anger empowers them to establish boundaries and assert unmet needs. As these responses are integrated, the client can begin the process of grieving past hurts and letting go of emotional burdens.

Through this emotional processing, the client experiences relief from chronic distress and a renewed sense of personal agency and empowerment to help patient's concerns of helplessness. Therapeutic goals focus on disrupting maladaptive self-treatment patterns, building tolerance for core emotional experiences, meeting unmet needs through adaptive responses, and fostering healing through grieving and emotional integration. Externalization of self-critical comments was also incorporated in the same.

The current case also saw expansive behaviour (dancing in the inpatient department, singing songs in the outpatient department), grandiose ideas of becoming a singer (agency and empowerment), 'will you come to my concert?', and regressive voice changes along with silly laughing when addressing certain pleasurable activities like going to the mall or singing (letting go). Access to media and internet led to a certain amount of exasperation and hyper sexualization of events, also seen in the assessments conducted.

Case conceptualization using the transdiagnostic model of Timulak and Keogh [7] for the current case can thus be as follows:-

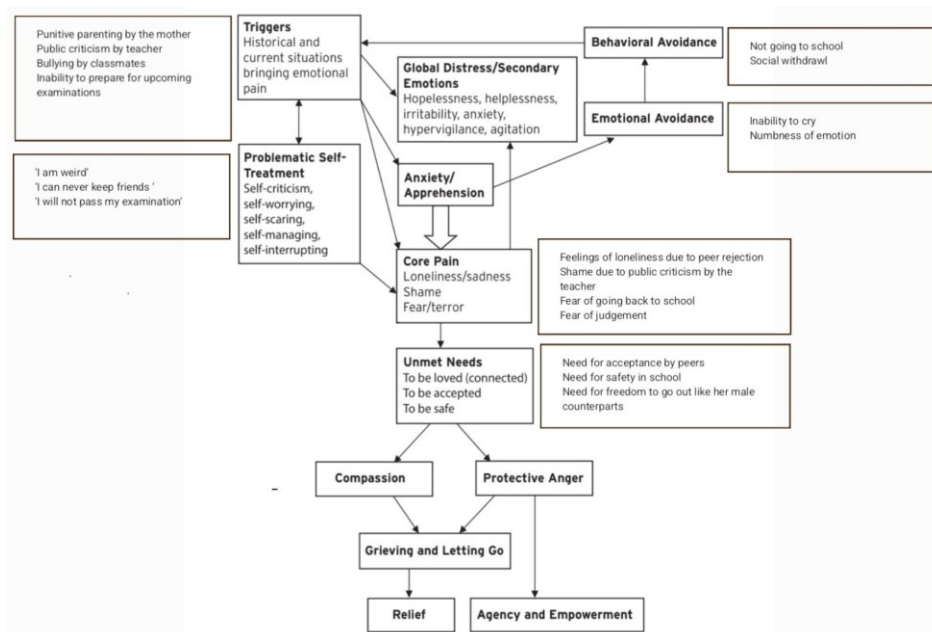


Figure 2 Case Conceptualization of the patient

## Psychotherapy

### Structure and Content

The therapy sessions were organized at a hospital setting in the inpatient department for 6 days and outpatient for 9 days after the patient was discharged. X was accompanied by her father. Adequate measures were taken to ensure the confidentiality of the obtained information.

The patient was psychoeducated about crisis management techniques along with the objectives of emotional focused therapy. The sessions were of 1 hour duration, which were provided weekly.

In addition, the therapy also incorporated grounding techniques, distress tolerance skills, and modulating emotional dysregulation. Parental psychoeducation in terms of gains as well as expressed emotions was provided. Parental skill training for using grounding techniques was also provided.

## Goals

As decided collaboratively with the patient, the short term goals were stabilization, providing space for ventilation and inducing relaxation. The long term goals involved forming a narrative of emotional pain, insight building and overcoming behavioural and emotional avoidance.

## Therapy Process

### *Initial phase*

#### Psychoeducation

The parents as well as the patient were provided with psychoeducation to help them understand the emotional difficulties. It was explained that emotional lability, anger outbursts, crying spells, and regression were not simply acts of defiance or immaturity, but were understood as expressions of deeper emotional pain and unmet needs. These behaviors were described as maladaptive secondary emotional responses that had likely developed as ways of coping with underlying primary emotions such as sadness, fear, or shame. The patient's anger was explained as a defense against feelings of vulnerability or emotional hurt due to humiliation by the teacher, while crying spells were seen as expressions of unacknowledged emotional pain, such as loss or rejection by peers. Regression behaviors were framed as an attempt to seek safety or comfort when she felt overwhelmed or emotionally threatened. Parents were reassured that these responses were common and were encouraged to respond with empathy, curiosity, and emotional validation. It was emphasized that, through support and therapeutic intervention, the adolescent could be helped to identify and process core emotions, and to gradually develop more adaptive emotional responses, such as healthy assertiveness and self-compassion (Figure 2).

#### Forming a therapeutic alliance

A strong therapeutic alliance was intentionally fostered with the patient by creating a safe, non-judgmental, and emotionally attuned space where their experiences were consistently validated. Efforts were made to build trust through genuine curiosity, empathy, and consistent emotional presence, allowing the patient to feel seen and understood. The therapist adopted a collaborative stance, gently exploring the patient's emotional world at their own pace while affirming their strengths and resilience. By attuning to their emotional needs and respecting their autonomy, the therapeutic relationship became a secure base from which deeper emotional exploration and healing could begin.

#### Grounding training

Grounding techniques were introduced to the patient to help manage episodes of emotional overwhelm and enhance their capacity for present-moment awareness. One key grounding strategy provided was the 5-4-3-2-1 technique, which was practiced in the initial sessions in the IPD. The patient was guided to focus on their immediate environment by identifying 5 things they could see, 4 things they could touch, 3 things they could hear, 2 things they could smell, and 1 thing they could taste (or imagine the taste of). This sensory-based exercise was framed as a way to anchor the patient in the present moment, she was especially during intense emotional states such as anger, anxiety, or dissociation. The technique was presented as a practical and accessible tool that could be used independently, and the patient was encouraged to practice it regularly to strengthen their emotional regulation skills outside of therapy. The parent (father) was also trained to do practice when needed with the patient.

#### Affective modulation and expression

Empathic holding - A soothing acknowledgement of the current experience was presented to the patient in a soothing voice while maintaining a close contact, 'I understand you feel rejected because your friend

won't talk to you and that makes you feel sad', 'You cannot understand whom to be angry at and it is making you uncomfortable so you feel like shouting'.

#### **Grounding and regulating**

Instructions to orient the patient to the present physical space as well as their emotions were given while building on empathetic holding 'you're sitting on the bed, holding the bed sheets and they feel soft and smooth in your hands'.

#### **Clearing a space task**

Through the practice of focusing, an experiential task was carried out by identifying markers of overwhelming and uncontrollable emotions. She was made to focus inward and describe the bodily aspects of the feeling along with naming and linking it to the current situation. An effort was made to externalize the feeling by 'putting it aside' and the practice was repeated.

### ***Middle phase***

#### **Overcoming avoidance**

To help the patient overcome emotional avoidance, the two-chair technique for self-interruption was introduced to address the internal conflict where one part of the self interrupts or blocks the expression of vulnerable emotions. The patient was invited to enact a dialogue between two parts of themselves: the "Interrupter"—the part that avoids or shuts down emotional experiences—and the "Experiencer"—the part that holds the underlying emotional pain. In the Interrupter chair, the patient expressed the thoughts or behaviors used to block feelings, while in the Experiencer chair, they were supported in exploring the emotional impact of being silenced or shut down. Through this dialogue, the patient was able to give voice to their core emotions, articulate unmet needs, and express how the Interrupter part may have developed as a protective mechanism. Over time, the goal was for the Interrupter to soften or transform, and for the Experiencer to gain permission to feel and express emotion freely. This process allowed the patient to move through avoidance and toward deeper emotional healing and integration.

#### **Access and transform emotional pain**

In therapy, the patient was gently guided to access their core emotional pain through a safe and supportive environment, the patient was encouraged to move beneath surface-level distress (anger outbursts and crying spells) and begin to explore the underlying primary emotions of sadness, fear, and shame. These emotions were carefully accessed through empathetic reflection and experiential techniques, such as imagery and two-chair dialogues, which helped the patient articulate the unmet needs embedded in their emotional pain (the need for connection with peers, acceptance, or safety at school). Once these core emotions were fully accessed and expressed, the patient was supported in generating adaptive emotional responses.

This included cultivating self-compassion in response to shame and protective anger in response to past invalidation or emotional injuries. As a result, emotional pain that had previously been overwhelming or avoided was gradually transformed, allowing the patient to experience relief, a greater sense of agency, and emotional integration.

#### **Interpersonal learning for EFT**

Interpersonal effectiveness was addressed by emphasizing the importance of emotional awareness and authentic expression in relationships. The patient was supported in identifying how unmet emotional needs (connection, acceptance, safety), were often masked by anger outbursts, withdrawal, or regression in interpersonal situations. The patient was guided to recognize and express their core emotions in a clearer, more constructive manner. Role-plays were used to help the patient practice setting boundaries and communicating needs with assertiveness and emotional honesty, rather than through reactive or avoidant behaviors. By fostering self-awareness and emotional clarity, the patient began to experience increased confidence and agency in their relationships, enhancing their ability to navigate interpersonal challenges with both vulnerability and strength.

### *Final phase*

#### **Consolidation of changes**

Through role plays and hypothetical situation setting, the patient's overcoming avoidance as well as adaptive reactions were practiced.

#### **Relapse prevention**

Relapse prevention for the patient focused on helping them recognize early warning signs of emotional dysregulation and re-engagement in avoidance strategies. Using Emotion-Focused Therapy principles, the patient was guided to develop increased awareness of their emotional triggers, patterns of problematic self-treatment (self-criticism or emotional suppression), and the internal cues that signaled a return to maladaptive coping. Together, a personalized plan was developed that included grounding techniques like the 5-4-3-2-1 method, compassionate self-dialogue, and continued use of adaptive emotional responses such as protective anger and self-soothing. The patient was encouraged to reflect on past therapeutic gains and to draw on moments of emotional resilience as evidence of their capacity to cope. Emphasis was placed on viewing setbacks not as failures, but as opportunities to re-engage with their emotional process, supported by strategies discussed in therapy and, when needed, seeking support through trusted relationships or further therapeutic contact.

### **Summary of Psychological Testing Results:**

#### *Draw a Person Test (DAPT)*

The patient showed a need for control or detachment from emotional or physical realities along with heightened curiosity or preoccupation with sexual themes. Feelings of inadequacy and expansive representation suggested the patient may be attempting to overcompensate for perceived deficiencies through self-aggrandizement or exaggerated self-importance. The patient may have a strong desire to assert themselves or prove their sense of control and autonomy. The patient tends to suppress their impulses or engage in self-restraint, possibly to the extent of emotional or social withdrawal. The patient may tend to withdraw from their environment, potentially due to difficulties in coping with external stimuli or a perceived lack of emotional resources to engage with the world effectively. There is also possible unresolved sexual issues or an overactive interest in sexuality reflecting neurotic tendencies around sexual expression or body image. Underlying feelings of vulnerability or a desire to project strength and control were noted. Ambitious or driven nature, possibly with a desire for control over one's environment, along with unresolved anger or distrust, contributing to the subject's social or interpersonal struggles. Feelings of dependency and helplessness, pointing to a potential need for support or a reliance on others to navigate challenges, were also noted.

#### *Rorschach Psychodiagnostics*

##### **Cognitive Findings of RIBT-(based on Information processing, mediation & ideation)**

The patient consistently views the world negatively, allowing this pessimism to permeate her thoughts and fostering doubt and a belief in inevitable failure. Instead, she likely integrates fleeting thoughts into a pre-existing, negative framework of thinking as a defense mechanism. Furthermore, when faced with stress, this patient has a strong inclination to escape into fantasy rather than confront reality, more so than typical individuals. Her thought processes are likely significantly impaired, and this level of impaired conceptualization often results in poor reality testing. Her thinking tends to be disorganized, contradictory, and frequently characterized by flawed judgment. While she does engage in some self-reflection, which could promote a more accurate self-perception, her overall self-image is tainted by negative self-attributions, leading to a more pessimistic view of herself. This difficulty in processing information accurately seems to be a pervasive issue, not just limited to ambiguous situations, suggesting a deliberate defensive distortion of reality. She may also frequently disregard social norms to prioritize her own desires, increasing the likelihood of unusual or inappropriate behaviors. She tends to process information with minimal effort,

which could indicate a lack of self-assurance or an unwillingness to engage in new experiences. This patient also demonstrates a consistent approach to processing new information and may set unrealistically high goals, increasing the potential for failure and subsequent frustration. Her scanning habits are characterized by haste and randomness, often causing her to miss crucial details, leading to flawed decisions and actions. Finally, she experiences notable difficulties in shifting her attention, and while her processing quality is generally adequate, it can sometimes decline in effectiveness or maturity.

**Personality Findings of RIBT- (based on control and stress tolerance, affect, self perception and situation related stress)**

The patient appears to be suffering from emotional deprivation, possibly stemming from a long-standing unmet need for closeness that exceeds typical interpersonal boundaries. She is currently overwhelmed by intense emotions that disrupt her thinking and can lead to impulsive actions. She tends to oversimplify complex or ambiguous situations by denying their existence and demonstrates inconsistency in problem-solving and decision-making, as well as in managing her emotions, which can fluctuate between overcontrol and inappropriate lack of control.

Furthermore, this patient often blends her feelings with her thoughts when trying to cope. While somewhat adaptable and occasionally willing to use a more logical approach to decision-making, she is currently experiencing distress and actively avoids emotional stimuli, feeling uncomfortable when dealing with emotions, which leads to social withdrawal. She strongly avoids emotional confrontation, suggesting a lack of trust in her own ability to cope using emotional experiences as a guide. This indicates the presence of significant, generalized anger that impacts her attitude towards her surroundings and can interfere with decision-making, coping strategies, and the ability to maintain meaningful relationships. Her psychological makeup appears less complex than expected, and she struggles behaviorally in complex emotional situations. She is often confused by emotions or emotional situations, experiences feelings more intensely than others, and this confusion can be disruptive, making it harder to resolve issues and increasing her distress. She is likely experiencing increased demands due to situational stress, which may lead to less organized decisions and behaviors. This mild to moderate stress is causing psychological disruption, affecting both her thinking and emotions, and she feels considerable discomfort due to a sense of helplessness related to her current situation, resulting in significant emotional confusion.

**Interpersonal findings of RIBT (based isolation index and interpersonal interest)**

The patient assumes a more passive, though not necessarily submissive role in interpersonal relations. The patient may prefer to avoid responsibility for decision making and is less prone to search out new solutions to problems, or initiate new patterns of behavior, especially when the possibility exists that others will assume the necessary responsibilities.

The patient is more conservative than might be anticipated in close interpersonal situations, especially those involving tactile exchange. The patient is more likely to be concerned with personal space, and much more cautious about creating or maintaining close emotional ties with others.

The patient has a strong interest in other people, but the patient does not understand people very well. This lack of understanding can often lead to unrealistic expectations concerning relationships and/or social blunders that alienate others.

The patient is unsure about her integrity in interpersonal situations and is prone to become defensively authoritarian as a way of fending off perceived challenges to the self that arise in those situations. The patient may be regarded by others as rigid or narrow-minded and, as a consequence she often has difficulties in maintaining close relations, especially with those who are not submissive to them.

### *Millon Clinical Multiaxial Inventory (MCMI-III)-*

MCMI-III indicate deep-seated feelings of inadequacy and sadness, leading to social withdrawal and a lack of assertiveness. Possible difficulties in forming close relationships due to eccentric behaviors and mistrust. It also suggests challenges with emotional regulation and interpersonal relationships.

(Avoidant-112, Depressive-99, Dependent-100, Masochistic-100, Schizotypal-111, Borderline-97, Paranoid-100, Anxiety-97, Bipolar Manic-95, Major Depression-109, Delusion Disorder-90).

### *Sacks Sentence Completion Test (SSCT)-*

The patient generally views themselves favorably and possesses a solid sense of identity. The results in areas of guilt feelings, past, future, and goal-related items reflected a balanced approach to emotional experiences, with room for deeper exploration or development in these areas. Findings show signs of emotional ambivalence in relationships, particularly with family members and the opposite sex. There may be unresolved issues from the past and a need for emotional security from relationships or those who have provided care.

### *Hamilton Anxiety Rating Scale*

16 indicating mild anxiety

### *Hamilton Depression Rating Scale*

16 indicating mild to moderate depression

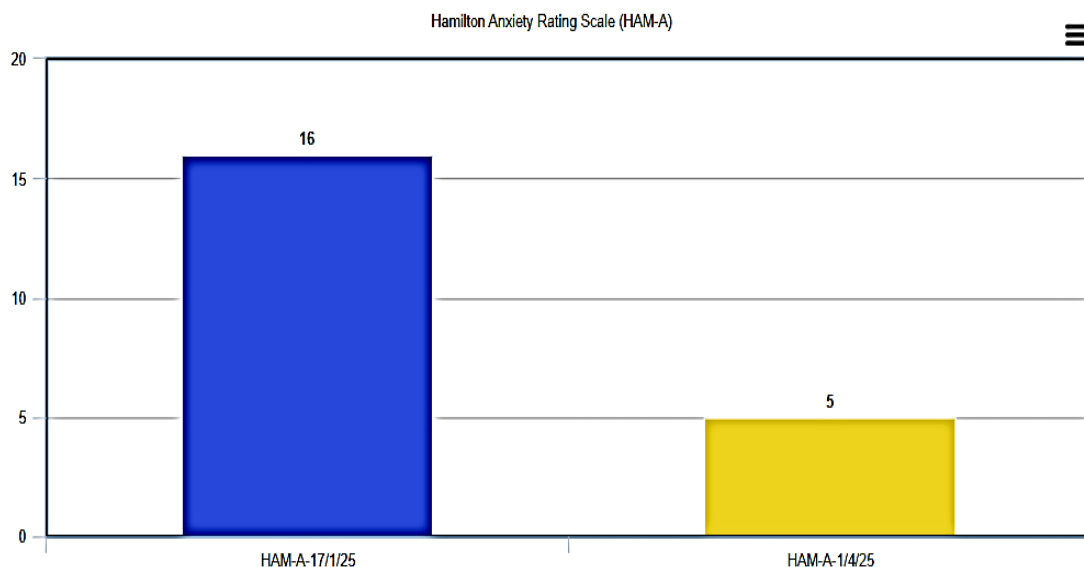
### *Young Mania Rating Scale*

A score of 23 suggests the presence of mild manic symptoms.

Overall, the testing indicates substantial psychological distress with significant cognitive distortions, severe difficulties in emotional regulation and personality functioning, and marked impairments in interpersonal relationships. The findings suggest difficulties with reality testing and potential for maladaptive behaviors under stress.

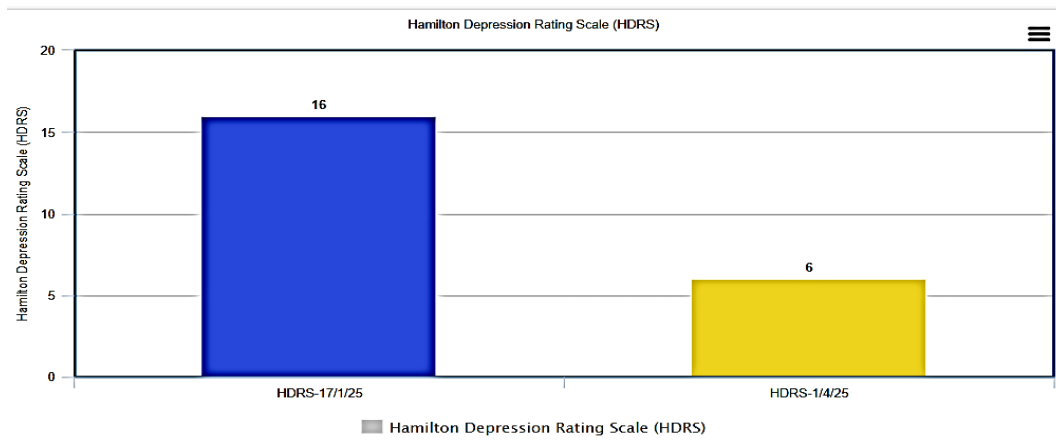
## **Therapy Outcome**

The assessments conducted post-therapy indicated significant improvement.



**Figure 3 Patients' anxiety levels as measured by HAM-A**

Figure 3 showing patient's anxiety levels, as measured by the Hamilton Anxiety Rating Scale (HAM-A), showed significant improvement. The pre-test score on January 17th was 16, indicating Mild Anxiety. By the post-test on April 1st, the score had reduced to 5, which falls within the normal range.



**Figure 4 Patients' anxiety levels as measured by HDRS**

Figure 4 showing patient's anxiety levels, as measured by the Hamilton Depression Rating Scale (HDRS), showed significant improvement. The pre-test score on January 17th was 16, indicating Mild Anxiety. By the post-test on April 1st, the score had reduced to 6, which falls within the normal range.

### Therapist's Reflection

The EFT techniques were provided to the patient with some modifications with respect to the sequence of the components, however, the core elements remained the same as in the manual. Since the patient was an Indian adolescent from a lower socio-economic strata, the analogies used to make her understand the techniques were modulated to her culture and age.

The patient could understand and practice emotional regulation techniques by understanding the underlying causes of behaviours deemed inappropriate by her peers and how unmet needs could lead to such consequences. She could further externalize through clearing a space task and approach the emotions that were being avoided in the past.

She reported significant improvement using grounding techniques. Additionally, on a trip during the middle phase of therapy, the parent also reported performing 5-4-3-2-1 technique when the patient reported feeling apprehensive in a crowd followed by box breathing exercise.

Overall, the patient as well as the informant, i.e., father, asserted significant improvement concerning shouting impulses, emotional regulation, and conduction of appropriate social behaviour (Figure 4).

### Barriers and Challenges Faced in Therapy

Diagnostic vagueness-Symptoms involved shouting attributed to anger, grandiose behaviour, regressive behaviour entailing voice changes but with no criteria for dissociation being met. Further, mood dysregulation varied from time to time depending upon primary gains. Violent outbursts in the OPD setting entailing hitting hands on the tables and kicking the almirah were also followed by guilt during the episode wherein she was apologizing as she was hitting the furniture. No derealization or depersonalization was reported, rather the patient asserted wanting to control said behaviour while it was happening but failing to do so. This intact reality orientation led to further investigation into primary gains and voluntary components of the symptomatology (Figure 3).

Boundary setting-In her initial admission to the emergency department of the hospital, she reportedly hugged and wrote a 'love letter' to her attending doctor. This was followed by wanting to hug and kiss attending doctors and therapists and asserting a desire to 'kiss' them. Frequent compliments were also observed wherein a voice change with a regressed child like tone was noted. Repeated requests to take

pictures were also made. Boundary setting and social norms were reinforced regularly and the therapists were mindful of not providing her with any positive reinforcement or gains with respect to these desires.

Violent outbursts-The therapeutic process was hindered by regular outbursts in the inpatient and outpatient department when she had a reality orientation of her desires (I want to do a concert) was emphasised.

Tertiary gains of mother-As reported by her father, when the patient has an episode of shouting and uncontrollable anger, her mother has similar symptoms of shouting, mood dysregulation and weakness. For the same, they were psychoeducated about expressed emotions.

Faith healing-Due to cultural beliefs and coercion by community members, the patient was taken to a religious place for 'jhaad phuk', which led to an episode entailing possession as described by her older sister. However, no similar episodes were reported before or after the same. This also led to an increased need for awareness among parents about mental health and its management.

## **Conclusion and Future Implications**

This case study highlights the multifaceted nature of adolescent emotional dysregulation arising from cumulative psychosocial trauma and developmental vulnerabilities, presented with overlapping affective and personality symptoms. A transdiagnostic, emotion-focused therapy approach allowed for the exploration and transformation of core emotional pain, resulting in notable clinical improvement in affect regulation, interpersonal functioning, and overall distress levels. However, the therapeutic process was not without challenges, diagnostic ambiguity, boundary testing behaviors, regressive tendencies, and socio-cultural influences, such as faith healing and family dynamics involving secondary and tertiary gains, posed significant barriers to therapeutic progress. These issues required sustained clinical vigilance, consistent psychoeducation, and reinforcement of therapeutic boundaries. Despite these hurdles, the intervention was effective in facilitating emotional insight and behavioral change. This case underscores the need for comprehensive, culturally attuned, and trauma-informed mental health care tailored to the developmental needs of adolescents in the Indian context. It further calls attention to the importance of clinician adaptability, family engagement, and systemic awareness in navigating complex therapeutic landscapes.

The case underscores the clinical utility of transdiagnostic frameworks, such as Emotion-Focused Therapy, in managing adolescents presenting with complex affective and personality-related symptomatology. There is a pressing need for trauma-informed assessment and intervention protocols within adolescent mental health services, particularly for individuals with histories of bullying, abuse, or emotional invalidation. Culturally sensitive adaptations of psychotherapeutic techniques are essential when working with Indian adolescents from diverse socioeconomic backgrounds, to enhance therapeutic alliance and effectiveness. Parental psychoeducation and involvement must be integrated into treatment plans, especially in cases where family dynamics contribute to the maintenance of emotional dysregulation or reinforce maladaptive behaviors. Clinicians must remain vigilant about boundary-setting and therapeutic neutrality, particularly in cases involving regressive behaviors, transference, or testing of social norms by the adolescent. The case highlights the impact of community beliefs and mental health stigma, such as faith healing practices, on treatment continuity and calls for public mental health awareness initiatives targeting caregivers and community stakeholders. Finally, the importance of comprehensive, multi-method psychological assessment is reinforced as a foundation for accurate diagnosis and individualized, evidence-based treatment planning in adolescent care.

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