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From the Desk of the Chief Editor

It gives me great pleasure to introduce the latest edition of our multidisciplinary journal, The NEHU Journal, Vol. XXI, No. 1 & 2, 2023 (2025) which continue to celebrate and disseminate the tradition of intellectual inquiry across diverse academic domains. In a world that is increasingly interconnected and technologically marching ahead, the value of interdisciplinary research has never been more evident. This journal stands as a testament to our collective commitment to fostering collaboration, innovation, and the relentless pursuit of knowledge. As scholars, educators, and innovators, we are entrusted with the responsibility of not only advancing knowledge within our respective fields but also bridging the gaps between them. The challenges we face today—climate change, public health crises, technological disruption, and socio-economic inequalities—demand collaborative and integrated solutions. They cannot be adequately addressed in disciplinary silos. This journal serves as a vibrant forum where ideas from science, technology, humanities, social sciences, and the arts intersect, challenge one another, and converge into meaningful dialogue.

This edition features a compelling collection of eight research papers that enrich our understanding of the world and demonstrate the power of convergent thinking.

I would like to commend the guest editors, Prof. Nirmalendu Saha, Department of Zoology, NEHU and Dr. S. Majaw, Department of Biotechnology and Bioinformatics, NEHU as well as our editorial team for their tireless efforts in maintaining the academic rigor and integrity of this publication. I also extend my heartfelt appreciation to the authors and reviewers whose expertise and commitment are the backbone of this journal. Their work inspires future research and contributes meaningfully to the broader academic discourse.

At the institutional level, we remain committed to creating an environment that encourages interdisciplinary dialogue and innovation. Our goal is to cultivate a research culture that creates cross-pollination of ideas and societal impact. Let us question assumptions, explore unfamiliar territories, and collaborate beyond conventional boundaries. In doing so, we not only enrich our academic pursuits but also fulfill our greater responsibility to society.

Prof. Fameline K. Marak
Chief Editor

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Human Evolution: A Review of Insights from Fossils, Genetics, and Archaeology

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Abstract

The evolutionary journey of Homo sapiens spans millions of years, shaped by morphological, behavioral, and genetic transformations. This review synthesizes fossil, genetic, and archaeological evidence to outline key stages in human evolution. Early hominins, such as Sahelanthropus tchadensis[~7 megaannum (Ma)] and Australopithecus afarensis (~3.9–2.9 Ma), developed bipedal locomotion, laying the foundation for later Homo species. The emergence of Homo habilis and Homo erectus (~2.4 Ma) marked significant brain expansion and tool use. Homo sapiens appeared by ~300 kiloannum (ka), displaying cognitive advancements and symbolic behavior, followed by global dispersal (~60–10 ka) and interbreeding with archaic hominins. Future research should focus on refining human migration models, understanding genetic adaptations to diverse environments, and exploring how cultural evolution shapes biology. Advances in ancient DNA analysis, machine learning, and bioarchaeology will further unravel the complexities of human evolution, offering deeper insights into our ancestry and adaptive potential.

Keywords: Behaviour, evolution, *Homo sapiens*.

Introduction

The evolutionary history of *Homo sapiens* is a multifaceted process shaped by genetic, environmental, and cultural factors. Over millions of years, early hominins gradually evolved key traits such as bipedalism, larger brain size, and advanced cognitive abilities. Fossil evidence suggests that species like *Sahelanthropus tchadensis*[~7 Megaannum (Ma)]and *Australopithecus afarensis* (~3.9–2.9 Ma) exhibited early forms of bipedal locomotion, setting the foundation for later members of the genus *Homo* (Brunet *et al.* 2002). With the emergence of *Homo habilis* and *Homo erectus*, significant advancements in tool-making and adaptive strategies became evident. By ~300 kiloannum (ka), *Homo sapiens* had appeared in Africa, eventually dispersing across the globe and interbreeding with other hominin species, such as Neanderthals and Denisovans (Stringer 2016). While much progress has been made in understanding human evolution, several research gaps remain.

One major gap is the unresolved phylogenetic relationships among early hominins, particularly in the Middle Pleistocene, where fossil evidence is limited (Harvati *et al.* 2022). Additionally, the genetic and environmental factors that drove key adaptations, such as increased brain size and complex social behaviors, are not fully understood (https://biologyinsights.com/. accessed on the 8th March 2025). The origins of language and symbolic thought, crucial to human culture, remain speculative due to limited archaeological evidence. Furthermore, the precise routes and timing of human migration out of Africa continue to be debated. The influence of climate change on human evolution and the ongoing microevolutionary changes in modern populations also require further investigation. Addressing these gaps through interdisciplinary research will provide deeper insights into our evolutionary past and future trajectory.

Evolutionary stages of *Homo sapiens*: fossils, genetics, tools, and adaptations

This review examines the evolution of *Homo sapiens* through distinct stages, highlighting key developments based on fossil, genetic, and archaeological evidence.

Early hominins (~7–2.5 Ma)

The evolutionary journey of *Homo sapiens* begins with early hominins, diverging from a common ancestor with chimpanzees around 6–7 million years ago (Ma). *Sahelanthropus tchadensis* (7 Ma), discovered in Chad, represents one of the earliest potential hominins, with a cranial morphology suggesting bipedalism (Brunet *et al.* 2002). This is followed by *Australopithecus afarensis* (3.9–2.9 Ma), exemplified by the "Lucy" specimen, which displays a small brain (~400 cm^3) but clear bipedal adaptations in its pelvis and femur (Johanson and Edey 1981). These early hominins inhabited savannah environments, with bipedalism likely evolving as an adaptation for efficient locomotion and foraging. While lacking advanced tool use, their skeletal features lay the groundwork for later genus *Homo* traits (Wood and Lonergan 2008).

Emergence of the Genus Homo (~2.4–1.8 Ma)

The transition to the genus *Homo*is marked by *Homo habilis* (2.4–1.4 Ma), often dubbed the "handy man" due to its association with rudimentary Oldowan stone tools. Fossils from East Africa reveal a brain size of ~600–800 cm³, larger than australopithecines, indicating enhanced cognitive capacity (Tobias 1991). Following this, *Homo erectus* (1.9 Ma–110 ka) emerged as a pivotal species, with a cranial capacity of ~900–1200 cm³, long legs for endurance, and evidence of controlled fire use by ~1 Ma (Rightmire 1990). This species dispersed widely across Africa, Asia, and Europe, with fossils like those from Zhoukoudian, China, showcasing adaptability to diverse climates. These advancements in tool technology (Acheulean) and behavior suggest a foundation for later sapiens-specific traits.

Archaic Homo sapiens and Middle Pleistocene developments (~781–126 ka)

During the Middle Pleistocene, archaic *Homosapiens*evolved, often linked to *Homo heidelbergensis* (700–200 ka), a species with a robust build and brain size (1100–1400 cm³) approaching that of modern humans. Fossils from sites like Broken Hill, Zambiaand Sima de losHuesos, Spain, indicate a mix of primitive and derived features (Rightmire 1990). The Jebel Irhoud remains from Morocco (~315 ka) further refine this stage, showing a modern-like face but an elongated, archaic skull, suggesting a pan-African mosaic evolution (Hublin *et al*. 2017). Genetic divergence within African populations during this period, as inferred from ancient DNA, points to structured populations that gradually coalesced into modern *Homo sapiens* (Schlebusch *et al.* 2017).

Anatomically Modern Homo sapiens (~300–50 ka)

Anatomically modern Homo sapiens emerged in Africa by 300 ka, with key fossils from OmoKibish, Ethiopia (195 ka), and Herto, Ethiopia (160 ka), exhibiting a high forehead, rounded skull, and reduced brow ridges—hallmarks of modernity (Stringer 2016). Genomic evidence, including mtDNA studies, supports an East African origin around 200–300 ka, followed by population expansions (Cann*et al*. 1987). By ~70–50 ka, modern humans began dispersing out of Africa, reaching the Levant, Europe, and Asia. This stage is marked by refined tools (e.g., microliths) and early symbolic behavior, such as ochre use at Blombos Cave, South Africa (75 ka), hinting at cognitive sophistication (Henshilwood and Marean 2003).

Global dispersal and admixture (~60–10 ka)

The global expansion of Homo sapiens saw interactions with archaic hominins, notably Neanderthals and Denisovans. Genetic analyses reveal interbreeding events 60–50 ka, with non-African populations retaining 1–2% Neanderthal DNA, conferring adaptive traits like immune system enhancements (Green *et al.* 2010; Reich *et al.* 2010). Fossils from Skhul and Qafzeh, Israel (120–90 ka), document early forays into Eurasia, while sites like Denisova Cave, Siberia, highlight Denisovan contributions (Pääbo 2014). The Upper Paleolithic (~50–10 ka) in Europe, with cave art (e.g., Chauvet, ~36 ka) and complex tools, reflects a cultural explosion tied to a brain size of ~1350 cm³ and social cooperation (Holloway *et al.* 2004).

Modern human evolution (10 ka–Present)

Since the Neolithic (~10 ka), *Homo sapiens* evolution has been shaped by cultural and environmental pressures rather than major morphological shifts. Adaptations like lactase persistence in pastoralist populations and skin pigmentation changes in response to UV radiation reflect ongoing natural selection (Schlebusch *et al.* 2017). Genomic studies of ancient and modern DNA continue to uncover subtle shifts, with population bottlenecks and expansions tied to migrations and agriculture (Welker *et al.* 2016). Today, *Homo sapiens* represents a single, globally distributed species, with its evolutionary history elucidated by an ever-growing synthesis of paleontology, genetics, and anthropology.

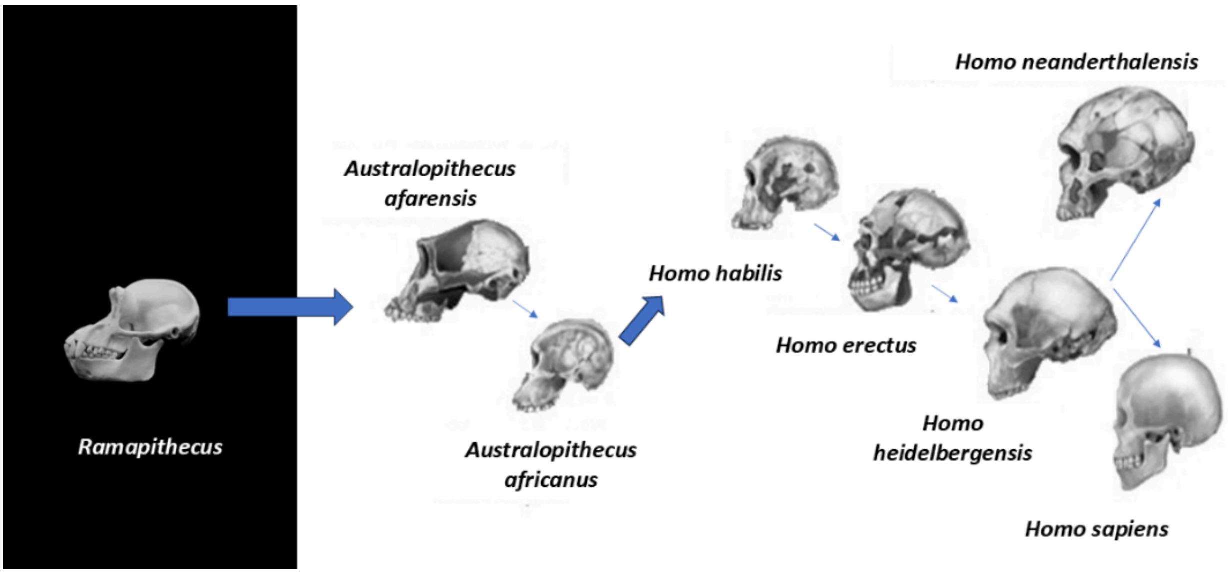

Fig. 1. Illustration of the stages of Human evolution in three key genera.

This stage-wise breakdown traces the trajectory from early hominids to modern humans, emphasizing key morphological, behavioral, and genetic milestones. Thus, the evolutionary stages leading to *Homo sapiens* include key genera in this evolutionary line: *Ramapithecus, Australopithecus* and several species of the genus *Homo* (**Fig. 1**). This progression encompasses a period from the late Miocene to the present, encompassing critical transitions in locomotion, brain development, tool use, and social behavior.

Ramapithecus (~14–7 Ma)

Ramapithecus, once considered a direct precursor to *Homo sapiens*, is now largely understood as a late Miocene ape more closely related to the orangutan lineage (*Sivapithecus*), rather than a hominin. Fossil evidence, primarily comprising jaw fragments and dental remains from sites in India and Pakistan, suggests that *Ramapithecus* exhibited robust mandibles and thick dental enamel, indicative of a diet consisting of tough vegetation (Pilbeam 1982). Initially, its dental morphology, particularly the similarity in tooth structure to later hominins, led to hypotheses that it represented an early hominid. However, molecular and cladistic studies have since clarified that it is a member of the broader hominoid clade, preceding the human-chimpanzee divergence (Begun 2004). Despite its early prominence in discussions of human origins, *Ramapithecus* is no longer considered a direct ancestor of modern humans (Wood and Lonergan 2008), but rather an important, though more distantly related, taxon in hominoid evolution.

Australopithecus (~4.2–2 Ma)

The genus *Australopithecus* marks the first unequivocal emergence of hominins and provides critical insight into the origins of bipedalism and human-like features. *Australopithecus* species appeared in East and South Africa around 4.2 Ma, with notable fossils including *A. afarensis*, represented by the famous "Lucy" skeleton (3.9–2.9 Ma). *A. afarensis* exhibits clear evidence of bipedalism, such as a forward-shifted foramen magnum, a valgus knee, and a pelvis adapted for upright locomotion (Johanson and Edey 1981). Despite these adaptations for bipedality, *A. afarensis* retained many primitive traits, including a small cranial capacity (400–450 cm^3) and ape-like facial features.

Earlier *Australopithecus* species, such as *A. anamensis* (4.2–3.9 Ma), show a mosaic of primitive and derived traits, with some evidence of climbing adaptations in the form of curved phalanges. Later forms, such as *A. africanus* (~3–2 Ma), exhibit a reduction in canine dimorphism, suggesting social shifts and possibly more complex social structures (Ward *et al.* 1999). While *Australopithecus* species did not produce tools, their locomotor adaptations are viewed as setting the stage for the genus *Homo* (Klein 2008).

Homo habilis (~2.4–1.4 Ma)

Homo habilis, often referred to as the "handy man", represents the earliest species of the genus *Homo*and emerged in East Africa around 2.4 Ma. Fossils from key sites such as Olduvai Gorge reveal that *H. habilis* had a larger brain than its australopithecine predecessors, ranging from 600 to 800 cm^3 (Tobias 1991). Additionally, *H. habilis* exhibited a reduced degree of prognathism and smaller teeth, reflecting dietary shifts from the more generalized feeding strategies seen in earlier hominins. Associated with *H. habilis* are simple Oldowan tools, such as choppers and flakes, indicative of rudimentary tool-making abilities. These tools suggest that *H. habilis* was likely engaged in scavenging and foraging, marking a shift from the behavior of earlier hominins. Despite these advances, *H. habilis* retained some arboreal traits, including relatively long arms and a more ape-like limb proportion, which positions it as a transitional species between *Australopithecus* and more advanced members of the genus *Homo* (Wood and Collard 1999).

Homo erectus (~1.9 Ma–110 ka)

Homo erectus represents a significant leap in hominin evolution, with the earliest evidence dating to approximately 1.9 Ma. The species, exhibiting a brain size between 900–1200 cm^3, demonstrates a fully upright posture and a reduction in sexual dimorphism, marking a major transition toward modern human-like morphology (Rightmire 1990). Fossil discoveries such as "Turkana Boy" (1.6 Ma) from Kenya and the remains from Zhoukoudian in China reveal an expansive geographic range, highlighting the species' adaptability across diverse environmental contexts.

The material culture of *H. erectus* includes Acheulean tools, such as bifacial handaxes, which represent a more sophisticated tool-making tradition compared to the Oldowan. Evidence suggests that *H. erectus* was also capable of fire use (~1 Ma) and may have employed rudimentary hunting strategies (Roebroeks and Villa 2011). These technological and behavioral advancements, combined with its anatomical traits, mark

H. erectus as a key ancestor of later *Homo* species, including both *H. sapiens* and the Neanderthals.

Homo heidelbergensis (~700–200 ka)

Homo heidelbergensis, which thrived during the Middle Pleistocene (~700–200 ka), is widely regarded as the common ancestor of both *Homo sapiens* and Neanderthals. Fossils from sites such as the Heidelberg jaw in Germany and the Broken Hill cranium in Africa indicate a species with a brain size ranging from 1100 to 1400 cm³, bridging the anatomical gap between earlier *Homo* species and later forms (Rightmire 2009). *H. heidelbergensis* exhibits a robust skeletal structure, with prominent brow ridges and other archaic features, but also displays a larger cranial vault, consistent with the development of more advanced cognitive capabilities.

The archaeological record suggests that *H. heidelbergensis* engaged in the production of more sophisticated tools, such as prepared-core flakes. Evidence from sites like Atapuerca in Spain also suggests the possibility of symbolic behaviors, including burial practices (Arsuaga*et al.* 1997). These cultural developments point toward an emerging capacity for social organization and the development of complex behaviors. The wide geographic distribution of *H. heidelbergensis,* from Africa to Europe, reflects its ecological versatility and positions it as a critical ancestor of both the Neanderthals in Europe and *Homo sapiens* in Africa.

Homo sapiens (~300 ka–Present)

Homo sapiens first appeared in Africa approximately 300 ka, with key fossil discoveries such as those from Jebel Irhoud, Morocco (315 ka), and OmoKibish, Ethiopia (195 ka), illustrating the gradual emergence of anatomically modern humans. These fossils reveal a suite of modern traits, including a high forehead, rounded skull, and reduced facial robusticity, coupled with a brain size of approximately 1350 cm³ (Hublin*et al.* 2017; Stringer 2016). This period also marks the onset of more sophisticated tool-making, as evidenced by the Upper Palaeolithic technology and symbolic artifacts that date to approximately 50 ka (Henshilwood and Marean 2003).

Genomic studies further confirm the African origin of *H. sapiens* and highlight subsequent migrations out of Africa beginning around 60–50 ka, with evidence of admixture from archaic populations such as Neanderthals and Denisovans, which contributed to the adaptability of modern humans (Green *et al.* 2010). Post-Neolithic adaptations, such as lactase persistence in certain populations, exemplify the ongoing evolutionary process that has shaped the diversity of the modern human population (Schlebusch *et al.* 2017). This continuous evolution underscores the dynamic nature of human adaptation and the complex interplay of cultural, environmental, and genetic factors in the development of *Homo sapiens*.

Perspectives and future directions

The evolutionary trajectory of *Homo sapiens* reveals a complex interplay of genetics, environment, and culture. While significant progress has been made, future research must focus on refining phylogenetic relationships, decoding the genetic basis of cognition, and tracing early language origins. Advances in ancient DNA analysis, AI-driven fossil reconstruction, and interdisciplinary collaborations will enhance our understanding. Predictive models in evolutionary genetics may reveal ongoing microevolutionary trends, while space colonization could drive future human adaptations. Integrating genomics, archaeology, and climate studies on platforms like the Human Origins Initiative will be pivotal in unveiling the next chapters of human evolution.

In our views, the role of *Homo sapiens* in the broader narrative of human evolution is deeply intertwined with the cultural, genetic, and adaptive diversity that has defined our species. The migrations out of Africa, alongside the interactions with Neanderthals and Denisovans, present an essential, yet complex, chapter in our story. These interactions were not merely about competition or replacement; they were also about adaptation, exchange of genes, and even survival strategies. But what is often left underexplored is the profound influence of environmental and climatic factors on the evolutionary changes that shaped our ancestors. For instance, the shifting climate of the Pleistocene. It's likely that the erratic climate changes, with their cycles of ice ages and warmer interglacial periods, played a central role in shaping the physical and behavioral traits of hominins. *Homo erectus,* for instance, might have had to adapt to colder climates as they spread across Eurasia, while *Homo sapiens*, emerging in a more diverse range of environments, had to develop advanced tools, cultural practices, and social strategies to survive. We would argue that the interplay between climate and culture was not just a backdrop, but a driving force for adaptive innovation.

Furthermore, while we often focus on the genetic aspect of evolution, we believe it's crucial to highlight the social and behavioral dimensions that set apart early *Homo* species, particularly *Homo sapiens*. It's tempting to assume that sophisticated social structures, cooperation, and symbolic communication emerged solely with *Homo sapiens*, but this view oversimplifies the story. Evidence suggests that earlier species, like *Homo erectus* and even

Neanderthals exhibited forms of social cooperation and possibly rudimentary symbolic communication, though they may not have been as complex or widespread as those seen in modern humans.This raises an interesting point: how did these social behaviors evolve? Was cooperation, for instance, always a part of hominin behavior, or did it become more pronounced and complex with the appearance of *Homo sapiens*?. We inclined to think that while *Homo sapiens* may have refined social structures to an unparalleled degree, the roots of cooperation, social interaction, and even symbolic communication may have emerged long before. It's possible that what we see in modern human societies—our intricate cultural systems, our art, our rituals—might have had much earlier precursors in the evolutionary timeline.

Conclusion

In conclusion, the story of *Homo sapiens* is more than just a tale of survival; it's one of adaptation, cultural evolution, and an ever-deepening complexity in our relationships with each other and the world around us. Environmental factors, social structures, and even the exchange of ideas between species all played a role in shaping who we are today. The full picture of human evolution is one that requires us to look beyond genetic changes and into the subtle, often unseen forces that shaped the development of our behaviors, cultures, and societies.

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Computational Advances in Drug Design: An overview of structure-based and AI-Driven Approaches

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Abstract

Computational approaches have radically improved in the field of drug discovery. The conventional method often takes more time and is non-economical and uncertain. Computer-aided drug discovery (CADD) has emerged as a powerful tool in pharmaceutical research, which is further classified into structure-based drug design (SBDD) and ligand-based drug design (LBDD). The target proteins are obtained through X-ray crystallography, cryo-electron microscopy, or NMR spectroscopy to design molecules with high binding affinity and specificity. Techniques like pharmacophore modeling, quantitative structure-activity relationships (QSARs) and artificial intelligence (AI) have improved drug screening and optimization and accelerated early-stage in drug discovery. Integration of AI further enhances the toxicity prediction of lead, target identification, and de novo drug design making drug design more efficient. This review highlights the application of SBDD and LBDD, emphasizing their importance in modern-day drug discovery and their potential to facilitate the development of novel therapeutic medicines.

Keywords: Docking, machine learning, MD simulation, structure-based drug design, virtual screening.

Introduction

Over the last few decades, the application of computational approaches in drug discovery has been consolidated. The lack of approved drugs or vaccines continues to be a challenge and further necessitates the discovery of new therapeutic molecules. Small molecule designing with drug-like properties remains a challenge in both fundamental and biopharmaceutical research. Traditional drug development methods are known for being time-consuming, expensive and less efficient, often taking around a decade. This method of drug development process follows a pipeline consisting of target identification, lead discovery, pre-clinical testing, clinical trials and regulatory approval which leads to many errors (Kiriiri *et al.* 2020).

The use of CADD techniques in preliminary studies by leading pharmaceutical companies and research groups has helped to expedite the drug discovery and development process minimizing the costs and failures in the final stage. CADD has several success stories and continues to play a vital role in the drug discovery process. This approach has been utilized in proposing drug candidates against coronavirus disease 2019 (COVID-19) (Tarighi *et al.* 2021). The advancement of computational biology is very evident from a comprehensive review that covers MERS-CoV, covering epidemiology, genome analysis, pathogenesis, diagnostics, vaccine development and predictive modelling (Ganesh *et al.* 2021; Chakrabartty *et al.* 2022). CADD can be broadly divided into structure-based and ligand-based drug design approaches, both have been widely used in the drug discovery process in the identification of suitable lead molecules (Gurung *et al.* 2021; Mouchlis *et al.* 2021; Isert *et al.* 2023).

Structure-based drug design (SBDD)

SBDD is a computational approach that uses protein 3D structures to predict potential drug molecules (Pant *et al.* 2022). It aims to design small-ligand molecules that bind with high affinity and specificity to pre-determined protein targets. Understanding the principles by which small molecules recognize and interact with macromolecules is of great importance in pharmaceutical research and development (Gohlke *et al.* 2002). SBDD systematically uses the structural data, such as macromolecular targets or receptors, gained by experimentation or computational homology modeling (Bajad *et al.* 2021). Recent advances in geometric deep learning, especially in modeling 3D structures of biomolecules, provide a promising direction for SBDD. Despite significant advances in the application of deep learning as surrogate docking models, the deep learning-based design of ligands that bind to target proteins remains a major difficulty in molecular modeling (Sumathi *et al.* 2023). By utilizing structural data, which is acquired by X-ray crystallography, cryo-electron microscopy, or NMR spectroscopy, SBDD makes it possible to generate compounds that selectively interact with the target protein's active region (Bajad *et al.* 2021; Cebi *et al.* 2024). The rational design of inhibitors, modulators, or activators with enhanced efficacy and selectivity is made easier by this approach which is essential to contemporary drug discovery. SBDD makes extensive use of methods including molecular docking, molecular dynamics simulation and free energy computations to forecast binding affinities and maximize drug-like characteristics (Anwar *et al.* 2021; Rakshit *et al.* 2022).

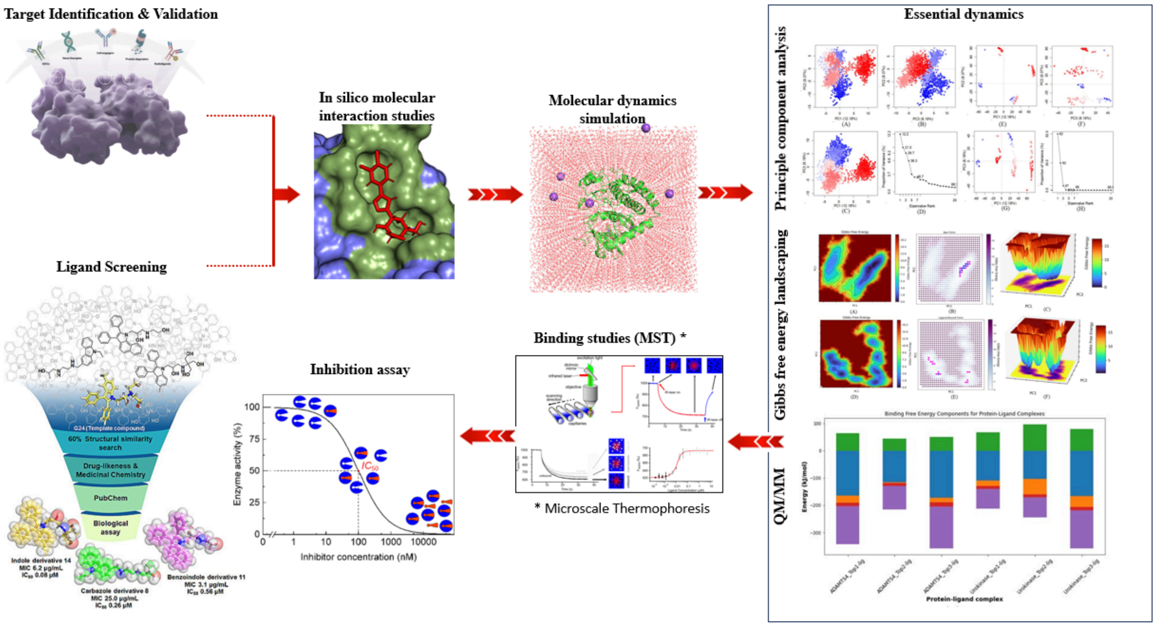

Fig. 1. Showing the general pipeline of structure-based drug discovery.

(Picture courtesy: Adapted from Smirnovienė *et al.* 2021; Jerabek-Willemsen *et al.*2014; Pakamwong *et al.* 2024).

Ligand-based drug design (LBDD)

LBDD is another widely used approach in computer-aided drug discovery and is employed when the three-dimensional structure of the target protein is not available (Ajjarapu *et al.* 2022; Yadav *et al.* 2022). Information from a set of active compounds against a specific target protein receptor reveals key structural properties linked to biological function based on their similarities(Yadav *et al.* 2022). This approach helps in screening virtual compound libraries, optimising lead compounds and accelerating the early stages of drug discovery. Some common techniques used in ligand-based virtual screening approach include pharmacophore modeling, quantitative structure-activity relationships (QSARs) and artificial intelligence (AI) (Murugan *et al.* 2022). QSAR methods help to evaluate the activity of a large number of compounds virtually, reducing the time and labor costs required for the chemical synthesis and experimental determination. This method increases the efficacy of drug discovery (Wang *et al.* 2021). Integration of AI in drug discovery has become a prominent part of modern pharmaceutical research. It helps to automate, assure quality, improve drug efficacy polypharmacology and personalized manufacturing with the least detected error. Furthermore, AI has a wide range of applications in drug discovery, including prediction of protein folding, protein-protein interaction, virtual screening, QSAR, evaluation of ADMET characteristics, and de novo drug design (Gupta *et al.* 2021). By using the capabilities of AI models and large databases, researchers are expediting the prediction, identification and validation of potential drug targets.

Virtual screening

Virtual screening is a computer-based approach developed for comparative analysis of multiple leads, evaluating chemical, biological features and protein-ligand interactions to determine which compounds have the greatest binding to a target protein (Lavecchia 2013). Virtual screens are often done utilizing libraries of substances that can be purchased cheaply and do not require specialised synthesis (Irwin *et al.* 2020). Using computer models of complexes, structure-based virtual screening seeks to identify compounds that generate favourable interactions with biological macromolecules (Carlsson and Luttens 2024). A virtual screening is a fast in silico approach that combines scoring and ranking methods to screen massive database compounds against a biological target. (Giordano *et al.* 2022). High-throughput screening is a widely used technique for identifying potential medication candidates (Maia *et al.* 2020). However, filtering millions of molecules still requires a significant amount of time and energy.

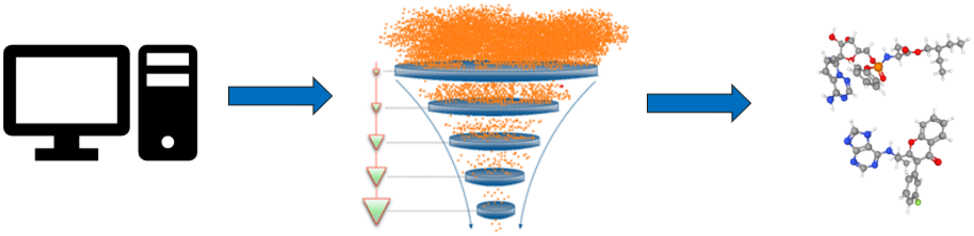

Fig. 2. Showing the different stages of virtual screening of compounds

(Picture courtesy:Adapted from Pyzer-Knapp *et al.* 2015).

An automated screening of compounds using cellular assays, typically carried out robotically, is what distinguishes high-throughput screening from virtual screening (Maia *et al.* 2020). A high-throughput screening is more expensive than a virtual screening (Maia *et al.* 2020). Although virtual screening holds promise for streamlining the drug discovery process, it has its drawbacks. False positives can occasionally emerge from virtual screenings, and the various technologies available for virtual screenings can yield disparate findings using the same data (Maia *et al.* 2020). Nonetheless, virtual screenings are currently being used extensively because of the anticipated time and efficiency savings in the drug discovery process. Acute toxicity, carcinogenicity, hepatotoxicity, Lipinski's rule of five, ADME and blood-brain barrier (BBB) penetration are some of the filtration criteria used to eliminate molecules with undesirable biological availability from the database of ligands (Yamashita and Hashida 2004).

Molecular Docking

Molecular docking usually serves to predict the energy and geometry of receptor-ligand binding by simulating the interaction between the ligand and the receptor's active region (Peluso *et al.* 2019). Molecular docking of small molecules to a biological target involves a creative sampling of potential ligand poses in the designated pocket or groove of the target candidate to determine the best binding shape (Mukesh and Rakesh2011; Guedes *et al.* 2014; Agarwal *et al.* 2015; Seeliger and de Groot 2010). This can be done with the docking software's user-defined fitness or score feature. AutoDock (Morris *et al.* 1998), GLIDE (Friesner *et al.* 2004), Dock (Allen *et al.* 2015) and GOLD (Verdonk *et al.* 2003) are popular programs for docking.

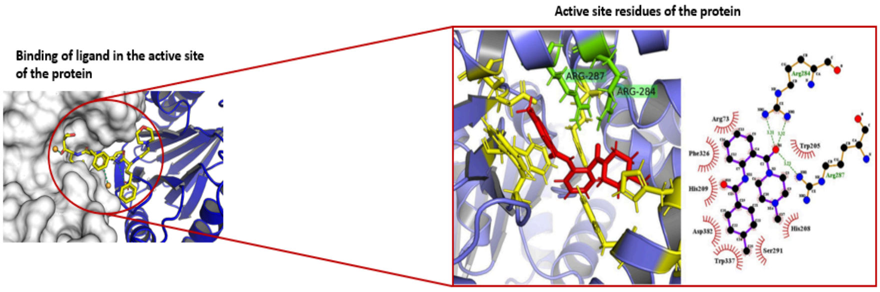

Fig.3. Showing the molecular interaction between the target protein and ligand (Picture courtesy:Adapted from Ferreira *et al.* 2015; Ganguly *et al.*2023).

Conformational search using different algorithms and scoring or ranking of the docked poses are the two main processes in a docking procedure. Scoring functions take as input a candidate binding pose and score the energetic favourability of the ligand binding to the target at that pose. Most docking software use empirical scoring functions (Eldridge *et al.*1997). Empirical scoring functions are made up of words that represent various protein-ligand interactions that are known to be essential for determining binding energy. Scoring functions are designed to maximise the accuracy with which they predict binding postures, binding energies, or the best binders from a set of compounds (Adeshina *et al.* 2020; Li *et al.* 2019). Depending on the goals of the docking simulations, there are many types of molecular docking processes that use either flexible or stiff ligand/target combinations. Flexible ligand docking, which includes the target as a hard molecule. This is the most typical method for docking. stiff body docking involves keeping both the target and ligand molecules stiff. Flexible docking involves both interacting molecules (Guedes *et al.* 2014; Shoichet *et al.* 2002; Gschwend *et al.* 1996). The highest-ranked complex by docking is carried for MD simulation, which explores the complex in detail from a dynamic perspective (Salmaso and Moro 2018).

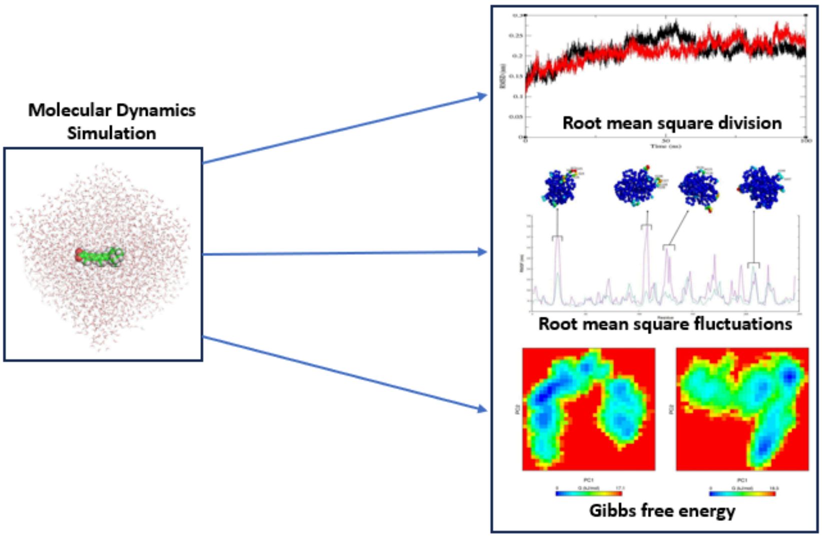

Fig. 4. Depicting the MD simulation between the target protein and ligand and its analysis (Picture courtesy:Böselt *et al.* 2021;Ganguly *et al.* 2022).

Molecular dynamics (MD) simulations

MD is a simulation that demonstrates how molecules move, vibrate, diffuse, and interact over time, inside a sufficiently simulation box, where their movements are governed by traditional Newton's laws of motion (Salo-Ahen *et al.* 2020). Several computer programs have been made available, and today regularly used programs for MD simulations include AMBER (Case *et al.* 2005), CHARMM (Brooks *et al.* 2009), GROMACS (Hess *et al.* 2008), and NAMD (Phillips *et al.* 2020). The MD algorithm incorporates Newton's equations of motion to mimic the motions of atoms over time, and common integration methods include the verlet and leapfrog algorithms (de Oliveira *et al.* 2008). The verlet algorithm is popular because it is simple and time-reversible, despite the fact that it does not explicitly calculate velocities, which can be obtained separately. The leapfrog algorithm, as an alternative, estimates velocities in half-time steps, enhancing velocity calculation accuracy. Choosing an appropriate time step is critical to balance accuracy and computing economy; higher time steps increase sampling efficiency, but can cause errors if too large (González 2011; Hollingsworth and Dror 2018). The force field is a set of equations and constants that represent the potential energy of a system, often split into bound interactions including bond stretching, angle bending, and dihedral torsions and non-bonded interactions such as van der Waals and electrostatic forces (Edeling *et al.* 2024; González 2011).

Hardware advancements, such as specialised supercomputers and graphics processing units (GPUs), have enabled microsecond to millisecond simulations of protein folding, conformational changes, and ligand binding (Durrant and McCammon 2011, Lazim *et al.* 2020). Molecular dynamics (MD) simulations have become essential for researching protein movements at the atomic level, yet they have numerous significant drawbacks. One key challenge is the accuracy of force fields, which are the mathematical models used to represent the forces between atoms; imperfections in force field parameters can lead to large errors in the simulated protein conformations and dynamics (Hollingsworth and Dror 2018; Ormeño and General 2024). The computing expense of MD simulations remains a considerable barrier, especially for long-timescale simulations aimed at capturing physiologically important events happening on millisecond to second durations. Although developments in hardware, such as the use of GPUs and specialised supercomputers, have permitted lengthier simulations, they still demand large resources and typically give restricted sampling due to the enormous configurational space of proteins (Lazim *et al.* 2020; Rácz *et al.* 2022)

Validation of protein-ligand interactions and structural determination

To confirm and characterize protein-ligand interactions, a range of biophysical, structural, and functional techniques are utilized. These methods help determine binding affinity, kinetics, conformational changes, and the biological impact of ligand binding. Each technique provides unique insights, and together, they offer a comprehensive understanding of molecular interactions (Arumugam *et al.* 2024).

Biophysical Techniques for Protein-Ligand Binding Analysis

Microscale Thermophoresis (MST)

MST is a powerful and versatile method used to quantify binding affinity between proteins and ligands by measuring changes in molecular movement within a temperature gradient. When a ligand binds to a protein, it alters the molecule's hydration shell, charge distribution, and size, affecting its thermophoretic mobility. This change is detected using fluorescence, enabling precise determination of dissociation constants (Kd). MST is particularly advantageous due to its low sample consumption, ability to work in native buffer conditions, and capacity to detect weak to strong interactions. Unlike immobilization-based techniques, MST allows interactions to be studied in free solution, preserving the native environment of biomolecules (Picchi 2023; Brunner 2020).

Surface Plasmon Resonance (SPR)

SPR provides real-time insights into binding interactions by detecting changes in the refractive index when a ligand interacts with an immobilized protein on a sensor chip. This technique enables the determination of crucial binding kinetics parameters, including the association rate (ka), dissociation rate (kd), and equilibrium dissociation constant (Kd). SPR is particularly valuable in studying protein-protein interactions, antibody-antigen binding, and small-molecule drug interactions (Ritzefeld *et al.* 2012; Puiu, 2016). It is highly sensitive, allowing the detection of transient and high-affinity interactions. The real-time nature of SPR makes it one of the most effective tools for understanding dynamic binding events, making it indispensable in drug discovery and biomolecular research.

Circular dichroism (CD) spectroscopy

CD Spectroscopy provides valuable information on the secondary structure and conformational stability of proteins upon ligand binding.CD measures the differential absorption of left- and right-circularly polarized light, generating spectral signatures characteristic of α-helices, β-sheets, and random coils. Ligand-induced structural changes can lead to shifts in the CD spectrum, allowing researchers to assess protein folding, stability, and ligand-induced conformational alterations (Pelton *et al.* 2020; Micsonai *et al.* 2022). This technique is particularly useful for evaluating how small molecules, peptides, or mutations affect protein integrity and stability. It plays a significant role in drug discovery, especially when screening compounds that induce conformational changes in target proteins.

Structural determination techniques

X-ray crystallography (XRD)

X-ray crystallography remains the gold standard for high-resolution structural determination of protein-ligand complexes (Srivastava *et al.* 2018**;** Majorek *et al.* 2020). This technique involves crystallizing the protein-ligand complex and using X-ray diffraction patterns to elucidate atomic-level details of molecular interactions. XRD provides precise insights into binding pockets, hydrogen bonding, steric interactions and overall molecular architecture. However, successful crystallization can be challenging, requiring optimization of conditions specific to each protein-ligand system. Despite its limitations, XRD is crucial for structure-based drug design, as it allows for the rational modification of ligands to enhance binding affinity and specificity.

Cryo-electron microscopy (Cryo-EM)

Cryo-EM has revolutionized structural biology by enabling the determination of near-atomic resolution structures of large, dynamic protein-ligand complexes without the need for crystallization. In this technique, biomolecules are rapidly frozen in a thin layer of vitreous ice, preserving their native conformations (Haymaker, 2024). Electron beams are then used to capture multiple projections of the complex, which are computationally reconstructed into three-dimensional structures. Cryo-EM is particularly advantageous for studying flexible proteins, large macromolecular assemblies, and transient interactions that are difficult to crystallize. This method has become an essential tool for visualizing conformational changes upon ligand binding and understanding protein dynamics at the molecular level.

Nuclear magnetic resonance (NMR) spectroscopy

NMR spectroscopy provides structural information for proteins in solution, offering insights into their dynamics and conformational flexibility. However, conventional solution-state NMR is generally limited to proteins smaller than 30 kDa due to signal overlap and spectral complexity (Ikeya *et al.* 2018). Recent advancements, such as solid-state NMR and small-angle X-ray scattering (SAXS), have expanded its applicability to larger biological macromolecules. By integrating SAXS data as constraints, researchers have successfully solved the structures of proteins beyond 20 kDa, improving resolution and accuracy (Delhommel *et al.* 2020).

Functional and mutagenesis studies

Enzyme inhibition assays

Beyond structural techniques, functional assays such as enzyme inhibition assays are crucial for assessing the biological activity of protein-ligand interactions (Riccardi*et al.* 2018; Khan*et al.* 2025). These assays measure whether ligand binding modulates enzymatic function, providing insights into inhibitory potential and mechanism of action. Enzyme activity can be assessed using various methods, including colorimetric assays that detect changes in substrate or product concentration, fluorescence-based assays that utilize fluorogenic substrates, and radioactive assays that offer high sensitivity. Key parameters such as the half-maximal inhibitory concentration (IC_{50}) and inhibition constant (Ki) are determined to evaluate the effectiveness of potential inhibitors. These assays are indispensable in drug discovery, particularly for identifying compounds that target key enzymatic pathways (Fienberg 2017; Tanwar *et al.* 2024).

Mutagenesis studies

Mutagenesis studies further contribute to the validation of protein-ligand interactions by identifying key residues involved in binding. Site-directed mutagenesis, where specific amino acids are substituted, allows researchers to determine the functional significance of individual residues within the binding pocket (Anand *et al.* 2014). Alanine scanning mutagenesis, a commonly used approach, systematically replaces amino acids with alanine to assess their contribution to binding affinity and stability (Moreira *et al.* 2017). More advanced techniques, such as deep mutational scanning, involve the simultaneous screening of multiple mutations to map interaction hotspots comprehensively (Verkhivker *et al.* 2023). These studies are instrumental in designing improved therapeutic proteins, enhancing ligand specificity, and understanding resistance mechanisms in drug-target interactions.

AI and machine learning in structural biology

AI and machine learning have significantly accelerated protein structure determination and ligand binding analysis. In X-ray crystallography, AI-driven algorithms assist in interpreting electron density maps, automating atomic placement, and refining structural models (Vollmar*et al.* 2021). In NMR spectroscopy, AI enhances the assignment of resonance peaks to specific atoms, improving the accuracy of chemical shift predictions and protein folding analysis (Shukla *et al.* 2023). Cryo-EM has particularly benefited from deep learning methods that improve image processing, particle detection, and three-dimensional reconstruction (Vilas *et al.* 2022). Convolutional neural networks (CNNs) have been instrumental in automating key steps such as particle classification and heterogeneity analysis, while generative models have been developed to reconstruct high-resolution 3D structures from heterogeneous samples (Da Wang *et al.* 2021).

The integration of AlphaFold's predictive models with experimental data exemplifies how AI can complement traditional structural biology techniques. AlphaFold has provided highly accurate models of protein structures, which researchers have used as starting points for further refinement through experimental methods (Jumper *et al.* 2021). Moreover, AI-driven approaches are now being applied to solve inverse problems in structural biology, such as refining cryo-EM density maps into atomic models using diffusion-based techniques. The combination of physics-based models with generative learning has proven superior to traditional posterior sampling methods, marking a significant advancement in structural refinement **(**Tiwary *et al.* 2024; Sil *et al.* 2024**)**.

Conclusion

Computational methods have revolutionized drug discovery by reducing costs and increasing efficiency. SBDD utilizes 3D protein structures to design highly specific molecules, enhanced by advances in structural biology and deep learning. LBDD identifies bioactive compounds through structural and physicochemical similarities, aiding virtual screening and lead optimization. Virtual screening rapidly evaluates large compound libraries, while molecular docking predicts ligand-receptor interactions, and molecular dynamics simulations provide insights into molecular stability and conformational changes. Despite challenges like false positives and computational costs, these techniques are essential in modern drug discovery, evolving with AI and high-performance computing. Biophysical methods (MST, SPR, CD spectroscopy) assess binding affinity, while high-resolution techniques (XRD, Cryo-EM) reveal atomic structures. Functional assays confirm biological relevance. AI-driven structural biology further enhances precision, creating a robust framework for drug discovery and targeted therapeutics.

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Pesticides and human health: a double-edged sword in modern agriculture

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Abstract

Without pesticides, global crop yields could decline significantly, as pests, fungi, and weeds threaten food production. Farmers rely on pesticides to prevent large-scale crop losses, ensuring food security and stable prices. While biopesticides have gained attention in recent years, chemical pesticides remain the primary defence against agricultural pests and diseases, playing a crucial role in sustaining global food supplies. Although many harmful pesticides have been restricted and modern formulations are considered safer for non-target species, extensive research indicates that pesticide residues can still pose long-term health risks to humans, animals, and ecosystems. Numerous research has associated pesticide exposure with increased rates of chronic diseases, including various cancers, diabetes, neurodegenerative disorders such as Parkinson's and Alzheimer's, birth defects and reproductive issues. This review explores the classification, mechanisms, benefits, and negative impacts of pesticides on human health and the environment, along with some strategies to mitigate their toxicity. Future research should prioritize innovative farming practices that reduce chemical pesticide use.

Keywords: Crop Protection, chronic disease, non-target organisms, pesticides, toxicity.

Introduction

With the increase in human population, there is a rising demand for food, making efficient agricultural production essential. Food crops are in competition with different weed species, worms and plant-eating insects. Thus, farmers turn to other ways and means to safeguard their crops. The most common thing farmers use to safeguard their crops is agricultural chemicals – pesticides and fertilizers. It is estimated that without pesticides, more than half of the world's crops could be lost to insects, diseases and weeds. Annually, 26% to 40% of potential crop yields are lost as a result of these factors.Without preventive measures, these losses could potentially double (Pesticide Facts 2017), resulting in a significant increase in global food prices.Pesticides enable farmers to grow more food on less land, thus increasing productivity per hectare. By mitigating pests and diseases, pesticides also minimize exposure to food polluted with harmful microorganisms and naturally occurring toxins, helping prevent food-related illnesses (USEPA2013).

According to USEPA (2014), pesticide is any substance or mixture designed to prevent, eliminate, repel, or control pests. Pesticides include insecticides, herbicides, fungicides, rodenticides, and plant growth regulators used to manage pests, diseases, and unwanted species in agriculture (**Fig. 1**). They help preserve agricultural products, ensuring safe storage and transportation, while reducing the risk of contamination. Additionally, they regulate plant growth, enhance yield and safeguard farm products from deterioration, contributing to food security.

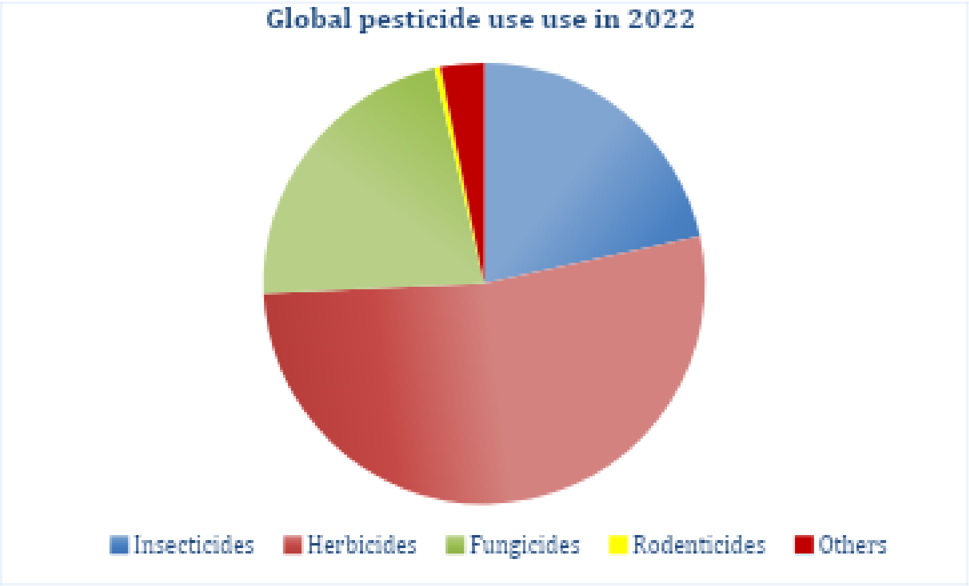

Fig. 1. Global pesticide use in 2022 according to different categories.

(**Source**: Pesticides use and trade. 1990–2022 by the Food and Agriculture Organization of the United Nations.)

The practice of using pesticides contributes to various environmental issues and poses significant risks to the well-being of both humans and animals. Over 98% of sprayed insecticides and 95% of herbicides disperse beyond their intended targets, affecting non-target species, air, water and soil (Saravi and Shokrzadeh 2011). The Green Revolution has significantly amplified global pesticide consumption over the past few decades. By 2022, worldwide agricultural pesticide usage had risen steadily, reaching 3.69 million metric tons. The leading countries in agricultural consumption of pesticides are listed in **Table 1**. In India, pesticide usage remains significantly high, with approximately 55,236 tonnes consumed during 2023–24. Uttar Pradesh, Maharashtra, Punjab, Telangana and West Bengal emerged as the leading consumers, collectively driving the pesticide demand (**Fig. 2**). Pesticides contribute to water pollution and soil contamination, with some classified as persistent organic pollutants. These chemicals are non-biodegradable, accumulating within the environment and entering the human food chain through contaminated food, water, and air. Despite regulatory controls, they persist in ecosystems, often detected in measurable amounts, including in marine life. Their long-term presence poses environmental and health risks, making pesticide exposure a significant concern (Kaur *et al*. 2019).

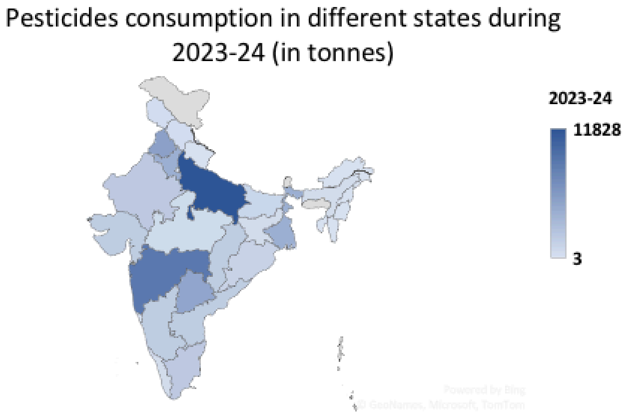

Fig. 2. Pesticides consumption in different states of India during 2023-24 (in tonnes) {**Source**: State-wise Consumption of Pesticides (Technical Grade) in India (2023-2024) by Indiastat.}

Pesticides extend beyond agriculture to significantly enhance public health by controlling vector-borne diseases (**Table 2**). They help reduce waterborne and insect-transmitted diseases such as malaria, Lyme disease and West Nile virus, preventing disease outbreaks by controlling rodent and insect populations. Pesticide application has greatly decreased the spread of diseases to humans, preventing an estimated seven million deaths since 1945 (Himeidan*et al*. 2012). The use of insecticides, a primary tool in vector control, has been pivotal in malaria control programs, leading to substantial declines in disease incidence. The use of pesticides in vector control is not limited to malaria; they are also used to combat vectors responsible for dengue, leishmaniasis, and Chagas disease (van den Berg *et al*. 2012). The strategic use of these chemicals has contributed to the decrease in morbidity and mortality associated with these illnesses.

Table 1. Leading countries in agricultural consumption of pesticides worldwide from 2018-2022 (in tonnes).

	Year				
Country	**2018**	**2019**	**2020**	**2021**	**2022**
Argentina	172928	204559	241294.2	241520	262506.9
Brazil	549280	620538	685746	719507	800652.2
Canada	87632	78893	92960	97692	97692
China	294682.5	273569.5	258757.9	244869.5	235760.4
Colombia	37773	69862	36711	39324	78230.75
France	83983.13	54303.71	64789.22	69697.52	67874.94
India	40093.69	40093.69	40093.69	40093.69	40093.69
Indonesia	261305.8	274421	213036.3	283297.1	294670.4
Russian Federation	74671.56	77306.76	90534.96	97018.45	97018.45
USA	457385.4	495672.7	474707.5	433770.9	467676.6
Vietnam	139416	134741.8	89334.84	148668.7	161908.1

(**Source**: Pesticides use and trade. 1990–2022 by the Food and Agriculture Organization of the United Nations.)

Table 2. Reported annual insecticide use in vector control operations in 2010–2019, by region and insecticide class, expressed in metric tonnes of active ingredient.

WHO region	**OC**	**OP**	**C**	**PY**	**NN**
African	337	390	335	34	35
Americas	0	1104	287	76	0
Eastern Mediterranean	0	15	47	25	1
European	0	1	0	0	0
South-East Asia	2977	92	3	31	0
Western Pacific	0	23	6	29	0
All	3314	1625	677	194	36

(**Source**: Global insecticide use for vector-borne disease control by World Health Organization, 2021) (**Note:** C, carbamates; NN, neonicotinoids; OC, organochlorines; OP, organophosphates; PY, pyrethroids)

Widespread pesticide use increases human exposure, causing acute effects like skin irritation, dizziness, and nausea, while chronic exposure is linked to cancer, respiratory issues, and diabetes. Pesticides contaminate food, water, and air, affecting vulnerable groups. Persistent chemicals bioaccumulate, heightening risks. This review examines health effects, toxicity mechanisms, epidemiological findings, regulations, and alternatives to minimize human exposure.

Benefits of organophosphate pesticides (OPs)

The four major groups of pesticides are organochlorine, organophosphate, carbamate and pyrethroid insecticides (Kaur *et al*. 2019). Among these, OPs remain prevalent due to their effectiveness and affordability, despite concerns about their harmful effects on human health and the environment. Market trends in pesticide use are influenced by agricultural demand, regulatory changes and advancements in pesticide formulation aimed at reducing toxicity and environmental impact (Metatech Insights 2025). Following are some of the main reasons why farmers choose OPs over other pesticides:

1. **Effectiveness**: Organophosphates are highly effective in controlling insect populations quickly, making them a valuable tool in managing severe pest infestations in agricultural settings and residential areas (Metatech Insights 2025). They work by inhibiting acetylcholinesterase, an enzyme crucial for neurotransmission, leading to the paralysis and death of target insects (Iyer *et al*. 2015).
2. **Low persistence**: Unlike some other pesticides, such as organochlorines, organophosphates break down rapidly in the environment. This reduces the risk of long-term environmental pollution and accumulation in ecosystems (Awe *et al*. 2022). Abiotic degradation, including hydrolysis and photodegradation, occurs rapidly under favourable conditions, especially in aerobic soils and sunlit waters. Malathion degrades quickly, reducing its environmental persistence. Biotic degradation further accelerates organophosphate breakdown, with microorganisms metabolizing diazinon and chlorpyrifos into less toxic by-products, significantly reducing their persistence in freshwater systems (Bondarenko *et al.* 2004).

Economic benefit: Organophosphates play a significant role in maintaining crop yields and preventing economic losses due to pest damage. Their use is particularly important in crops like broccoli, oranges, almonds and alfalfa, where alternative pest control methods may be less effective (Zhang *et al*. 2012). Other reason why OPs are commonly used is also that they are very low cost (Metatech Insights 2025).

3. **Disease vector control**: Organophosphate pesticides are widely used for controlling disease vectors, particularly in public health sectors. These insecticides are effective against a variety of pests and vectors of diseases such as malaria, dengue, and leishmaniasis (van den Berg *et al*. 2012). In vector control, organophosphates are used for residual spraying, space spraying, and larviciding (van den Berg *et al.* 2012). They are especially prevalent in regions like South-East Asia, where they are used intensively for malaria control (Hassan *et al.*2021).
4. **Resistance management**: Organophosphates have a low likelihood of inducing resistance in target pests, making them a reliable long-term solution for pest management (Khan *et al*. 2020). Additionally, the use of multiple organophosphate compounds with different potencies, such as chlorpyrifos and omethoate, has been shown to help manage resistance. For example, in field studies on Halotydeus destructor mites, omethoate remained effective even when resistance to chlorpyrifos was observed. Combining OPs or rotating them with other classes of pesticides can also delay resistance development (Umina *et al*. 2023).

Toxic effects of OPs on humans

Pesticides enter humans directly and indirectly, primarily through food (Kalyabina*et al*. 2021). Crops grown on contaminated soil accumulate pesticides in edible and inedible parts, reaching levels that pose health risks to humans and animals. Pesticides can enter the human body through the skin, ingestion, ocular contact, and inhalation. The routes of exposure include ingestion, inhalation, and dermal absorption (Kaur *et al*. 2019). In humans and animals, pesticides can be stored in fatty tissue or they may be metabolized into more toxic substances (Ray and Shaju 2023). Distinct from other organisms, humans are exposed to pesticides in three main ways namely intentional (accidental/suicidal), occupational and non-occupational exposures (Sabarwal *et al*. 2018).

Acute toxicity

Acute toxicity describes the harmful effects that occur following a single exposure or multiple exposures to a substance within a short duration, usually less than 24 h. Pesticides, designed to kill pests, can also pose a risk to human health through acute exposure. The severity of the effects depends on factors like the pesticide type, the dose, the route of exposure (inhalation, ingestion, skin contact), and individual susceptibility (Sabarwal *et al.* 2018).

Clinical symptoms of acute pesticide poisoning range from mild to severe. Mild exposure may cause skin or eye irritation, headaches, dizziness, nausea and vomiting, though severe cases can result in respiratory distress, muscle weakness, convulsions, unconsciousness, or even death. Recently, reports highlighted 17 deaths in a village in Jammu and Kashmir, with OPs identified as the primary cause (Sharma 2025).

Acute exposure to pesticides is often observed in individuals involved in pesticide application, especially without proper protective equipment. Organophosphate and carbamate pesticides inhibit acetylcholinesterase, leading to excessive airway secretions and bronchoconstriction, worsening conditions like asthma and chronic obstructive pulmonary disease (COPD) (Mamane *et al.* 2015).

Cholinesterase inhibition by organophosphate and carbamate pesticides triggers a cholinergic crisis with excessive salivation, sweating, tremors, and respiratory failure. Children are particularly vulnerable to the acute toxicity of pesticides as a result of their smaller body size, developing organs, and behaviours like putting things in their mouths (Buralli *et al.* 2020). Agricultural workers and pesticide applicators are also more vulnerable because of occupational exposure.

Chronic effects

Respiratory disorders

Exposure to pesticides has been increasingly recognized as a significant contributor to respiratory disorders. Pesticide exposure has been increasingly linked to respiratory disorders, affecting both agricultural workers and the general population. Pesticides, particularly organophosphates, carbamates and pyrethroids, contain volatile compounds which, when inhaled, may cause acute and chronic respiratory effects.

Chronic exposure is associated with long-term lung function decline and increased susceptibility to respiratory diseases. Research has connected pesticide exposure to an elevated risk of asthma, bronchitis and COPD (Mamane *et al*. 2015). Epidemiological studies have established a correlation between pesticide exposure and heightened incidences of respiratory conditions including asthma, COPD and lung cancer (Tarmure *et al*. 2020). Prolonged pesticide inhalation can induce oxidative stress, inflammation, and airway remodelling, contributing to irreversible lung damage. Additionally, pesticide-induced oxidative stress may impair immune responses, making individuals more vulnerable to respiratory infections (Doust *et al*. 2014). Occupational exposure is a significant concern, particularly among agricultural workers. Studies revealed that individuals in these professions exhibit more frequent cases of respiratory symptoms, including chronic bronchitis and asthma, compared to non-exposed populations (Mamane *et al*. 2015). Moreover, environmental exposure affects vulnerable populations such as children. Research indicates that children residing in proximity to farms utilizing pesticides like elemental sulfur experience reduced lung function and increased asthma-related symptoms (Buralli *et al*. 2020). Epidemiological studies indicate a higher incidence of asthma and reduced lung function in agricultural workers exposed to pesticides. Additionally, non-occupational exposure through contaminated air, water, and food poses respiratory health risks to the general population (Sabarwal *et al*. 2018).

The underlying pathophysiological processes behind these associations are complex. Certain pesticides, notably organophosphates, possess anti-cholinesterase activity, leading to laryngeal and bronchial constriction and can worsen asthma episodes and other respiratory symptoms. Additionally, these chemicals may trigger oxidative stress, overwhelming cellular detoxification processes and contributing to respiratory pathology (Salameh *et al*. 2006).

Developmental defects

Direct or indirect exposure to pesticides by pregnant mothers, can pose significant health risks. Exposure to pesticides during critical periods of human development has shown a connection with an increased risk of birth defects and developmental toxicity (Rappazzo *et al*. 2016). Prenatal pesticide exposure during the first and second trimesters is linked with diminished cognitive performance in infants, while third-trimester exposure is linked to delayed expressive communication and impaired fine motor skills (Suwannakul *et al*. 2021).

Studies have shown connections between parental pesticide exposure and congenital anomalies, including neural tube defects. For instance, research suggests that prenatal exposure to certain pesticides may elevate the risk of neural tube defects and anencephaly (Felisbino *et al*. 2024).

A study by Bouchard *et al.* (2011) showed that prenatal OPs exposure during pregnancy has been linked to lower cognitive abilities in children at age 7, with a 7-point IQ deficit observed in the highest exposure group. The study suggested that the developing foetal nervous system is particularly susceptible to OPs due to critical neurodevelopmental processes, including synapse formation and myelination.

Furthermore, in this study, researchers found a linear relationship between prenatal contact with organophosphate pesticides and cognitive decline in children. This means that as exposure increases, cognitive abilities decrease gradually and consistently, rather than deteriorating only at high exposure levels. The absence of a threshold implies that there is no known safe level of OPs exposure during pregnancy. Even minimal exposure levels were correlated with reduced IQ scores in children (Bouchard *et al*. 2011).

Research indicates that OPs can cross the placental barrier, potentially disrupting foetal development and increasing the risk of neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) and cognitive impairments (Chen *et al*. 2021; Choi *et al*. 2021).

Many pesticides function as endocrine disruptors (Singare 2016), as such they can interfere with hormonal pathways essential for normal foetal development. The routes of exposure are diverse, encompassing occupational contact among agricultural workers, residential proximity to treated areas, and dietary intake of pesticide residues (Rani *et al.* 2021). Notably, pesticides have been detected in maternal serum, placenta, and umbilical cord (Felisbino *et al.* 2024), indicating foetal exposure during gestation. This prenatal exposure underscores the vulnerability of developing foetuses to environmental toxins.

Neurodegeneration

Emerging research indicates a notable correlation between pesticide exposure and the development of neurodegenerative diseases like amyotrophic lateral sclerosis (ALS), Parkinson’s and Alzheimer’s disease. These diseases, which progressively damage the nervous system, pose significant socio-economic challenges (Yu *et al*. 2021). Pesticides are recognized neurotoxins, and their exposure has been linked to various neurodegenerative disorders, including mild cognitive impairment and dementia (Yan *et al*. 2016). A notable study by researchers at UCLA Health and Harvard identified ten pesticides that disrupted neurons associated with Parkinson's disease, providing new insights into how environmental toxins contribute to this condition (Paul *et al*. 2023).

Epidemiological studies have further supported these findings. For instance, research has demonstrated that exposure to pesticides is associated with at least a 50% increased risk of developing neurodegenerative diseases (Gunnarsson and Bodin, 2019). In addition, a study examining lifelong cumulative exposure to pesticides identified a significant link between this exposure and neurodegenerative diseases in the elderly. Specifically, past occupational exposure to pesticides showed an association with reduced cognitive performance and an increased risk of developing Alzheimer's disease and Parkinson's disease among older adults (Baldi *et al.* 2003).

Also, Hernández *et al.* (2016) established a strong link between prenatal organophosphate exposure and neurodevelopmental disorders, including altered mental or psychomotor development, pervasive developmental disorder and ADHD in children. Neuropathy and other brain damage are among the few signs of the patients exposed to OPs for a long time (Figueiredo *et al*. 2018; Uwaifo and John-Ohimai 2020). Prolonged exposure results in seizures, damages the amygdala most severely, followed by other brain regions, including the hippocampus and cortex (Figueiredo *et al*. 2018).

The mechanisms by which pesticides may induce neurodegeneration include the inhibition of acetylcholinesterase, leading to the accumulation of acetylcholine and subsequent neuronal damage (Kalyabina *et al*. 2021). Additionally, certain pesticides can generate oxidative stress, resulting in neuronal injury (Yan *et al*. 2016). Epigenetic modifications have also been implicated, where pesticide exposure leads to changes in gene expression without altering the DNA sequence, potentially triggering neurodegenerative processes (Yu *et al*. 2021).

Cancer

Pesticide use has been associated with a higher risk of various cancers, raising concerns about their impact on public health. The relationship between pesticide exposure and cancer development has been a subject of extensive research. Epidemiological studies have identified associations between pesticide exposure and various cancers, including leukaemia, lymphoma, and cancers of the different organs like brain, breast and skin (Cavalier *et al*. 2023). It is crucial to highlight that while occupational exposure to pesticides, such as in agricultural settings, has been associated with a higher incidence of cancer risks, the evidence for cancer risk from low-level exposure in the general population is less conclusive. For instance, Cancer Research UK states that there is no strong evidence that exposure to pesticides at low levels causes cancer (Cancer Research UK 2019). A study evaluating the consequences of pesticide use on cancer development in the United States found positive associations between pesticide use and increased rates of non-Hodgkin's lymphoma, leukaemia, colon, bladder, pancreatic, and lung cancer. The study indicated that the cancer risk from pesticide usage is equivalent to tobacco smoking (Gerken *et al*. 2024). Specific pesticides like atrazine, boscalid, dimethomorph, Dicamba, Dimethenamid, Dinotefuran, Glyphosate, Imazethapyr, and metolachlor were identified as primary contributors in areas with a high incidence of malignancies and colon cancers. Dimethomorph is linked to high risks of non-Hodgkin lymphoma and leukaemia. Glyphosate, a common herbicide, has been classified as a probable carcinogen by the International Agency for Research on Cancer (Gerken *et al*. 2024). Commonly used pesticides like OPs are also related to cancer (Sabarwal *et al*. 2018).

A study compared women with breast cancer to healthy women among farm workers, revealing that medium malathion use was linked to a higher breast cancer risk, though not significantly different across all cases. It was also discovered that exposure to any organophosphate, particularly chlorpyrifos, was associated with an elevated risk of breast cancer, especially in postmenopausal women. Repeated exposure to malathion further increased this risk, while a one-time exposure did not have a similar impact (Yang *et al*. 2020).

Diabetes

Pesticide exposure has been increasingly linked to the development of diabetes, marking it as a significant public health concern. Emerging research indicates a notable correlation between pesticide exposure and a greater risk of developing diabetes, particularly type 2 diabetes mellitus (Tyagi *et al*. 2021). Organochlorine pesticides (OCPs) may induce endocrine disruption, potentially leading to the development of diabetes (Tyagi *et al*. 2021). Epidemiological studies have identified a positive correlation between exposure to various classes of pesticides—including insecticides, herbicides, fungicides, rodenticides, and molluscicides—and the prevalence of diabetes. Research conducted in Thailand found that exposure to specific pesticides like endosulfan, mevinphos, carbaryl/Sevin, and benlate significantly increased the risk of diabetes. These findings align with previous epidemiological and animal studies (Juntarawijit and Juntarawijit 2018).

Glyphosate exposure has also been connected with adverse outcomes, with many epidemiological studies linking pesticide exposure to obesity and type 2 diabetes (Li *et al.* 2023). Leso *et al.* (2017) identified a probable link between pesticides, particularly organochlorines and organophosphates, and Diabetes Mellitus. Furthermore, studies demonstrate that individuals with higher pesticide exposure levels, such as agricultural workers, exhibit a greater risk of developing type 2 diabetes, gestational diabetes, and obesity. Conversely, consuming organic foods, which typically contain lower pesticide residues, has been linked to a reduced risk of diabetes and obesity (Poulia *et al.* 2024).

The mechanisms through which pesticides cause abnormal glucose regulation (AGR) and diabetes is thought to be by inducing oxidative stress, inflammation, and insulin resistance, as evidenced by studies on organophosphates, neonicotinoids, and persistent organic pollutants (POPs) (Kim *et al.* 2022). Exposure to chlorpyrifos, diazinon, and organochlorine pesticides has been associated with an elevated risk of diabetes. A meta-analysis revealed a 2.30 times higher risk of developing type 2 diabetes linked to these pesticide exposures (Kim *et al.* 2022). Glyphosate (commonly known as Roundup) seems to influence the gut microbiome, which has been associated with diabetes (Walsh *et al.* 2023).

The underlying mechanisms by which pesticides may contribute to diabetes involve disruptions in metabolic processes. Certain pesticides can interfere with nuclear receptors that regulate lipid and carbohydrate metabolism, such as peroxisome proliferator-activated receptors (PPARs). Alterations in PPAR activity can lead to insulin resistance and impaired glucose homeostasis, both of which are key factors in the development of type 2 diabetes (Hernández-Valdez *et al.* 2023).

Mitigation strategies

Organophosphates are chemicals widely used as pesticides and to control vector borne diseases, but they pose significant risks to human health due to their ability to inhibit acetylcholinesterase, an enzyme critical for nervous system function. Mitigating their effects requires a multifaceted approach involving regulation, exposure reduction and medical interventions. Some of the methods to mitigate are as follows.

1.**Regulatory measures**: Regulatory agencies must enforce rigorous testing of OP compounds before approval, ensuring they do not cause severe neurotoxic effects, as seen with compounds evaluated using the hen test for delayed polyneuropathy (Ehrich and Jortner 2010). Limiting OP use in agriculture and household products can reduce exposure. For example, alternatives to OPs-based sheep dips should be promoted (Kozawa *et al.* 2009).

2.**Reducing Exposure**: Workers handling OPs should wear personal protective equipment (PPE), such as gloves and respiratory masks, to minimize skin absorption and inhalation. Training programs for farmers and pesticide applicators can teach safe handling techniques, reducing risks during activities like orchard spraying or sheep dipping. Monitoring OP residues in food and water supplies is essential to prevent chronic low-level exposure in the general population.

3. **Medical monitoring and treatment**: Regular health checks for workers exposed to OPs can identify early signs of toxicity. Biomarkers like acetylcholinesterase activity levels can be monitored to assess exposure (Ehrich and Jortner 2010). Acute OP poisoning should be treated promptly with antidotes like atropine and oximes, which counteract the inhibition of acetylcholinesterase (Iyer *et al.* 2015). Individuals with chronic effects from OP exposure may require neurological and psychological support, as long-term complications include peripheral neuropathy and neuropsychological abnormalities (Iyer *et al.* 2015). In case of suspected poisoning, immediate medical attention is crucial, along with decontamination measures like removing contaminated clothing and washing the affected skin.

4. **Research and Public Awareness**: Continued research into the mechanisms of OP toxicity and safer alternatives is critical. Public awareness campaigns can educate communities about the risks of OP exposure and safe practices. By combining regulatory oversight, preventive measures, medical intervention, and education, the adverse effects of OPs on humans can be significantly mitigated.

Conclusion

Pesticides play a key role in controlling harmful organisms, enhancing crop yields, improving quality, and preventing economic losses by reducing commodity deterioration. They also aid in disease control. However, their potential health risks have become a growing concern. Occupational exposure, particularly among farmers and agricultural workers, is especially worrying, as they frequently handle significant quantities of these chemicals.

The growing body of evidence suggests that pesticides are involved and linked to the different diseases mentioned above and this has driven scientists to investigate how pesticides contribute to chronic diseases. While much remains to be explored, several mechanisms and pathways have been identified. These mechanisms often interact rather than act independently and may even heighten risks in genetically susceptible individuals. Given the substantial research linking pesticide exposure to chronic illnesses, it is increasingly recognized as a significant risk factor. With chronic diseases being a major global health concern, it is imperative to adopt preventive strategies.

Although there are downsides, such as potential harm to the environment and the development of pesticide-resistant pests, pesticides remain vital for maintaining current levels of food production and preventing widespread malnutrition. Therefore, while pesticides remain a cornerstone in the fight against pests and vector-borne diseases, it is crucial to implement integrated pest management strategies. This includes reducing pesticide use,minimizing dependency, and identifying safer alternatives to mitigate health risks associated with agrochemicals. Such approaches combine chemical and non-chemical methods, ensuring the sustainable and effective control of both agricultural pest and disease vectors.

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Risk factors fueling Meghalaya's cancer incidence

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Abstract

Nestled in the heart of Northeast India, Meghalaya, meaning "Abode of the Clouds," is a state renowned for its lush landscapes and unique cultural heritage. Despite the state's natural beauty and cultural richness, Meghalaya faces a range of healthcare challenges, particularly in the realm of cancer. Many factors have been shown to contribute to the limited access to quality cancer care in the region. The state faces a high prevalence of preventable risk factors, including tobacco and alcohol use, poor diet habits like unhealthy consumption of areca nuts and smoked meats coupled with dietary deficiencies arising from malnutrition and limited access to diverse, nutrient-rich foods. This review paper delves into the multifaceted factors contributing to the high cancer rates in Meghalaya, examining the interplay of lifestyle choices, socio-economic disparities, limited healthcare access and low awareness levels.

Keywords: Betel nut, cancer, Meghalaya, smoke meat, tobacco.

Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide, with its prevalence continuing to soar as the global population ages. As per the Global Cancer Observatory (GLOBOCAN) estimates, 2020 saw a total of 19.3 million new cancer cases globally (Sung *et al*. 2021). India is the third country with the most cancer cases in the world, after China and the United States. It is expected that the number of cancer cases in India will increase by 57.5% between 2020 and 2040, reaching 2.08 million cases (Sathishkumar *et al*. 2023). The northeast region of India bears the highest cancer burden in the country due to a lack of awareness, socio-economic disparities and limited access to diagnosis and treatment facilities, highlighting a geographic disparity compared to more urbanized areas (Mohan *et al*. 2018).In the last few years, the Northeast part of India, especially Meghalaya, has seen a troubling rise in cancer rates, which is causing a big public health crisis. This increase is due to a mix of environmental, lifestyle and socio-economic factors that make people more at risk for different types of cancer.Meghalaya has an alarmingly high incidence of certain cancers compared to other regions in India. Tobacco consumption remains a significant contributor to cancer incidence in Meghalaya (Vaiphei and Sisodia 2020). Additionally, old customs like betel nut chewing and diets high in preserved foods are connected to higher cancer risks (Shanker *et al*. 2021).There is also a very low level of awareness about cancer prevention and screening, with only a few women getting the screenings they need, highlighting a strong need for focused education efforts (Oswal *et al*. 2020).

Meghalaya is a state in Northeastern India known for its breathtaking natural beauty and diverse tribal culture. The state has lush green landscapes, stunning waterfalls, and unique living root bridges. It is also home to the vibrant Khasi, Garo and Jaintia tribes, each with its own distinct traditions and languages(Roy and Tomar 2001). Limited access to healthcare facilities, particularly cancer screening and treatment centers, poses a significant obstacle for the population. The rising global cancer burden puts a tremendous strain on healthcare systems around the world, requiring significant investment in infrastructure and resources. Despite limited data and research specifically on cancer incidence in Meghalaya, this review aims to highlight the key risk factors contributing to the growth of cancer cases within the state.

Overview of cancer incidence in Meghalaya and its significance

In recent times, cancer data from Meghalaya has shown alarming trends that highlight the area's public health issues. In Meghalaya, esophageal cancer is the most common type of cancer for both men and women. Among men, hypopharynx and stomach cancer are the second and third most common, while for women, cervix-uteri and mouth cancer are the second and third most common. A large majority of cancers in both men (67%) and women (43%) are related to tobacco use, with esophageal cancer being the leading tobacco-related cancer for both genders (ICMR-NCDIR 2021). In addition, the very low screening rates, just 0.2% for women and even less for menshow a lack of early diagnosis efforts that could greatly enhance survival rates (Shanker *et al*. 2021). Moreover, complex socio-economic issues, like poor access to health care and high tobacco use, worsen the cancer situation in the area (Vaiphei and Sisodia 2020). Therefore, grasping these factors is essential not only for individual health but also for developing effective public health strategies to address this escalating crisis in Meghalaya.

Cancer facilities in Meghalaya

Currently, cancer care and services in Meghalaya are primarily provided by Shillong Civil Hospital, NEIGRIHMS (North Eastern Indira Gandhi Regional Institute of Health and Medical Sciences), and Tura Civil Hospital. According to a study on the development of cancer care in Northeast India (Harris *et al*. 2022) the first cancer treatments in the state were offered at Shillong Civil Hospital in 1995. Subsequently, NEIGRIHMS has offered chemotherapy services since 2006. Notably, to this day only NEIGRIHMS is the sole government-operated hospital providing radiation therapy to cancer patients within Meghalaya. The state's limited healthcare infrastructure and relatively small population often result in inadequate facilities and specialized expertise. Consequently, many patients seeking advanced treatment or specialized care are compelled to travel outside the state, particularly to neighboring Assam, where larger hospitals and cancer centers offer a wider range of services.While Meghalaya has some hospitals providing cancer care, the number of specialized cancer centers remains limited. A significant increase in financial resources is crucial to improve infrastructure. While the state has a network of Primary Health Centers (PHCs) and Community Health Centers (CHCs), there is a significant shortage of medical professionals, including specialists and radiographers. This shortage, coupled with the state's difficult terrain, poses a barrier to accessing appropriate healthcare. PHCs are the first point of contact for many people in Meghalaya, especially in rural areas. Strengthening PHCs by improving infrastructure, ensuring adequate staffing, and providing essential resources can significantly enhance healthcare access. Establishing more dedicated cancer hospitals or comprehensive cancer care units within existing hospitals can improve access to specialized treatment and diagnostics.Existing cancer care facilities like NEIGRIHMS and Civil Hospital can be further strengthened by increasing bed capacity, upgrading equipment, and ensuring the availability of essential medicines and supplies.The government should also prioritize investment in crucial medical equipment, such as diagnostic tools and advanced treatment technologies.By implementing these strategies, Meghalaya can significantly improve its healthcare infrastructure and ensure accessible and quality healthcare services for all its citizens.

Current cancer statistics and trends in Meghalaya

Current data on cancer shows a concerning situation in Meghalaya, with high rates of occurrence and significant death figures. The state has some of the highest cancer rates in India, with cancers like esophageal, stomach, and cervical cancer being especially common among the people (Shanker *et al.* 2021). East Khasi Hills district in Meghalaya has the highest rates of various cancers, particularly those related to the mouth, throat and esophagus, especially among men**.** This region also has the highest incidence of Human papillomavirus(HPV)-related oropharyngeal cancers in India. Key factors causing this increase seem to be linked to lifestyle habits, including high tobacco use, which is common among both men and increasingly among women (Vaiphei and Sisodia 2020). Unfortunately, many people in Meghalaya face late cancer diagnosis, which significantly reduces their chances of survival. This is largely due to the weak healthcare system and lack of screening programs. A shockingly low 0.2% of women had cancer screenings during that period (ICMR-NCDIR 2021).

Despite the Meghalaya government's efforts to implement public health interventions to combat cancer, the effectiveness of these programs has been limited due to various challenges.One major public health intervention is the National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NPCDCS), implemented nationwide in 2010 (Muksor and Parmar 2022). This program focuses on strengthening infrastructure, human resource development, health promotion, early diagnosis, management, and referral for non-communicable diseases, including cancer. It has led to the establishment of NCD clinics, but data on its impact on cancer screening and incidence reduction is limited due to challenges like vacant health staff positions and low community awareness.Another significant initiative is Ayushman Bharat – Health and Wellness Centres (HWCs) incorporate screening for oral, breast, and cervical cancers as a key service. Despite the establishment of over 173,000 HWCs across India, data on their impact on cancer screening in Meghalaya needs further evaluation (Ministry of Health & Family Welfare 2024). While studies suggest HWCs have increased NCD detection and encouraged public healthcare facility use, their specific impact on cancer screening in Meghalaya requires further assessment. Public health interventions in Meghalaya also face challenges due to sociocultural barriers, such as traditional beliefs and stigma associated with cancer, which hinder prevention and early detection. These beliefs can lead to delays in seeking medical attention and a preference for traditional remedies over conventional treatment (Dkhar *et al.* 2024). Furthermore, deeply ingrained cultural practices like betel nut chewing and tobacco use contribute significantly to cancer risk, and these habits are often concealed, making it difficult to address them effectively through public health campaigns. Cancer treatment can be expensive and many people in Meghalaya face financial barriers to accessing quality care. While health insurance schemes exist, their coverage and effectiveness in addressing the financial burden of cancer treatment needs improvement. Limited healthcare access, lack of awareness about cancer risk factors and prevention strategies, and financial constraints further compound the challenges in effectively countering cancer in Meghalaya.

Lifestyle and environmental risk factors

The mix of how people live and what they are exposed to in the environment plays a big role in the increasing number of cancer cases in Meghalaya, which is shown by local eating habits and substance use.Like other Northeastern states in India, Meghalaya experiences high rates of tobacco and alcohol use, consumption of fermented and smoked meat and chewing of smokeless tobacco. The region also tends to have a lower intake of fruits and vegetables (Shanker *et al*. 2021). Also, many people eat preserved and smoked foods, which can lead to a higher risk of different cancers because of harmful chemicals used in making these foods(Govindasamy *et al*. 2018). Moreover, tobacco use is still very common, especially among young people, where society often accepts it, allowing continued use even though many know about the health risks (Harris *et al*. 2022). With nearly 70% of cancer cases in India linked to avoidable risk factors, these insights highlight the critical need for public health programs focused on education and changing behaviors (Lyngdoh *et al*. 2024). Dealing with these lifestyle and environmental risks through specific actions will be essential to reducing the growing cancer problem in Meghalaya.

Tobacco and betel nut use as major contributors to cancer

Research indicates a significantly higher prevalence of tobacco use among adults in Meghalaya, with 47.0% of adults using tobacco products, surpassing the national average of 28.6%. This breakdown includes smokers (26.7% in Meghalaya vs. 7.2% nationally), smokeless tobacco users (15.4% in Meghalaya vs. 17.9% nationally) and dual users (4.9% in Meghalaya vs. 3.4% nationally) (Biswas *et al*. 2021). Tobacco use has been identified as a significant factor in about two-thirds (67%) of cancer cases in males and 43% in females in Meghalaya. The most common cancer sites related to tobacco use were the esophagus, with 31.0% in males and 22.3% in females, and other prominent sites included the hypopharynx and stomach in males, and cervix-uteri and mouth in females (Shanker *et al*. 2021). The long-standing popularity of tobacco use within the community reflects a complex mix of tradition and modernity. Practices like betel nut chewing and smokeless tobacco are often seen as normal in social situations, especially among young people. With a high rate of tobacco use among both adults (47%) and young people (33.6%), these habits are deeply ingrained in community identity and family traditions. This is especially clear when you see how much parents' tobacco use influences young people starting to use tobacco themselves (Ladusingh *et al.* 2017). Research indicates that the state has the highest rates of esophageal cancer in the country, with a substantial fraction of these cases linked to tobacco usage. Moreover, tobacco-related deaths in Meghalaya exceed 8,000 annually, underscoring the pressing public health crisis (Government of Meghalaya 2023). Among the over 7,000 chemicals present in tobacco smoke, approximately 70 are classified as carcinogens, including polycyclic aromatic hydrocarbons, nitrosamines, and formaldehyde. These compounds directly contribute to genetic mutations within human cells, leading to the development of malignancies. Notably, tobacco smoke is a well-established cause of lung cancer, accounting for approximately 85% of cases, but its impact extends beyond the lungs. Cancers of the mouth, throat, esophagus, pancreas, bladder, kidney, and cervix are also linked to tobacco use, with the synergistic effects of other risk factors, such as alcohol consumption, exacerbating these risks (Hecht 1999). Failure to implement comprehensive tobacco control measures will perpetuate the cycle of health deterioration associated with tobacco use, necessitating urgent action to protect the future generations of Meghalaya.

Betel nut chewing has been a part of Southeast Asian cultures for centuries. It is more than just a habit; it is a tradition that brings people together. In India, especially in Meghalaya, betel nuts have a long history,it's often used in rituals and symbolizes friendship and welcome (Shanker *et al.* 2021). In Meghalaya, high prevalence rates of betel nut use correlate with a troubling rise in oral and esophageal cancers. Many young people start chewing betel nuts because their friends and family do it. They often think it helps with digestion and makes them feel good. Betel nuts are easy to find and are often accepted. In fact, 72.1% of young people chew betel nuts without tobacco. Moreover, an alarming number of users are either unaware of the associated health risks, including its strong correlation with oral cancers, or mistakenly believe such risks are negligible or restricted to tobacco users (Snigdha *et al.* 2021). The consumption of areca nut, a primary component of betel quid, has been linked to a spectrum of health issues, notably oral squamous cell carcinoma (OSCC). The International Agency for Research on Cancer (IARC) has classified betel nut as a Group 1 carcinogen, signifying sufficient evidence for its cancer-causing properties in humans (Warnakulasuriya and Chen 2022). Research indicates that chronic exposure to areca nut compounds leads to significant alterations in chromosomal regions associated with cancer progression, particularly the 9p21 locus, which harbors critical tumor suppressor genes such as CDKN2A. Additionally, its mind-altering effects can lead to addiction, making it difficult to quit and encouraging continued use among young people (Rai *et al*. 2012). The complicated chemical makeup of betel nut includes a variety of bioactive compounds, most notably arecoline, which has been linked to its cancer-causing properties. Arecoline is a powerful alkaloid that can affect different metabolic processes and potentially cause DNA damage, a precursor to cancer development, and contributes to the development of oral premalignant lesions. These lesions, such as leukoplakia and oral submucous fibrosis, can progress to oral cancer if betel nut use persists (Pankaj 2011). Understanding the carcinogenic properties of betel nut, which is classified as a Group 1 human carcinogen by theWorld Health Organization (WHO), can provide crucial insights into its association with the increasing rates of oral cavity and pharyngeal cancers in places like Meghalaya, where an estimated 75% of the population engages in betel nut chewing(Senevirathna *et al*. 2023). The alarming statistics indicating that areca nut chewing contributes to roughly 9.4% of all oral cancers in India reveal an urgent need for focused research to address the health risks associated with its consumption(Garg *et al*. 2014). Despite the ongoing efforts, betel nut use remains widespread in Meghalaya. Studies indicate a high prevalence of betel nut chewing among both adults and youth, with a higher female-to-male ratio. This highlights the deeply rooted nature of the habit and the need for sustained interventions. Alarmingly, children in Meghalaya are becoming familiarized with betel nuts at a young age (10-12 years), emphasizing the need for early intervention and prevention programs (Snigdha *et al*. 2021). Betel nut chewing stands as the primary cause of oral cancer in Meghalaya. The scientific evidence unequivocally links betel nut consumption to an increased risk of this devastating disease. While cultural factors contribute to the widespread prevalence of this habit, public health interventions are crucial to mitigate the oral cancer burden.

Dietary habits and their impact on cancer risk

Over 85% of Meghalaya's population consumes meat (Tripathi *et al*. 2019). A study on dietary habits in Meghalaya reveals that the average monthly meat consumption per consumer unit is significantly higher than the national average. Specifically, rural areas report consuming 0.856 kg, urban areas consume 0.892 kg, while the national average sits at a much lower 0.468 kg (Govindasamy *et al*. 2018). Meghalaya's rugged terrain, dense jungles, and limited access to modern preservation techniques have shaped traditional food practices. The challenging landscape makes frequent food gathering difficult, leading communities to rely on smoking as a means of preserving meat. This age-old method allows them to extend the shelf life of their limited food resources, ensuring sustenance amidst the challenging geographical conditions. However, these culinary practices raise substantial health concerns due to the presence of carcinogenic compounds, notably polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs), which are formed during the smoking process (Lu *et al*. 2017). The interaction of heat with organic matter during smoking processes is pivotal to the formation of PAHs, a group of hazardous substances known for their mutagenic potential. During smoking, the incomplete combustion of wood and the pyrolysis of fat contribute significantly to PAH production, as volatile compounds from the burning materials interact with the meat. Studies have highlighted that various factors including temperature, smoking duration, and the type of wood used affect PAH concentrations in smoked products, presenting considerable health risks when these compounds are ingested (Roshandel *et al*. 2012). Nitrosamines, which are well-known cancer-causing compounds, are thought to play a significant role in the cancer risk associated with red and processed meats (Xie *et al*. 2023). Nitrosamines, such as N-nitroso dimethylamine (NDMA), arise through reactions between nitrites commonly used as preservatives in cured meats and amines during the smoking process (Iko *et al*. 2021). Nitrosamines, found in these products, are not only carcinogenic but are also recognized as one of the three most potent cancer-causing agents globally. Their danger lies in their genotoxic nature, meaning they can damage DNA, cause mutations, and ultimately lead to an increase in the risk of colorectal cancer (Diallo *et al*. 2018). According to one study on colorectal cancers in India, the incidence rate of colon cancer in Meghalaya peaked among individuals aged 60-65, with a negligible rate in younger females. The lifetime risk of developing colon cancer was highest in the Northeast region for both sexes, reaching 1 in 167. Dietary factors such as beef consumption, pungent spices, and red meat may have contributed to this higher incidence (Asthana *et al*. 2021). Consuming smoked meat and living in a poorly ventilated smoky environment was also shown to increase the risk of nasopharyngeal carcinoma (NPC), which has been shown to be the highest in the Northeastern regions (Kataki *et al*. 2011). These findings underline the necessity of strict monitoring and informed dietary practices, as excessive intake of smoked meats laden with toxic substances could significantly contribute to the global burden of cancer.

Alcohol use and other risk factors

In recent years, patterns of alcohol consumption in Meghalaya have garnered attention due to their alarming implications for public health, particularly regarding cancer risk. The prevalence of alcohol use in this state is notably higher than the national average, where approximately 60% of men engage in drinking, significantly exceeding the national average of 38% in India (Yadav *et al.* 2017). This trend is concerning, as studies have established a robust link between alcohol intake and various cancers, including those of the oral cavity, pharynx, and liver cancers that are already widespread in Meghalaya due to regional tobacco use (Bhattacharjee *et al.* 2020). In Meghalaya, nearly 12% of the population reportedly consumes alcohol, positioning it along with tribal practices that may contribute further to this trend (Swargiary 2023). Alcohol consumption poses significant health risks, particularly with various cancers. Among these, liver cancer stands out, with heavy alcohol intake leading to cirrhosis—an established precursor to hepatocellular carcinoma. In India, alcohol accounts for 6.5% of male cancer cases and about 0.8% in females, underscoring the pervasive nature of this issue, especially in resource-limited regions like Meghalaya (Mehrotra *et al.* 2022). Furthermore, studies indicate that breast cancer risk rises with alcohol use, driven by ethanol’s role in increasing estrogen levels and its potential to promote tumorigenesis(Blot 1992).The risk of esophageal cancer, notably high in heavy drinkers, increases significantly with combined alcohol and tobacco use. This is especially relevant in Meghalaya, which has the highest rates of tobacco consumption (Simila *et al.* 2023). Given these correlations, the alarming increase in alcohol consumption in Northeast India, coupled with the socio-cultural acceptance of drinking, raises concerns about the rising incidence of these cancers, highlighting the need for targeted interventions and public health strategies to mitigate these risks.

Other contributing factors to the high prevalence of cancer cases in the state are the low consumption of fruits and green vegetables and more towards a meat-based diet that has been commonly seen within the Northeastern states. Although Meghalaya has been blessed with a lush green forest harboring a wide variety of fruits and vegetables it has been found that there has been a large gap in their intake across each district in the state(Kumar *et al.* 2024). Consumption of many different fruits and vegetables has been shown to reduce the incidence of cancer, and so significant emphasis has been directed in recent years to dietary implications for cancer prevention (Reddy *et al.* 2003). Malnutrition is also another significant concern contributing to the increased risk of cancer in the region.In Meghalaya, high rates of undernutrition, particularly among rural indigenous hill communities, exacerbate the public health crisis (Bhagat 2021). The nutritional landscape in the state presents a significant public health concern, characterized by alarmingly high rates of malnutrition among various demographics. Studies indicate that approximately 31% of children in the region are classified as underweight, with stunting and wasting affecting 57% and 10% of the child population, respectively. Severe anemia also impacts a staggering 68% of children and 83% of women, highlighting pervasive deficiencies in essential micronutrients. Furthermore, the reliance on staples such as rice, combined with inadequate consumption of fruits and vegetables only representing 10.7% of consumer expenditure exacerbates dietary deficiencies and elevates the risk of nutrition-related diseases, including cancer (Nongbri *et al*. 2020). Malnutrition significantly exacerbates cancer risks, establishing a complex interplay between inadequate nutrition and cancer progression. The alarming rates of malnutrition, particularly among vulnerable populations, have critical implications for cancer patients, as noted by research illustrating that 30% to 85% of these individuals face nutritional deficiencies, particularly in settings like Meghalaya where dietary choices are limited (Lyngdoh *et al.* 2024). This nutritional inadequacy is compounded by specific cancer types that induce cachexia, a syndrome characterized by severe weight loss and muscle wasting, which further compromises the immune system and overall health (Argiles 2005). Lastly, in Meghalaya, many people still deeply trust traditional healers for their healthcare. While these healers are important figures in the community, this trust sometimes means people delay or interrupt conventional cancer diagnosis and treatment. Sadly, this often leads to people seeking help when their cancer is more advanced, which makes treatment less effective and lowers the chances of survival (Lyngdoh 2022).

Socio-economic and educational influences

Socio-economic factors play a big role in the increase in cancer rates in Meghalaya. Research shows a clear link between low income and poor access to healthcare resources. Being financially unstable often leads to late diagnoses and insufficient treatment options, making cancer outcomes worse. A local study found that only certain groups understood cancer risk factors, with those who had more money and education being more aware (Oswal *et al.* 2020). This gap in education makes health communication less effective, leaving many at-risk groups not knowing about the importance of prevention and early detection. Moreover, traditional eating habits, shaped by economic conditions, have been connected to higher cancer rates, especially due to the common use of tobacco and preserved foods (Ladusingh *et al*. 2017). Therefore, tackling these socio-economic and educational challenges is important to improving cancer awareness, lowering risk factors, and boosting health outcomes in Meghalaya.

Awareness and knowledge gaps regarding cancer and its risk factors

Even with high cancer rates in Meghalaya, many people still lack awareness and knowledge about its risk factors and ways to prevent it. A cross-sectional study was conducted in the Northeast Region of India, involving 1,400 participants from Assam, Meghalaya and Nagaland. Results revealed limited awareness of cancer types, especially cervical cancer. Understanding of risk factors, symptoms, and signs was also low. While 34% were aware of cancer screening, only a small fraction had undergone any screening. Media was the primary source of cancer information (Oswal *et al.* 2020). This lack of awareness is worrying, especially since preventable risk factors like tobacco and alcohol use are common in the area. Additionally, even though most people understand the importance of early detection and screening, actual participation in these measures is very low (Sathishkumar*et al.* 2021). Limited access to information, along with social and cultural issues, continues to widen these gaps, often resulting in late-stage cancer diagnoses when treatment options are limited (Dutta *et al.* 2022).

Impact of socio-economic status on cancer prevalence, early detection, and treatment access

Socio-economic factors play a big role in cancer rates and treatment access, especially in places like Meghalaya. People from lower income levels often find out they have cancer at more advanced stages because they cannot get the cancer screenings and healthcare they need. For example, the alarming statistic that only 0.2% of women have had screenings for cervical and breast cancer shows how serious these socio-economic gaps in health services are(ICMR-NCDIR 2021). These gaps are made worse by a lack of awareness and education; many people do not know about cancer risk factors and how to prevent them.The rates of certain cancers, like those related to tobacco such as oral and lung cancers, are much higher in poorer communities because these individuals are more likely to take part in risky behaviors without the help to quit (Shanker *et al.* 2021). Therefore, tackling the socio-economic factors affecting health is crucial to improving early detection, treatment access, and overall cancer results in Meghalaya.

Government policies and initiatives play a vital role in cancer control. These include implementing tobacco control measures, promoting healthy diets and ensuring affordable access to cancer treatment.Early cancer detection is critical for successful treatment and improved survival rates, and Meghalaya has implemented various programs to facilitate early screening. Several cancer screening programs operate in Meghalaya, including the "Megh

CAN Care" program, which focuses on community engagement and early detection of prevalent cancers, having screened over 32,000 beneficiaries as of December 2023 (World Economic Forum 2024). Additionally, the nationwide NPCDCS and "Ayushman Bharat – Health and Wellness Centres" include cancer screening as part of their services. Still, data on the number of people screened in Meghalaya is not readily available. Despite these programs, cancer screening in Meghalaya faces challenges such as limited data on long-term impact, hindering proper evaluation and improvement. Sociocultural barriers, including traditional beliefs and stigma, can also hinder screening uptake. Additionally, limited infrastructure and workforce shortages, particularly in rural areas, pose challenges to accessing screening services.

To enhance the effectiveness of cancer screening programs in Meghalaya, it is crucial to strengthen data collection and monitoring, address sociocultural barriers through culturally sensitive awareness campaigns, improve healthcare access, especially in rural areas, and ensure financial support for screening to reduce financial barriers.

Role of education in promoting cancer prevention and early detection

Education is essential for improving cancer prevention and early detection, especially in places like Meghalaya, where awareness is very low. A cross-sectional study on senior school and college-going girls in Shillong revealed that despite a high awareness of breast cancer, a significant portion of respondents were unsure or believed that early detection did not guarantee a favorable prognosis. Breast self-examination (BSE) was less familiar, with only a small percentage of respondents having performed it. While most girls expressed interest in learning BSE, fewer were willing to advocate for its practice in the community showing a lack of serious knowledge of Breast Cancer among senior and college-going girls in Meghalaya (Biswas *et al.* 2020). A significant proportion of women in Meghalaya are diagnosed with breast and cervical cancer at advanced stages (III and IV). This late detection is compounded by the sociocultural context in the tribal community, where open discussions about sexual and reproductive health, including issues related to sexual organs, are often limited in both private and public spheres (Dkhar*et al.* 2024). Better educational programs can give people information about cancer risk factors—like tobacco use, which is closely related to high cancer rates, and promote good health habits, such as regular check-ups (Shanker *et al.* 2021). Additionally, including cancer education in school programs can help teach healthy behaviors from an early age, encouraging changes in how think about cancer prevention. Successful examples, like those in cities with better education levels, show the need for focused outreach programs(Brawley 2017). In the end, using education to create informed communities is crucial for improving early detection, which may help decrease the overall cancer burden in Meghalaya.

Conclusion

The rising cancer cases in Meghalaya discussed in this review show a serious public health issue that needs prompt and thorough action.The cancer situation in Meghalaya shows a worrying pattern where certain types of cancer are more common due to local lifestyles and environmental influences. Data shows that oral cancer makes up many cases, mainly caused by the high use of tobacco and areca nut, worsened by poor oral hygiene(Chaturvedi 2012).Breast cancer rates are increasing among urban women. Still, knowledge about risk factors and screening is very low, with only a small part of the population practicing preventive methods like BSE. Also, the data shows a worrying trend of late-stage cancer diagnoses, increasing death rates, especially for cervical cancer, where screening is very low at just 0.2% in the region. The combination of lifestyle habits, especially tobacco and betel nut use, is an important point in looking at cancer rates in Meghalaya, India. These products are deeply tied to local culture but also play a major role in the high rates of oral and esophageal cancers in the area. Research shows that tobacco accounts for about 67% of cancer cases in men and 43% in women, underscoring its widespread nature as a cancer-causing agent.Even though many people are aware of health issues, there are still gaps in knowledge about how these habits relate to cancer, with only 63% aware of key symptoms for diseases like cervical cancer. Important awareness campaigns should focus on encouraging healthier, nutrient-rich foods rich in fruits and vegetables which have been shown to have a wide range of anti-cancer properties (Kapinova *et al.* 2017). Consequently, targeted educational initiatives that consider socio-economic factors could be instrumental in curbing tobacco use and enhancing overall public health. Integrating awareness campaigns in schools, particularly for younger populations, serves as an essential strategy to boost knowledge and ultimately reduce tobacco-related health risks. The high incidence of tobacco-related diseases highlights the necessity for stringent enforcement of existing regulations such as the Cigarettes and Other Tobacco Products Act (COTPA) to restrict access to tobacco among minors. Some European countries have successfully implemented strict tobacco control laws, resulting in decreased smoking rates and lower incidences of lung cancer (Gredner *et al*. 2021). These strategies include high tobacco taxes, plain packaging, graphic health warnings, and smoke-free public places, demonstrating the potential for similar policies to reduce tobacco-related cancers in Meghalaya effectively. Implement stricter regulations on the sale, advertising and consumption of tobacco products, similar to those for betel nuts, with increased taxation and penalties for violations. Engaging local tribal leaders and influencers in anti-tobacco and anti-betel nut initiatives can be highly effective in promoting behavior change within communities. By actively engaging in health promotion campaigns, they can disseminate crucial information about cancer risk factors, prevention strategies, and the importance of early detection. Their involvement can help overcome sociocultural barriers and encourage community members to adopt healthier lifestyles, participate in screening programs, and seek timely medical attention.By understanding the risk factors contributing to cancer incidence and implementing comprehensive cancer control strategies, the state can strive to reduce its cancer burden and improve the health and well-being of its population. Ultimately, a collaborative framework involving local government, healthcare providers, and educational institutions is crucial for fostering a cancer-free environment in Meghalaya.

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De novo **organogenesis in** ***Citrus latipes*** **(Swingle) Yu. Tanaka:*in vitro*** **root and shoot development from cotyledonary explants**

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Abstract

This study investigates the effects of different concentrations of Naphthaleneacetic Acid (NAA) and 6-Benzylaminopurine (BAP) on the morphological growth parameters of Citrus latipes (Swingle) Yu. Tanaka, with a focus on cotyledon as the potential explant. The optimal concentrations for individual application were identified as 0.4 µM for NAA and 0.3 µM for BAP. Combination of both regulators resulted in enhanced growth, with cotyledon explants demonstrating superior responsiveness and uniform morphological development. Heatmaps were utilized to visualize the effects of various concentrations, while network plots illustrated the influence of each treatment on growth parameters. Additionally, the Bray-Curtis index of dissimilarity was applied to assess correlation between treatments and morphological traits. The findings emphasize the crucial role of cotyledon explants in optimizing in vitro propagation protocols. This study provides valuable insights into the hormonal interplay governing organogenesis in C. latipes, contributing to the refinement of micropropagation strategies for conservation and agricultural applications.

Keywords: Cotyledons, *Citrus latipes* (Swingle) Yu. Tanaka, *in vitro* propagation, shoot and root induction.

Introduction

The genus *Citrus* (family: Rutaceae) comprises several commercially and ecologically significant species cultivated worldwide for their fruits, medicinal properties, and economic value (Nassarawa *et al.* 2024). *Citrus latipes* (Swingle)Yu. Tanaka, commonly known as Khasi papeda, is a lesser-known but important species endemic to Northeast India (Upadhaya *et al.* 2016). It is valued for its genetic diversity, adaptability and potential use in breeding programs for disease resistance and stress tolerance (Arlotta *et al.* 2024). However, conventional propagation methods of *C. latipes*, including seed germination and vegetative propagation, are limited by factors such as long juvenility, low seed viability and susceptibility to biotic and abiotic stresses (Conti *et al.* 2021). Therefore, the development of efficient *in vitro* propagation techniques is crucial for its conservation, mass propagation and genetic improvement.

Tissue culture-based propagation offers a promising alternative for the multiplication and conservation of elite *Citrus* germplasm (Iqbal *et al.* 2019). Among the different explants used in *in vitro* regeneration, cotyledonary explants have shown potential in several plant species due to their high regeneration capacity and responsiveness to growth regulators (Kato 1986; Jhankare *et al.* 2011; Sharma and Srivastava 2014; Gambhir *et al.* 2017; Rajput *et al.* 2022). However, the morphogenetic responses of *Citrus* species cotyledonary explants to different culture conditions remain largely unexplored. The induction of adventitious shoots and roots from cotyledonary explants can provide an effective system for rapid multiplication, genetic transformation studies and cryopreservation.

The success of organogenesis in *Citrus* is largely influenced by the choice of explant, culture medium and the auxin-to-cytokinin ratio rather than the individual concentrations of plant growth regulators (PGRs). Previous studies on *Citrus* species have demonstrated that an optimal balance between cytokinins, such as 6-benzylaminopurine (BAP) and kinetin (Kn), and auxins, such as indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA), is critical for coordinated shoot and root induction (Sharma *et al.* 2021). However, the specific hormonal ratio required for efficient organogenesis in *C. latipes* remains to be optimized.

This study aims to establish an efficient *in vitro* regeneration protocol for *C. latipes* using cotyledonary explants. Specifically, it evaluates the effects of different concentrations and combinations of cytokinins and auxins on shoot and root induction. The findings of this study will contribute to the conservation and large-scale propagation of *C. latipes*, facilitating its potential application in genetic improvement programs and *ex situ* conservation efforts.

Methodology

Mature fruits of *C. latipes* were collected from Upper Shillong, Meghalaya, India. These fruits were then brought to the laboratory and the seeds were then excised. These seeds were then washed with labolene in a 500 mL beaker and then kept in running water for 45 min. After 45 min, the seeds were then transferred into a laminar air flow cabinet and were surface sterilized with 0.2% mercuric chloride for 2 min followed by repeated rinsing with double distilled water 3-5 times. This is then followed by treating the seeds with 70% ethanol for 5 min and repeated rinsing with double distilled water for 5-10 times. An incision was made on the seed coat and the two cotyledons were split. Cotyledon explants were initially cultured in Woody Plant Medium (WPM) supplemented with NAA or BAP individually at concentrations ranging from 0.1 to 0.5 µM to determine their individual effects. Based on the best-performing treatments, a combined treatment of 0.4 µM NAA + 0.3 µM BAP was also evaluated for potential synergistic effects. The pH of the medium was adjusted to 5.8 using 1N NaOH prior to autoclaving at 15 psi, 121°C for 15 min.

Table 1. Composition of Woody Plant Medium (WPM) (Lloyd & McCown, 1980).

Component	**Concentration (mg/L)**
Macronutrients	
NH_4NO_3 (Ammonium nitrate)	400
KNO_3 (Potassium nitrate)	2500
$CaCl_2\cdot 2H_2O$ (Calcium chloride dihydrate)	288
$MgSO_4\cdot 7H_2O$ (Magnesium sulfate heptahydrate)	370
KH_2PO_4 (Potassium dihydrogen phosphate)	170
Micronutrients	
$MnSO_4\cdot H_2O$ (Manganese sulfate monohydrate)	22.3
$ZnSO_4\cdot 7H_2O$ (Zinc sulfate heptahydrate)	8.6
H_3BO_3 (Boric acid)	6.2
KI (Potassium iodide)	0.83
$Na_2MoO_4\cdot 2H_2O$ (Sodium molybdate dihydrate)	0.25
$CuSO_4\cdot 5H_2O$ (Copper sulfate pentahydrate)	0.025
$CoCl_2\cdot 6H_2O$ (Cobalt chloride hexahydrate)	0.025
Iron Source	
$FeSO_4\cdot 7H_2O$ (Ferrous sulfate heptahydrate)	27.8
$Na_2EDTA\cdot 2H_2O$ (Disodium ethylenediaminetetraacetic acid dihydrate)	37.3
Organic Additives	
Myo-inositol	100
Thiamine-HCl (Vitamin B1)	1
Pyridoxine-HCl (Vitamin B6)	1
Nicotinic acid (Niacin)	1
Glycine	2
Carbon Source	
Sucrose	20,000.00
Gelling Agent	
Agar	7,000.00
pH	Adjusted to 5.2 – 5.5

The test tubes containing the cotyledons were initially kept in the dark for 7 days before transferring them to culture room conditions at 25±2°C and 16 h photoperiod at 50 µM $m^{-2}s^{-1}$ light intensity (Light intensity was measured using a Traceable™ Dual-Display Light Meter, Fisher Scientific, Mumbai, India). The regeneration rates as well as other morphological growth parameters were assessed 30 days after inoculation. On the other hand, time taken for regeneration was accessed every week. The emergence of miniature shoots or roots were taken as a sign of regeneration.Ten replicates were maintained and the experiment was repeated thrice.

The plantlets that emerged from these cotyledons were subcultured every 2 months by transferring them on to a freshly prepared WPM medium (**Table 1**) supplemented with the best combination of NAA and IAA three times.

Statistical analysis

All data obtained in this investigation were subjected to one-way ANOVA. Means of the data generated in this investigation were subjected to significance test employing Duncan's test of significance at significance level $p \leq 0.05$ using origin pro statistical software (PC version 8.1, Northampton, MA). All chemicals used in this study were of analytical grade and were procured from HiMedia Laboratories (**Table 2**).

Table 2. List of chemicals used in this study.

Chemical Name	**Catalog Number**	**Company Name**
6-Benzylaminopurine (BAP)	PCT0802	HiMedia Laboratories
Naphthaleneacetic acid (NAA)	RM3986	HiMedia Laboratories
Indole-3-butyric acid (IBA)	RM3987	HiMedia Laboratories
Murashige & Skoog Basal Medium	PT001	HiMedia Laboratories
Woody Plant Medium (WPM)	PT010	HiMedia Laboratories
Sucrose	GRM077	HiMedia Laboratories
Agar	RM026	HiMedia Laboratories
Sodium nitroprusside	RM577	HiMedia Laboratories
α-Tocopherol (Vitamin E)	RM2762	HiMedia Laboratories

Results and discussion

The results indicated that the optimal individual concentrations for shoot and root induction were 0.4 µM NAA and 0.3 µM BAP, respectively. To further assess potential synergistic effects, these concentrations were combined (0.4 µM NAA + 0.3 µM BAP), resulting in enhanced regeneration efficiency (**Table 3**). These findings align with previous studies that highlight the role of auxins and cytokinins in plant tissue culture, particularly in the regulation of cell division, elongation, and differentiation (Sosnowski*et al.* 2023; Hairuddin *et al.* 2023).

Table 3. Effect of varying concentration of Napthaleneacetic acid (NAA) and 6-benzylaminopurines (BAP) on several morphological growth parameters in *Citrus latipes* (Swingle) Yu. Tanaka.

Treatments	Concentration (µM)	Number of cotyledons assessed	Regeneration Percentage (%)	Number of leaves	Shoot length (cm)	Root length (cm)	Root number	Shoot number	Time taken for regeneration (in days)
NAA	0.1	30	41.25±0.05	4.21±0.22	5.65±0.33	9.63±0.24	4.22±0.07	2.24±0.08	17.25±0.11
	0.2		43.66±0.07	5.14±0.02	5.86±0.05	9.88±0.08	4.58±0.14	2.36±0.06	16.52±0.07
	0.3		47.85±0.02	5.36±0.07	6.25±0.01	10.85±0.02	4.98±0.05	2.48±0.08	16.02±0.05
	0.4		52.25±0.06	6.02±0.04	5.12±0.08	10.56±0.22	5.24±0.05	2.85±0.14	15.24±0.15
	0.5		49.35±0.02	5.56±0.01	5.02±0.05	10.14±0.06	5.02±0.08	2.74±0.10	15.88±0.16
BAP	0.1		36.68±0.02	4.65±0.02	4.85±0.04	7.52±0.05	3.25±0.14	3.36±0.02	18.56±0.11
	0.2		39.65±0.04	4.88±0.15	4.96±0.22	7.65±0.02	3.88±0.17	3.45±0.24	18.02±0.05
	0.3		49.25±0.12	6.66±0.11	5.35±0.06	8.12±0.25	4.25±0.06	4.21±0.25	16.24±0.08
	0.4		47.25±0.01	5.36±0.04	5.10±0.02	8.59±0.07	4.05±0.06	3.95±0.14	16.88±0.14
	0.5		43.44±0.05	5.68±0.05	5.02±0.04	8.18±0.05	3.95±0.07	3.66±0.15	17.52±0.01
NAA+BAP	0.4+0.3		61.36±0.04	7.15±0.11	6.88±0.14	11.55±0.02	4.98±0.02	4.55±0.11	14.45±0.22

Heatmaps were utilized to visually represent the effect of different molar concentrations of NAA and BAP on various growth parameters. This approach provided a comprehensive and intuitive analysis of the data, allowing for the identification of trends and interactions between the plant growth regulators. The heatmaps revealed a clear concentration-dependent response, where increasing concentrations of NAA beyond 0.4 µM and BAP beyond 0.3 µM led to diminishing returns or even inhibitory effects on growth (**Fig. 1**). This visualization highlights the importance of precise hormonal balance for optimal *in vitro* regeneration.

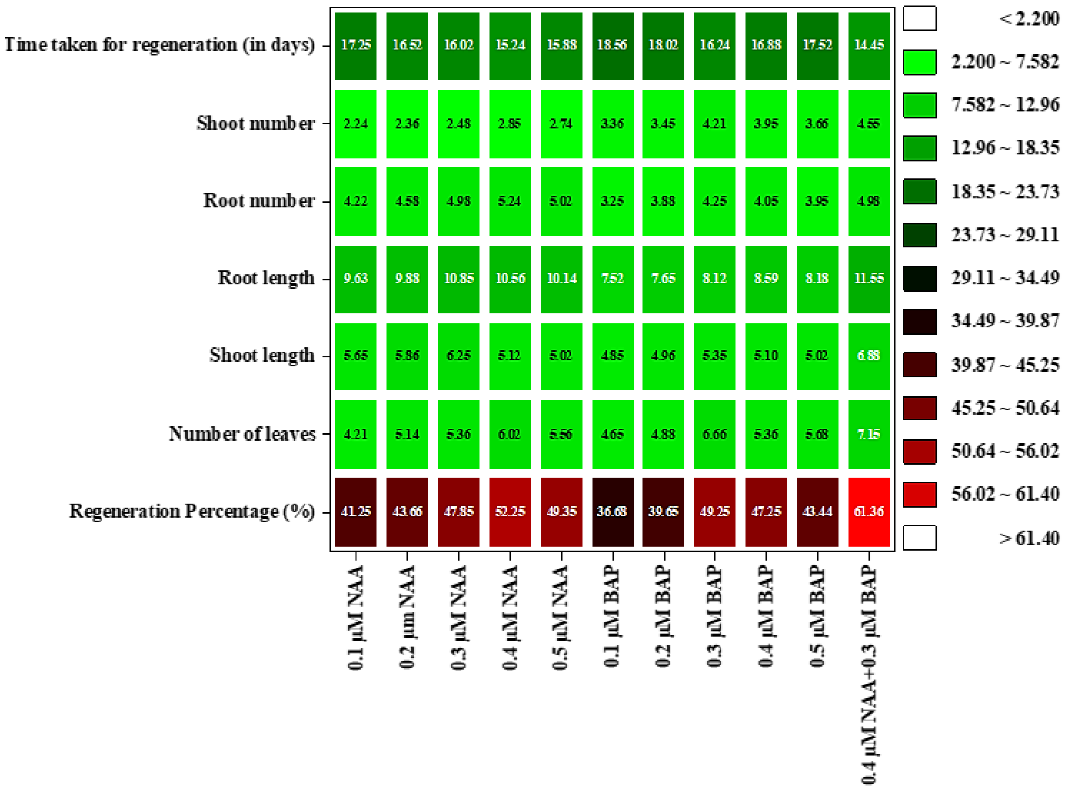

Fig. 1. Heatmaps showing a comparison of the effect of different molar concentration of Napthaleneacetic acid (NAA) and 6-benzylaminopurines (BAP) on several morphological growth parameters in *Citrus latipes* (Swingle) Yu. Tanaka.

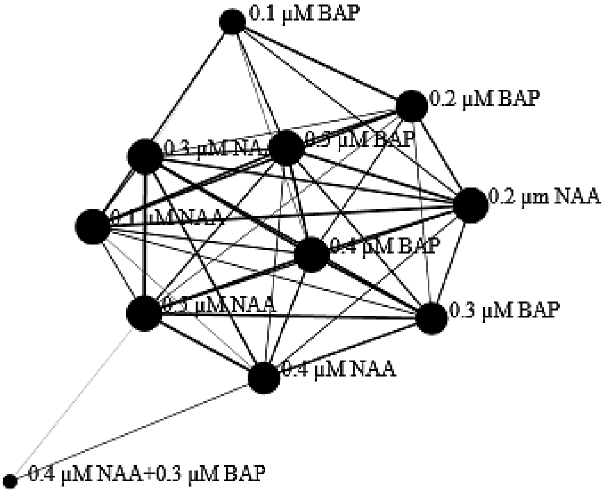

Fig. 2. Network plot analysis illustrating the influence and correlation between different concentrations (µM) of naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) on plantlet morphological growth parameters, including regeneration percentage, number of leaves, shoot and root length, shoot and root number, and time taken for regeneration in *Citrus latipes* (Swingle) Yu. Tanaka. The nodes (dots) represent specific NAA and BAP treatment concentrations. The bold lines indicate strong correlations between treatments and plant growth parameters, while the lighter lines represent weaker correlations.

In addition to heatmaps, a network plot was prepared to illustrate the level of influence of each treatment on the morphological growth parameters. This analysis helped in identifying key hormonal interactions and their relative contributions to growth responses. The network plot analysis (**Fig. 2**) revealed that irrespective of the compound used, when they are not combine, they independently often exhibit closely related pattern of growth. However, when these compounds are combined together there is a sharp increase in the overall plant development as evident from the treatment being an outlier (**Fig. 3**).

Fig. 3. Sequential development of *Citrus latipes* (Swingle) Yu. Tanaka plantlets in Woody Plant Medium (WPM) supplemented with 0.4 µM NAA in combination with 0.3 µM BAP. (A) 15-day-old cotyledon explant cultured in WPM. (B) Root emergence from cotyledon explants at 15 days after inoculation. (C) Shoot initiation from cotyledon explants at 30 days after inoculation. (D) 1.5-month-old plantlets developed in WPM. (E) 2-month-old plantlets showing further growth and development. (F) Well-developed roots of 2-month-old plantlets.

Furthermore, the correlational relationships between different treatments and growth parameters were analyzed using the Bray-Curtis index of dissimilarity. This analysis allowed for a quantitative assessment of the degree of similarity or divergence between treatment effects (Ricotta and Pavoine 2022), thereby highlighting the extent to which certain hormone concentrations elicit comparable or distinct morphological responses. The results revealed clusters of treatments that induced similar growth patterns, reinforcing the idea that specific NAA and BAP concentrations produce predictable and reproducible effects on plant development (**Table 4**).

Table 4. Correlation between different treatments using Bray-Curtis index of dissimilarity by taking all plantlets morphological growth parameters into account

Treatments	**0.1 µM NAA**	**0.2 µM NAA**	**0.3 µM NAA**	**0.4 µM NAA**	**0.5 µM NAA**	**0.1 µM BAP**	**0.2 µM BAP**	**0.3 µM BAP**	**0.4 µM BAP**	**0.5 µM BAP**	**0.4 µM NAA+0.3 µM BAP**
0.1 µM NAA	1.0000	0.9709	0.9338	0.9014	0.9256	0.9307	0.9565	0.9145	0.9374	0.9552	0.8358
0.2 µM NAA	0.9709	1.0000	0.9626	0.9281	0.9523	0.9089	0.9373	0.9350	0.9535	0.9645	0.8640
0.3 µM NAA	0.9338	0.9626	1.0000	0.9587	0.9782	0.8740	0.9022	0.9520	0.9607	0.9319	0.9010
0.4 µM NAA	0.9014	0.9281	0.9587	1.0000	0.9746	0.8520	0.8801	0.9495	0.9386	0.9133	0.9244
0.5 µM NAA	0.9256	0.9523	0.9782	0.9746	1.0000	0.8758	0.9040	0.9673	0.9615	0.9359	0.9015
0.1 µM BAP	0.9307	0.9089	0.8740	0.8520	0.8758	1.0000	0.9709	0.8852	0.9079	0.9359	0.7878
0.2 µM BAP	0.9565	0.9373	0.9022	0.8801	0.9040	0.9709	1.0000	0.9142	0.9368	0.9649	0.8161
0.3 µM BAP	0.9145	0.9350	0.9520	0.9495	0.9673	0.8852	0.9142	1.0000	0.9724	0.9487	0.9004
0.4 µM BAP	0.9374	0.9535	0.9607	0.9386	0.9615	0.9079	0.9368	0.9724	1.0000	0.9684	0.8783
0.5 µM BAP	0.9552	0.9645	0.9319	0.9133	0.9359	0.9359	0.9649	0.9487	0.9684	1.0000	0.8507
0.4 µM NAA+0.3 µM BAP	0.8358	0.8640	0.9010	0.9244	0.9015	0.7878	0.8161	0.9004	0.8783	0.8507	1.0000

The combined application of NAA and BAP resulted in even greater enhancement of morphological traits when cotyledon explants were used. This synergistic effect can be attributed to the interplay between auxins and cytokinins in regulating organogenesis. While auxins primarily influence root formation and cell expansion, cytokinins promote shoot proliferation and delay senescence (Revathi *et al.* 2020; Benedetto *et al.* 2023). The heatmaps and network plots further demonstrated that cotyledon explants showed a more responsive and uniform growth pattern under the optimized concentrations, reinforcing their suitability for *in vitro* propagation.

The enhanced response observed with cotyledon explants suggests that this tissue type may have a higher capacity for organogenesis under optimal hormonal conditions. Cotyledons often contain high levels of endogenous growth regulators, which could contribute to their enhanced response when exogenous NAA and BAP are applied (Ċosiċ *et al.* 2015). Additionally, the physiological state of the cotyledonary tissue might be more conducive to hormonal uptake and signal transduction, thereby amplifying the growth-promoting effects of the supplemented medium.

These findings emphasize the importance of optimizing plant growth regulators for *in vitro* propagation of *C. latipes*. The use of heatmaps and network plots as data visualization tools provided an additional layer of insight, enabling the identification of optimal concentration ranges, key treatment influences, and potential threshold effects. The application of the Bray-Curtis index of dissimilarity further strengthened the statistical evaluation, offering a robust framework for understanding treatment correlations. Future studies could further explore the molecular mechanisms underlying these interactions and evaluate the long-term effects of these hormonal combinations on plantlet acclimatization and field performance. Understanding these dynamics is crucial for refining micropropagation protocols, particularly for the conservation and mass propagation of economically and ecologically significant citrus species.

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Conflict of Interest

The authors declare that there are no known financial or any other conflicts of interest.

Data availability

All data presented in this investigation will be provided upon request.

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In-vitro **assessment for antioxidant activity and phytochemical analysis of *Garcinia pedunculata***

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Abstract

In this study, the fruit of Garcinia pedunculata was screened for the presence of phytochemical compounds and antioxidant properties of the fruit in different extracts (aqueous, methanol, ethyl acetate and butanol, were compared and evaluated. Preliminary qualitative phytochemical screening of various extracts of the fruit confirmed the presence of alkaloids, phenols and tannins, flavonoids, saponins, proteins and carbohydrates. Phytochemical quantification of various extracts showed the highest total phenolic content with total flavonoid content of ***15.95 ± 0.29*** *mg QE/g,* ***21.39 ± 0.34*** *mg GAE/g in the aqueous extract of the plant. The same was also observed for TAC with aqueous showing the highest (****19.19 ± 0.36*** *mg AAE/g) and anti-oxidant activity with* IC_{50} *of* ***1.19*** *mg/ml for DPPH. Aqueous extract also showed the highest reducing capacity of* ***0.123 ± 0.0023*** *for FRAP.*

Keywords: Antioxidants, Garcinia pedunculata, phytochemicals, natural plant.

Introduction

A high dietary intake of fruits and vegetables is highly recommended as they possess several health benefits. Studies have reported that consumption of plant based diet significantly reduces the risk of developing stress related disorders due to the presence of antioxidants. Fruits and vegetables have been proven to have concentrations of vitamin C and A, electrolytes and phytochemicals, which are being identified as antioxidants(Arabshahi-Delouee and Urooj 2007). Major components of phytochemicals include alkaloids, glycosides, polyphenols (flavonoids and phenolic compounds) and terpenes. Diet rich in fruits and vegetables have also been proven to lower the chances of developing cancer (Ames 1983; Slavin and Lloyd 2012).

Since time immemorial, plants have been extensively used as traditional treatment and cure for various ailments. The knowledge of plants and their medicinal properties has led to an increase in the development of plant-based medicines that offer unique advantages like little to no side-effects, less toxic, cost effective and therapeutic effects (Scartezzini and Speroni 2000). According to WHO (World Health Organisation), 65% to 80% of the population of developing countries rely on traditional medicines for treatment of various ailments. WHO also encourages the safe and rational use of plants as traditional medicines for treatment (WHO 2011).

The use of natural products has witnessed a remarkable growth over the past years for their potential as a novel approach to medicinal therapy via phytochemicals for development of drugs (Chaachouay and Zidane 2024). Despite the advancement in the modern medicinal field, the use of medicinal plants continues to thrive due to their easy access, low-cost and having minimal to no side effect on patients(Veeresham 2012; Karimi *et al.* 2015). People living in rural areas and villages are highly dependent on plant therapy for treatment and survival, with little to no access to modern healthcare (Garcia 2020). About 25% of modern drugs and 60% of chemotherapeutic drugs available are chemical compounds derived from natural plant products and have been extensively used for medicinal treatment since the beginning of time (Gordaliza 2007; Newman and Cragg 2012). The unique properties of plants possessing phytochemicals make them the perfect choice of research areas such as their roles in inhibiting cancer cell progression (Hosseini and Ghorbani 2015). In recent times, traditional medicines have gained immense popularity and have been aggressively marketed as diet supplements (Ekor 2014).

Garcinia pedunculata Roxb. (**Fig. 1.**) which belongs to Kingdom: Plantae; Phylum: Tracheophyta; Class: Magnoliopsida; Order: Malpighiales; Family: Clusiaceae; Genus: *Garcinia*; Species: *pedunculata*. *Garcinia pedunculata* (GP) also known as BorThekera in Assam, Taikor in Bangladesh, Amlavetasa in India and Soh Danei in Meghalaya, is widely distributed throughout Northeast region of India (Sawian*et al.* 2007; Mundugaru*et al.* 2014; Islam *et al.* 2015; Bhuyan *et al.* 2020). Traditionally, this plant has been used for the treatment of various ailments. Local practitioners use the fruits for treatment of obesity, digestive disorders, gastric problems, detoxification for food poisoning and body cleanse due to the use of wrong drugs (Sarma and Devi 2015). Methanolic and ethanolic extract of *Garcinia pedunculata* pericarp showed promising results as treatments for urinary tract infections (Zoliansanga and Lalfakzuala 2021). Methanolic extract of the fruit of *G.pedunculata* significantly decreased the levels of LDL-cholesterol and VLDL-cholesterol while increasing HDL cholesterol level in high fat induced rats, suggesting an efficient way to treat hyperlipidemia (Sarma *et al.* 2016). Other reported benefits of GP includes antibacterial, hepatoprotective, anti-inflammatory, cardioprotective, nephroprotective, antioxidant and anti-pyretic (Mundugaru*et al.*2016; Bhuyan *et al.* 2020; Basak *et al.* 2021, Zoliangsanga and Lalfakzuala 2021). Antimutagenic property of GP was first reported in the extracts of fruit rinds (Jayaprakasha *et al.*2006).

In the current study, we aim to determine the phytochemical compounds present in different solvent extracts and to also evaluate the antioxidant properties of *Garcinia pedunculata.*

Fig. 1. Fruit of *Garcinia pedunculata*.

Materials and methods

Plant material

Fresh fruits of *Garcinia pedunculata* were collected from Ri-Bhoi district of Meghalaya, India. The fruits were washed thoroughly with tap water and then distilled water. The fruits were peeled off, deseeded and cut into thin slices. The fruit slices were dried in an oven at less than 40 ºC for 5-7 days. The dried fruit slices were grounded using an electrical blender. The powdered form of the dried fruit was weighed and stored in an airtight bottle at 4 ºC for further analysis.

Extract preparation

The extracts were prepared by dissolving 10 g of powdered fruit material in 100 ml of solvent in a beaker, at the ratio of 1:10 (powder:solvent). The beaker was covered using an aluminium foil and the powder+solvent was subjected to magnetic stirring for 24 h. Themethanolic, ethyl acetate and butanolic filtrates were collected by filtering the extract through muslin cloth followed by Whatman # 1 filter paper. The filtrates were then concentrated by using a Rotary Evaporator and lyophilised to obtain the powdered form of the extract, whereas the aqueous extract was directly lyophilised. The lyophilised form of the extract was dissolved in normal saline solution at desired concentration and stored at 4℃ for further use.

Chemicals

Standards, such as DPPH, ascorbic acid, gallic acid and tannic acid were purchased from SRL. Folin-Ciocalteu's reagent, sodium carbonate, sodium nitrite, aluminum chloride, sodium hydroxide, potassium dihydrogen orthophosphate, sodium hydroxide, potassium ferricyanide, trichloroacetic acid, ferric chloride and others chemicals used in the study were of analytical grade and purchased from Sigma-Aldrich and HiMedia laboratory, India.

Phytochemical Screening

Qualitative analysis

The lyophilised samples were screened for the presence of phytochemical constituents such as flavonoids (Zhishen*et al.* 1999; Edeoga*et al.* 2005); saponins (Kokate 1999); tannins (Trease and Evans 1989; Ainsworth and Gillespie, 2007);alkaloids (Harborne 1980; Evans 1997); polyphenols (Ainsworth and Gillespie 2007); anthraquinones (Trease and Evans 1996).

Quantitative analysis

- ***Estimation of total flavonoid content* (TFC)**

The amount of TFC present in the plant extracts was determined spectrophotometrically by the aluminium chloride method with slight modifications (Arvouet-grand *et al.* 1994). An aliquot of 1 ml sample (0.1-1 mg/ml) was added to 1 ml of 2% aluminium chloride (dissolved in methanol). The reaction mixture was allowed to stand at room temperature for 1 h after which the absorbance was read at 415 nm against reagent blank. Quercetin was used as a standard reference. The TFC was expressed as mg Quercetin Equivalent/g dried weight of extract and calculated by the formula, TFC = (C x V)/M where, C is the concentration of quercetin (mg/ml), V is the volume of the extract in ml and M is the mass of the plant sample (g).

- ***Estimation of total phenolic content*** **(TPC)**

The amount of the TPCwas determined by the Folin-Ciocalteu method with slight modifications (Singleton*et al.* 1999). In brief, 1 ml of sample extract (0.1-1 mg/ml) was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted 1:10 with distilled water). This was allowed to stand for 5 min after which 4 ml of 7.5% sodium carbonate solution was added. The reaction mixture was incubated in the dark at room temperature for 2 h and the absorbance read at 740 nm against reagent blank. Gallic acid was used as a standard reference. The TPC was expressed as mg gallic acid equivalents per g dried sample and calculated by the formula , TPC = (C x V)/M, where C is the concentration of the gallic acid (mg/ml), V is the volume of the sample in ml and M is the mass of the sample extract in g.

- ***Estimation of total tannin content*** **(TTC)**

TTC was determined by a slightly modified Folin-Ciocalteu method (Ainsworth and Gillespie 2007).0.1 ml of sample extract was first dissolved in 7.5 ml distilled water followed by addition of Folin-Ciocalteu reagent (previously diluted 1:10 with distilled water). To this mixture, 1 ml of 35% sodium carbonate solution was added and the final volume of the solution was made to 10 ml. This was then incubated at room temperature for 30 min after which the absorbance was read at 700nm. Tannic acid was used as a reference standard. The TTC was expressed as mg tannic acid equivalent per g dried sample and calculated by the formula, TTC= (C x V)/M, where C is the concentration of tannic acid (mg/ml), V is the volume of sample extract(ml) and M is the mass of the sample (g).

In-vitro **Antioxidant Activity**

- ***DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay***

The ability of the plant extracts to scavenge the DPPH radical was determined *in-vitro* according to the method with slight modifications (Karadag *et al.* 2009). An aliquot of 1ml of plant extracts of varying concentrations (0.1-1 mg/ml) was added to 2 ml of DPPH reagent (0.004% dissolved in methanol). The reaction mixtures were incubated in the dark for 30 min after which the samples were read at 517 nm. A control was prepared without the sample. Ascorbic acid was used as the standard reference. The scavenging activity of the samples was expressed as % inhibition and calculated according to the formula below:

Scavenging activity/ % inhibition = [(absorbance$_{control}$ -absorbance$_{sample)}$/absorbance$_{control}$] x 100

A decrease in absorbance signifies higher DPPH scavenging activity.

- ***Total reducing power assay***

This assay is based on the ability of plant extracts to reduce Fe^{3+} to Fe^{2+} and the total reducing power was obtained by using the Ferric Reducing Antioxidant Power (FRAP) method (Deore *et al.* 2009). An aliquot of 1 ml of sample of varying concentrations (0.1-1 mg/ml) was added to 1 ml of phosphate buffer (0.2 M, ph 6.6) and 1 ml of 1% potassium ferricyanide [($K_3Fe(CN)_6$]. The mixture was incubated at 50℃ for 30 min followed by the addition of 1 ml of 10% trichloroacetic acid (TCA). This was then centrifuged at 3000 rpm for 10 min. 1 ml of the supernatant was taken and mixed with 1 ml distilled water and 0.2 ml of 0.1% fresh ferric chloride ($FeCl_3.6H_2O$). After 5 min, the absorbance of the mixtures was measured at 700 nm against a blank containing all components except samples. Ascorbic acid was used as a standard reference. An increase in absorbance indicates a higher reducing power.

- ***Phosphomolybdenum assay***

The total antioxidant capacity (TAC) of the plant extracts was determined using the phosphomolybdenum method with slight modifications (Prieto *et al.* 1999). An aliquot of 0.1 ml of sample of varying concentrations (0.1–1 mg/ml) was mixed with 1 ml of reagent solution containing 28mM sodium phosphate, 4mM ammonium molybdate and 0.6 M sulphuric acid. The mixture was incubated at 95℃ for 90 min. The mixture was allowed to cool down to room temperature after which the absorbance was read at 695 nm against a blank containing solvent and methanol. Ascorbic acid was used as a standard reference. A control was prepared by adding all the reagents except samples. The TAC was expressed in mg ascorbic acid equivalent/g dry weight of extract and calculated by the formula TAC = (CxV)/M where C is the concentration of the standard ascorbic acid, V is the volume of the plant extract in ml, M is the mass of the plant extract.

Statistical analysis

All experiments were done in triplicates and the results were expressed as mean values ±SEM. Linear regression analysis was used to calculate IC_{50} for each plant extract.

Results

Qualitative analyses

Preliminary phytochemicals screening revealed the presence of major classes of phytochemical compounds in the different extracts of the plant sample (**Table 1**). The phytochemical screening was performed with aqueous, methanol, butanol and ethyl acetate extracts of *Garcinia pedunculata.*

Table 1: Qualitative analyses of phytochemical substances in different extracts of *Garcinia pedunculata.*

Compound	**Aqueous**	**Methanol**	**Butanol**	**Ethyl acetate**
Alkaloids	+	+	-	+
Flavonoids	++	+	+	+
Phenolic compounds	++	+	+	+
Tannins	+	+	+	+
Saponins	++	+	-	+
Proteins	+	+	+	+
Carbohydrates	+	+	+	+

++: highly present, +: present, -: absent

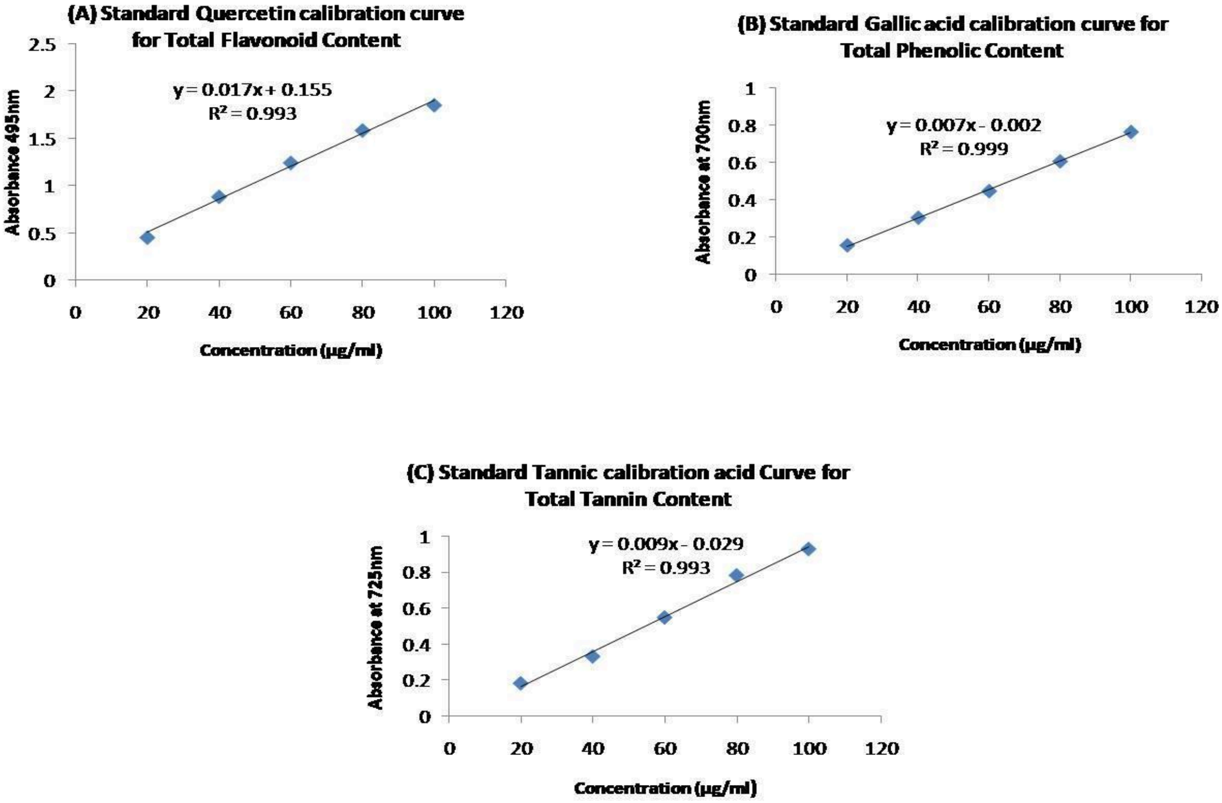


Fig. 2. Calibrative curve for Quercetin, Gallic acid and Tannic acid for TFC (A), TPC (B) and TTC (C) respectively.

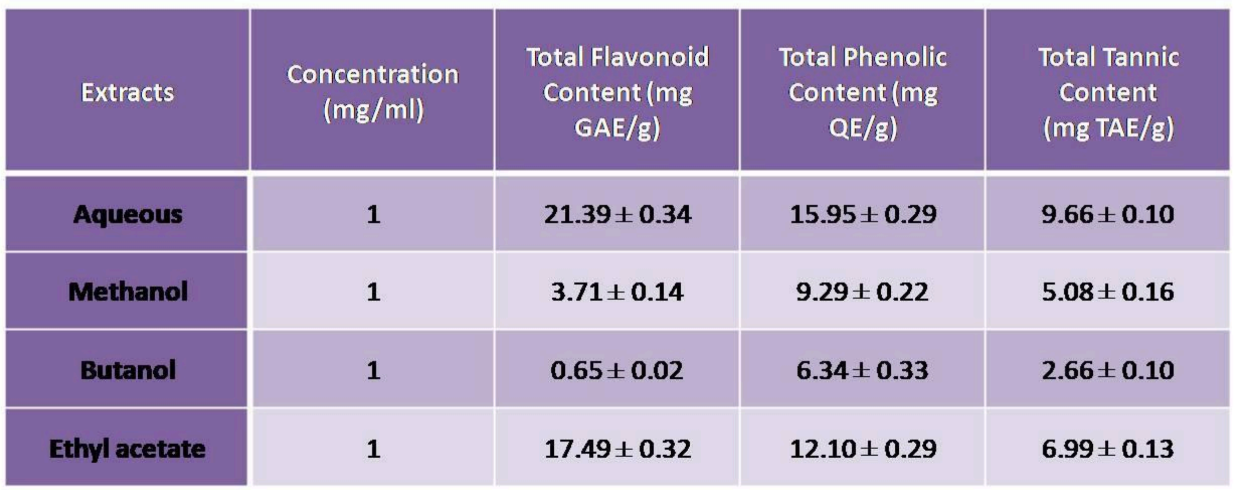
Quantitative analyses

The TFC, TPC and TTC content in all extracts were calculated using the linear equation obtained from the standard curve as shown in figure 1, where y is the absorbance and x is the amount of quercetin equivalent (QE), gallic acid equivalent (GAE) and tannic acid equivalent (TAE) for TFC, TPC and TTC respectively. The TFC (21.39 ± 0.34),TPC (15.95 ± 0.29) and TTC (9.66 ± 0.10) was found to be highest in aqueous extract with butanol extract showing the lowest TFC (0.65 ± 0.02), TPC (6.34 ± 0.33) and TTC (2.66 ± 0.10), as observed in table 2. The TFC, TPC and TTC of the four extracts were found in decreasing order of aqueous> ethyl acetate> methanol > butanol for all the analyses.

In-vitro Antioxidant Activity

- ***DPPH assay*** Ascorbic acid, widely used antioxidant standard, was used as a positive control and the calculated % inhibition values of both standards and samples was plotted against their respective concentrations as shown in **Fig. 3**. The results were expressed as IC_{50} which is the concentration of standard/sample required to scavenge 50% of the DPPH radical. Low IC_{50} value indicates the highest scavenging activity. The aqueous extract showed the highest antioxidant activity as it had the lowest IC_{50} when compared to the other solvents. **Table 3** shows the scavenging effect of samples on DPPH radical and were in the following order: aqueous>methanol> butanol> ethyl acetate.

Table 2: Total flavonoid content, total phenolic content and total tannic content (equivalent/mg dry weight of extract)of different extracts of *Garcinia pedunculata*. Results expressed as mg gallic acid equivalents/g dry weight, mg quercetin equivalents/ g dry weight and mg tannic acid.



Extracts	Concentration (mg/ml)	Total Flavonoid Content (mg GAE/g)	Total Phenolic Content (mg QE/g)	Total Tannic Content (mg TAE/g)
Aqueous	1	21.39 ± 0.34	15.95 ± 0.29	9.66 ± 0.10
Methanol	1	3.71 ± 0.14	9.29 ± 0.22	5.08 ± 0.16
Butanol	1	0.65 ± 0.02	6.34 ± 0.33	2.66 ± 0.10
Ethyl acetate	1	17.49 ± 0.32	12.10 ± 0.29	6.99 ± 0.13

Data represented as Mean ± SEM, where n=3.

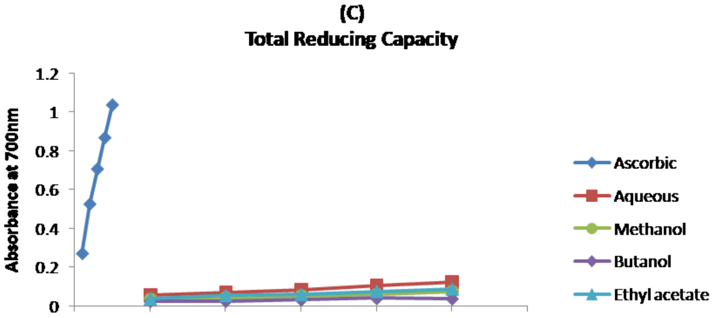


Fig. 3. Percentage inhibition of standard and various solvent extracts on DPPH (A), Absorbance of Total Antioxidant capacity of different extracts (B) and Absorbance of Total Reducing Capacity/FRAP (C), of *Garcinia pedunculata.**Phosphomolybdenum assay (Total antioxidant Capacity).***

The phosphomolybdate method is quantitative, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents (AAE). **Fig. 4.** shows the absorbance reading of various extracts of the fruit against standard ascorbic acid. The TAC of various solvent samples was found to decrease in this order: aqueous> methanol> ethyl acetate> butanol as shown in **Table 4**. All results showed activity in a dose dependent manner.

Table 3: DPPH percentage inhibition of ascorbic acid and different solvent extracts.

	Ascorbic acid	Aqueous	Methanol	Butanol	Ethyl acetate
Concentration (mg/ml)	0.1	1	1	1	1
% inhibition (Mean±SEM)	52.61 ± 0.65	44.61 ± 0.12	39.51 ± 0.67	27.06 ± 0.21	25.24 ± 0.25
IC50 (mg/ml)	0.095	1.19	1.61	3.10	3.26

- ***Total Reducing Power Assay***

Absorbance reading of the various extracts shows a close comparison (**Fig. 5.**). Ascorbic acid being the standard displayed the strongest reducing power but when compared among the solvents it is evident that aqueous extract showed the highest and the other solvents decreased in this order: aqueous>ethyl acetate> methanol> butanol (**Table 5**).

Table 4: Total antioxidant content of different solvent extracts of *Garcinia pedunculata.* Data represented as Mean ± SEM, where n=3.

	Aqueous	Methanol	Butanol	Ethyl acetate
Concentration (mg/ml)	1	1	1	1
TAC (mg AAE/G)	19.19 ± 0.36	12.42 ± 0.43	6.11 ± 0.73	9.44 ± 0.17

Table 5: Results of total reducing power of different solvent extracts of *Garcinia pedunculata.*

	Ascorbic acid	Aqueous	Methanol	Butanol	Ethyl acetate
Concentration (mg/ml)	0.1	1	1	1	1
Absorbance (Mean±SEM)	1.047 ± 0.0043	0.123 ± 0.0023	0.073 ± 0.0012	0.038 ± 0.0008	0.084 ± 0.0018

Discussion

The use of plants to treat sickness and diseases has been in existence since old age. This is because medicinal plants possess biologically active compounds such as alkaloids, flavonoids, phenolic compounds, saponins, tannins, etc. these compounds have been proven to possess activities such as antioxidant, anti-inflammation, anti-bacterial, anticancer, etc, each through different mechanisms (Bharti *et al.* 2012;Greenwell and Rahman 2015; Shingala *et al.* 2021; Wasihun *et al.* 2023).

Isolation of these biologically active compounds from their crude form is highly dependent on the type of solvent they are most soluble and displaying antioxidant activities through the different techniques. In this study, we have observed that aqueous extract showed the highest antioxidant activity.Phytochemical screening showed all compounds such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, proteins and carbohydrates to be present in the different solvents with the exception of butanol showing no presence of alkaloids and saponins. Quantitatively, aqueous extracts showed the highest yield for TFC (21.39 ± 0.34),TPC (15.95 ± 0.29) and TTC (9.66 ± 0.10) with butanol showing the lowest. The descending order of TFC, TPC and TTC for all solvent extracts are in the order of aqueous>ethyl acetate>methanol>butanol.

The ability of the solvent extracts to quench DPPH free radicals, converting the purple-colored DPPH solution to colorless product 2,2-diphenyl-1-picryl hydrazine displays the scavenging effects of the plant sample; the solvent extracts showed significant scavenging effects on the DPPH radical which was increasing with increase in the concentration of the extracts. In this study, we observed that the aqueous extract showed the highest DPPH scavenging activity amongst the four solvents chosen and were found to decrease in the order aqueous>methanol>butanol>ethyl acetate. The IC_{50} calculated also showed the anti-oxidant activity of aqueous extract to be the lowest, with the value of 1.19.

A similar trend is also observed when assayed for total antioxidant activity which measures the scavenging ability of the antioxidants in the extract for free radicals with aqueous extract demonstrating the highest antioxidant capacity for phosphomolybdate reaction and were found to decrease in the order of aqueous>methanol>ethyl acetate>butanol. Studies have shown that many flavonoids and polyphenols have contributed to the total antioxidant activity of medicinal plants (Pietta 2000; Pandey and Rizvi 2009).

The reducing power of the different solvent extracts of the plant was assayed and it was observed that higher the absorbance, the stronger is the antioxidant activity; thus, the reducing power of the extracts also increases with the increase in concentration. Amongst the solvent extracts, the aqueous extract showed the highest reducing power capacity while butanol showed the lowest. The reducing power capacity of the four solvents were in decreasing order of aqueous> ethyl acetate> methanol> butanol.

All assays supported the same trend as TFC,TPC and TTC which strongly suggests that aqueous solvent is an excellent choice for extraction, due to the presence of the biologically active phytochemicals, which are responsible for the antioxidant activity of *Garcinia pedunculata.*

Conclusion

The use of plants as herbal medicines has been proven to be highly effective and popular for treatment of several ailments as it is safe, low cost and with lesser side effects as compared to synthetic drugs.Herbal remedies and traditional medicines are popular worldwide, from as simple as adding ginger to tea aiding in digestion to novel anti-cancer research. They are being consumed by the majority population without even realizing it. The present study showed *Garcinia pedunculata* as a rich source of secondary metabolites/phytochemicals including flavonoids, phenolic compounds, tannins and alkaloids which contributed to the antioxidant activity exhibited by the plant. The study also compared different solvents for extraction with aqueous extract displaying the most prominent results as compared to methanol, butanol and ethyl acetate.*Garcinia pedunculata* thus proved to be a plant of interest with a diverse application in biological and pharmacological activities.

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A comprehensive review on ethnomedicinal and pharmaceutical properties of *Aerides*, a medicinally important genus of orchids

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Abstract

The genus Aerides consists of only a few species and these species are highly valuable for their ornamental flowers and therapeutic potential. Several orchids in this genus are used as traditional medicines in various cultures and areas based on their natural populations. Many bioactive substances, such as phenanthrenes, phenolic compounds, stilbenoids, and phenylpropanoids have been reported in Aerides orchids. A wide range of pharmacological activities, including anti-inflammatory, anticancer, antioxidant, neuroprotective, antimicrobial, and α-glucosidase inhibitory characteristics, are attributed to the presence of these secondary metabolites. The native populations of Aerides orchids are threatened by overexploitation due to extensive commercial demand for their ornamental and therapeutic properties. This review highlights numerous pharmacological and medicinal properties of Aerides orchids with an emphasis on their conservation and sustainable utilization.

Keywords: *Aerides,* Ethnomedicine, Phytochemistry, Pharmacology.

Introduction

Orchids belong to the family Orchideaceae, which comprises one of the largest and most diverse groups amongst the flowering plants, with over 750 genera and 28,000 species dispersed all over the world except Antarctica (Zhang *et al*. 2022; Castillo-Perez *et al*. 2024). Orchids' richness and distribution differ from place to place and severely rely on climatic conditions. In terms of orchid distribution, the Indo-Malaysian region and Colombia are the richest in the world (Paul and Kumaria 2017; Vibha *et al*. 2019). Because of their amazing variety of forms, colours and shapes, both cut flowers and potted plants of orchids are highly demanded in the world's floriculture industry (Bhattacharyya *et al.* 2014; Sarmah *et al.* 2024). Even though orchids are typically planted for their aesthetic qualities, they are also utilized as food and traditional medicines in many countries around the world (Hossain 2011; Ahmed *et al.* 2024). The populations of orchids in the wild are rapidly declining due to anthropogenic activities like massive deforestation and urbanization as well as overexploitation due to illegal trade triggered by the high commercial demand for attractive flowers and medicinal properties (Bhattacharyya *et al.* 2014; Debnath and Kumaria 2023). The IUCN Red List currently classified a total of 955 orchid species as threatened, 259 as critically endangered, 456 as endangered, 240 as vulnerable, 105 as nearly threatened, and 6 species as extinct (IUCN 2022; Monica *et al.* 2024).

Genus *Aerides* Lour

The genus *Aerides* Lour is a small genus of the family Orchidaceae, which includes 31 species distributed in tropical and subtropical regions of Asia, such as India, Nepal, Bhutan, China to Philippines, Sulawesi and Papua New Guinea (POWO 2024; Metusala 2024). This genus is epiphytic in nature, except for *Aerides krabiensis*, which grows in a boletus-like manner on the surface of limestone (Teoh 2021). The genus was established in 1790 by Loureiro. Since its initial classification**,** it has undergone several taxonomic modifications. Dozens of species once described in this genus have been assigned to other related genera (Chen *et al.* 2023; Tao *et al.* 2024).

The phytomorphology of the genus is described as monopodial, erect, freely branching, trunk surrounded by the base of bipartite, elongated and coriaceous leaves. Flowering is seasonal with several inflorescences bearing many fragrant pink flowers that open at the same time **(Fig.1A-C)**. Sepals and petals are free and widespread and the lip is three-lobed with a spur characteristic of the genus (Teoh 2021). Due to the fragrant nature of the flowers, many species of this genus are considered as a highly valuable source for creating artificial hybrids and variants (Kocyan *et al.* 2008). According to POWO (2024), *Aerides agasthiyamalaiana, A. crassifolia, A. crispa, A. emericii, A. maculosa, A. mcmorlandii, A. multiflora, A. odorata, A. ringens, and A. rosea* are among the 31 species that have been identified as existing in India.

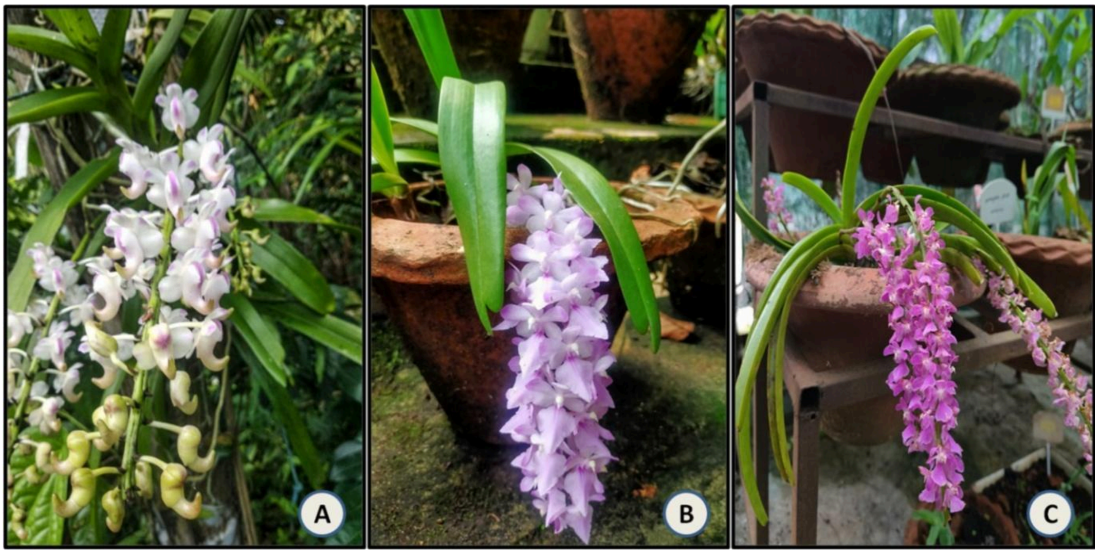

Fig.1. Aerides orchids in the greenhouse of Plant Biotechnology Laboratory, Botany Department, NEHU (A) *A. odorata* (B) *A. rosea* (C) *A. multiflora*

Ethnomedicinal Uses

Plants have been used as traditional medicines for thousands of years; and have evolved over the centuries among many different communities (Thapa *et al*. 2022). There is a long history of using orchids as traditional medicines around the world. Although orchids originated on earth 120 million years ago, the Chinese were the first to cultivate, describe and use orchids as a source of herbal remedies since 2800 B.C. **(**Jalal *et al*. 2008; Hossain 2011; Pant 2013). Apart from China, in many countries like America and some parts of Europe, Australia and Africa, orchids have been used as traditional drugs for a very long time (Bulpitt *et al*. 2007; Tsering *et al*. 2017). The oldest references to the Indian system of traditional medicine found in the ancient Sanskrit literature known as '*Veda*'. '*Ashtavarga*', known as the 'herb of immortality,' is an important ingredient of various classical Ayurvedic formulations like '*Chavyanprasa*', a combination of 8 herbs containing 4 species of orchids, namely**,** *Malaxis muscifera* (Jivaka), *Malaxis acuminate* (Rishbhaka), *Habenaria intermedia* (Riddhi), and *Habenaria edgeworthi* (Vriddhi) **(**Singh and Duggal 2009**;** Hossain 2011; Tsering *et al*. 2017; Choudhary *et al*. 2023; De 2023). In '*Sushrutasamhita*', one of the ancient Sanskrit literature sources, the use of orchid drugs under the name "*Rasna*" (*Acampe praemorsa* and *Vanda tessellata*) is mentioned. The *"Rasna Panchaka Quatha"* is an Ayurvedic formulation used to treat arthritis and rheumatism. The root of *Vanda tessellata* is an antidote against scorpion sting and a remedy for bronchitis. '*Rasna'* plant *Acampe praemorsa* belongs to the Aeridinae subtribe, which is very close to the *Aerides* genus (Hossain 2011; Bindiya Prakash and Bais 2016; Khan *et al.* 2019). Orchid species under the genera *Aerides, Coelogyne, Dendrobium* and *Vanda* possess both medicinal and ornamental properties and are the most commonly used medicinal orchids which have both high medicinal and ornamental values (Hegde 2005; Subedi *et al.* 2013; De and Medhi 2014).

Depending on the region it grows, the genus *Aerides* has various uses in traditional medicine in different parts of the world**.** *Aerides falcata* is commonly used as a tonic for weak infants in Vietnam, while leaf powder of *A. multiflora* is used in tonic preparations in Nepal. It has been reported that some species of *Aerides* are used traditionally as medicine for skin diseases. For e.g., in South Asian countries, the seeds of *A. falcata* are sprinkled on boils and used to promote healing of skin diseases. The traditional healers from Sikkim have used the stem powder of *A. odorata* to cure skin diseases (Panda and Mandal 2013; Teoh 2016; Rivai *et al.* 2023). Studies have shown that the hill tribes of Odisha, India used fresh roots of *A. odorata* mixed with *Saraca asoca* root powder, *Azadirachta indica* bark and regular salt to cure painful inflamed joints (Dash *et al.* 2008; Teoh 2016). The species, *A. maculosa* and *A. odorata* are used as herbal remedies for tuberculosis. The leaf juice of *A. odorata* has been used for the treatment of tuberculosis and among the *Bhilla* tribe of Maharashtra, India the use of infusion made from the roots of *A. maculosa* are used (Dash *et al.* 2008; Kamble *et al.* 2010). The use of orchid species belonging to the genus *Aerides* in the treatment of follems related to the ear and nose has been reported. For e.g., the leaf juice of *A. multiflora* is used in Sikkim to treat nasal congestion and otitis media. Also, boils in ears and nose are reported to have been treated with the juice obtained from leaves of *A. odorata* (Rao 2004; Panda and Mandal 2013; Teoh 2016). In southern parts of India, powder of *A. crispa* along with neem oil is used to cure pain and deafness (Rajendran *et al.* 1997). Leaves and mature capsules of *A. odorata* and leaf paste of *A. rosea*, *A. multiflora* are used for cuts and wounds in India and Nepal (Rao 2004; Joshi *et al.* 2009; Subedi *et al.* 2013; Teoh 2016; Ninawe and Swapna 2017; Gupta *et al.* 2024).

Phytochemical and Pharmacological Activities

Secondary metabolites which are low molecular weight organic compounds, are produced under stress conditions and play a critical role in medicinal orchids. Their diverse biological activities, high specificity and diversity make them a promising source of highly specialized active ingredients for the treatment of incurable diseases (Thakur *et al.* 2019; Gantait *et al.* 2021; Li *et al.* 2023). Alkaloids, flavonoids, tannins, phenanthrenes, steroids, stilbenoids and bibenzyls are some of the secondary metabolites which are responsible for imparting medicinal properties to the orchids (Raskoti and Ale 2021; Targu *et al.* 2024). Due to the presence of these phytochemicals, the tissue extracts of orchids have been found to have anti-inflammatory, antimicrobial, anti-aging, anti-HIV, anti-helminthic, anti-dandruff, analgesic, anti-rheumatic, diuretic, hypoglycemic, antibacterial, antiviral, antispasmodic, anticarcinogenic, relaxing and neuroprotective properties (Gutierrez 2010; Pant *et al.* 2022; Shukla *et al.* 2022; Bazzicalupo *et al.* 2023; Targu *et al.* 2023, 2024). Different types of compounds have been reported to be present in the species of *Aerides* (Table 1).

Further, medicinal orchids often contain phenanthrenes, a kind of substance with three-benzene rings, categorized as simple, dihydrophenanthrene, phenanthraquinone, phenanthrenefuran and phenanthrene dimer, found only in the subfamilies of orchids, Orchidoideae and Epidendroideae (Niu *et al.* 2017; Thant *et al.* 2021; Li *et al.* 2023). The genus *Aerides* has been reported as a source of novel phenanthrene compounds. Aeridin, also known as 2,7-dihydroxy-l,3-dimethoxy-9, 10-dihydrophenanthropyran, is a phenanthropyran derivative found in *Aerides crispa* **(Fig.2)**. It is known for its significant anti-inflammatory activities (Anuradha and Parkasa Rao 1998; Mridula *et al.* 2009). *Aerides rosea* is the source of two new phenanthrene derivatives: aerosin (3-methoxy-9,10-dihydro-2,5,7-phenanthrenetriol) and aerosanthrene (5-methoxyphenanthrene-2,3,7-triol) The species, *A. rosea* also yields 3,5-dimethoxyphenanthrene-2,7-diol and 3-methoxy-2,7-dihydroxy-5H-phenanthro[4,5-bcd]pyran (Cakova *et al.* 2015). Three unique phenanthrene compounds have been identified from *A.* *multiflora*, viz., aerimultins A, B, and C. All three have been found to exhibit α-glucosidase inhibitory activity, wherein aerimultins C shows the highest level of this activity (Thant *et al.* 2021). Rivai *et al.* (2023) have reported that two biphenanthrene derivatives, 2,7-dihydroxy-3,4,6-trimethoxyphenanthrene and aerifalcatin isolated from *A. falcata* show anti-neuroinflammatory and anticancer properties and can extensively reduce the release of IL-6 and TNF-α, two pro-inflammatory cytokines in the cell lines. Aerifalcatin has also shown a decrease in the glioblastoma and neuroblastoma cell migration and proliferation in *in-vitro* cell lines. Both the reported compounds hold promise for further exploration as therapeutic agents for central nervous system (CNS) diseases.

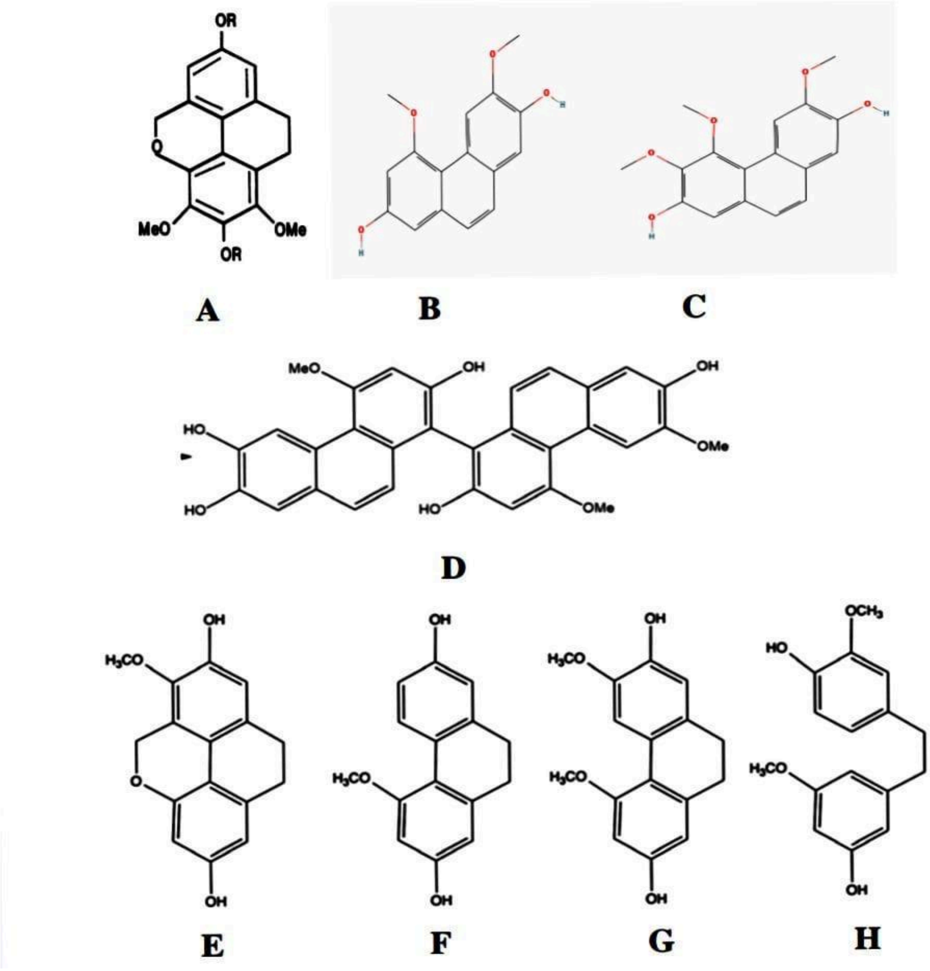

Fig.2. Some bioactive compounds identified from *Aerides* genus

(A) Aeriden; (B) 3,5-dimethoxyphenanthrene-2,7-diol; (C) 2,7-dihydroxy-3,4,6 trimethoxyphenanthrene; (D) Aerifalcatin; (E) Imbricatin; (F) Coelonin; (G) Methoxycoelonine; (H) Gigantol

Another group of compounds, the phenolics include hydroxyl groups. These phenolic compounds are classified into two groups according to the number of phenol units in their structure: simple phenols and polyphenols. The phenolic compounds namely 2-methyl-5(1,2,2-trimethycyclopentyl) phenol and phenyl(piperidin-3-yl) methanone reported in the species *A. odorata* showing anticancer properties

(Oon *et al*. 2015). Also stilbenoids such as gigantol, coelonin, methoxycoelonin and imbricatin which are one of the important secondary metabolites found in orchids. Out of these compounds, imbricatin has been reported to show antioxidant activity, whereas gigantol shows anti-inflammatory and anticancer effects. Both these compounds are reported to be present in *A. multiflora* and *A. rosea* (Simmler *et al*. 2010; Charoenrungruang *et al*. 2014; Cakova *et al*. 2015; Thant *et al*. 2021; Chowdhury *et al*. 2024).

Some other secondary metabolites such as the phenylpropanoid compound, dihydroconiferyl dihydro-p-coumarate, are also reported to show antioxidant activities and anti-inflammatory effects (Ahammed *et al*. 2021, 2023). This compound is found to be produced from the phenylpropanoid that is present in *A. multiflora* (Thant *et al.* 2021). Using cell lines, studies have shown that agrostonin reported from *A. multiflora* and *A. falcata* and syringaresinol from *A. falcata*, show significant anti-neuroinflammatory effects and strong cytotoxic activities in C6 glioblastoma and SH-SY5Y *in-vitro* cells, indicating a suppressive role of the compounds in brain cancer cells. Additionally, syringaresinol extracted from *A. falcata* is reported to exhibit anti-cancerous properties in leukaemia, breast cancer and prostate cancer (Rivai *et al*. 2023).

Conclusion and Future Perspectives

The medicinal species under the orchid genus *Aerides* contain bioactive compounds that may have antioxidant, anticancer and anti-inflammatory effects. However, *Aerides* orchids are still relatively unknown due to their small populations. There is a significant potential to discover novel compounds within this genus, which can be scientifically validated and used in pharmaceutical industries. To achieve this, further studies are needed to be carried out on the conservation of *Aerides* spp. and identification of bioactive compounds present in these orchids. This could involve the use of biotechnological approaches of conservation and sustainable utilization of the species under the genus *Aerides* so as to avoid the pressures on their natural populations.

Table 1. Bioactive compounds of *Aerides* genus with molecular structures and their biological activities.

Sl. no.	*Aerides* species	Compound name	Molecular formula	Phytochemical class	Biological activity	References
1	*A. crispa*	Aeridin	$C_{17}H_{16}O_5$	Phenanthropyran	Anti-inflammatory activity	Mridula *et al.* (2009)
2	*A. falcata*	Aerifalcatin	$C_{31}H_{24}O_8$	Biphenanthrene derivatives	Anti-neuroinflammatory and anticancer activity	Rivai *et al.* (2023)
3	*A. falcata*	2,7-Dihydroxy-3,4,6-trimethoxyphenanthrene	$C_{17}H_{16}O_5$	Biphenanthrene derivatives		
4	*A. falcata*	Syringaresinol	$C_{22}H_{26}O_8$	Polyphenol		
5	*A. falcata* *A. multiflora*	Agrostonin		Others		
6	*A. falcata*	Paprazine	$C_{17}H_{17}NO_3$	Phenylpropanoid	Anti-inflammatory and antioxidant activity	Rivai *et al.* (2023); Bakrim *et al.* (2024)
7	*A. falcata*	n-Trans-feruloyl tyramine	$C_{18}H_{19}NO_4$	Alkaloid	Antioxidant and α-glucosidase inhibitory activity	Soi-Ampornkul *et al.* (2022); Rivai *et al.* (2023)
8	*A. multiflora*	Aerimultin A		Phenanthrene	α-Glucosidase inhibitory activity	Thant *et al.* (2021)
9	*A. multiflora*	Aerimultin B				
10	*A. multiflora*	Aerimultin C				
11	*A. multiflora* *A. rosea*	Gigantol	$C_6H_{18}O_4$	Stilbenoid	Anti-inflammatory and anticancer activity	Charoenrungruang *et al.* (2014); Thant *et al.* (2021); Chowdhury *et al.* (2024)
12	*A. multiflora* *A. rosea*	Imbricatin	$C_{16}H_{14}O_4$	Stilbenoid	Antioxidant activity	Simmler *et al.* (2010); Cakova *et al.* (2015); Thant *et al.* (2021)
13	*A. multiflora*	Dihydroconiferyl dihydro-p-coumarate	$C_{19}H_{22}O_5$	Phenylpropanoid	Antioxidant and anti-inflammatory activity	Ahammed *et al.* (2021); Thant *et al.* (2021); Ahammed *et al.* (2023).

14	*A. odorata*	2-Methyl-5-(1,2,2-Trimethycyclopentyl) phenol	$C_{15}H_{22}O$	Phenol	Anticancer activity	Oon *et al.* (2015)
15	*A. odorata*	Phenyl(piperidin-3-yl) methanone	$C_{12}H_{15}NO$	Phenylpiperidines		
16	*A. odorata*	β-Selinene	$C_{15}H_{24}$	Sesquiterpenoid	Antioxidant and anti-inflammatory activity	
17	*A. odorata*	Squalene	$C_{30}H_{50}$	Triterpene	Antibacterial, antioxidant, anti-tumour, anticancer, immunostimulent, lipoxygenase inhibitory activity	
18	*A. rosea*	Coelonin	$C_{15}H_{14}O_3$	Stilbenoid	Anti-inflammatory activity	Jiang *et al.* (2019)

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A Green Solution: Microalgae for Soil health

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Abstract

Soil degradation in India, exacerbated by intensive agricultural practices and the Green Revolution, poses a significant threat to food security and environmental sustainability. This review highlights microalgae as a viable, eco-friendly solution for soil reclamation. Microalgae, particularly cyanobacteria, enhance soil fertility through nitrogen fixation, organic acid secretion, and biofilm formation, improving soil structure and nutrient availability. Additionally, phycoremediation—a process leveraging microalgae to remove contaminants—demonstrates potential in mitigating heavy metal pollution, salinization and erosion. This study synthesizes current research on microalgae-based remediation strategies and their practical applications, emphasizing their cost-effectiveness, adaptability and role in sustainable agriculture. By integrating microalgae into soil management practices, we can develop scalable, nature-based solutions to combat soil degradation, ensuring long-term agricultural productivity and environmental resilience.

Keywords: Soil health, Microalgae, Phycoremediation, Cyanobacteria, Sustainable agriculture.

Introduction

India, primarily an agricultural economy, relies heavily on healthy soil for sustainable food production. However, the Green Revolution, while increasing food production from 50 to 300 million tonnes, also led to significant soil degradation. This degradation is primarily due to erosion and waterlogging, which result in the loss of topsoil and salinization, respectively.

Soil reclamation is a crucial process that involves restoring degraded or contaminated soil to a healthy state capable of supporting plant growth. Maheswarappa*et al.* (2011) observed a decline in soil C-sustainability index following the Green Revolution, attributed to increased chemical input use. Additionally, excessive fertilizer application has led to nitrate accumulation and declining water tables. According to the National Bureau of Soil Survey and Land Use Planning, approximately 30% of India's land, or 146.8 million hectares, is degraded. This degradation results in substantial economic losses, estimated at around $3 billion annually due to salt-affected soils alone. With a significant population to feed and limited land resources, India faces a pressing need for effective soil reclamation strategies.

Soil reclamation aims to restore soil nutrients, fertility and structure to enhance agricultural productivity. Microalgae, particularly cyanobacteria and other algal species, have gained global attention for their potential in soil reclamation due to their ability to improve soil fertility, structure and contaminant removal. Various studies have demonstrated their effectiveness in restoring degraded lands across different regions.In China, a study has shown that cyanobacteria-based biocrusts play a crucial role in rehabilitating deserted lands by stabilizing soil, improving organic matter content and enhancing nitrogen fixation (Hu *et al.* 2021). Similarly, in Spain, microalgae-based biofertilizers, such as *Chlorella vulgaris*, have been found to improve soil microbial activity and increase crop productivity in degraded agricultural lands (González-Delgado andKhoshnevisan 2019). In arid regions of Africa, studies in Egypt and Algeria have demonstrated that algal biomass applications enhance soil organic matter, promote microbial diversity and boost crop yields under harsh environmental conditions (Essa *et al.* 2020). In developed nations such as the United States and European countries, research has explored integrating microalgae into organic farming practices. These studies highlight how algae-based amendments not only enrich the soil with essential nutrients but also reduce dependency on chemical fertilizers, making agricultural practices more sustainable (Gao *et al.* 2021). These global examples highlight the potential of microalgae in addressing soil degradation challenges across diverse environments. Given India's urgent need for sustainable soil reclamation solutions, leveraging microalgae-based technologies could provide an effective and eco-friendly approach to enhancing soil health, increasing agricultural productivity and ensuring long-term environmental resilience.

Causes of Soil Degradation

Soil degradation, a critical environmental issue, stems from both natural processes and human activities, profoundly impacting soil health and ecosystem stability. In India, as food grain production escalated over the decades, soil nutrient deficiencies have also risen alarmingly. While nitrogen (N) was the sole deficient element in Indian soils in 1950, by 2005–2006, the number of deficient nutrients had surged to nine, including nitrogen (N), phosphorus (P), potassium (K), sulphur (S), boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) (Ministry of Agriculture 2006). This trend underscores the increasing strain on soil resources due to intensive agricultural practices and insufficient replenishment of essential nutrients (FAO 2015).

Natural processes, such as wind and water erosion, alongside human-induced activities like deforestation and improper land management, are leading causes of soil erosion. Erosion, particularly through water, remains the most significant degradation issue in India. It not only removes the nutrient-rich topsoil but also alters the terrain, making it less hospitable for plant growth (Pimentel and Burgess 2013). The combined impact of these factors has serious implications for agricultural productivity and ecological balance (Lal 2001).

Natural Causes of Soil Degradation

Natural phenomena are a substantial contributor to soil degradation. Events such as earthquakes, tsunamis, droughts, avalanches, landslides, volcanic eruptions, floods, tornadoes, and wildfires can cause significant soil displacement and loss of fertility. For instance, floods and landslides wash away topsoil, exposing subsoil layers that are often less fertile (FAO 2015). Similarly, droughts and wildfires can reduce organic matter and alter soil structure, leading to compaction and reduced permeability (Lal 2001). The frequency and intensity of such natural events, often exacerbated by climate change, highlight the vulnerability of soils to environmental changes (Pimentel and Burgess 2013).

Human-Induced Causes of Soil Degradation

Human activities are a major driver of soil degradation, with several factors contributing to the issue. Unsustainable land clearing and deforestation are significant contributors, stripping the soil of protective vegetation cover and exposing it to erosion (FAO 2015). Similarly, inappropriate agricultural practices such as excessive tillage, the use of heavy machinery and imbalanced application of inorganic fertilizers lead to structural damage and nutrient depletion (Lal 2001). Poor irrigation practices and pesticide overuse further exacerbate the problem by disrupting soil microbial communities and contributing to salinization (Pimentel and Burgess 2013). The inadequate incorporation of crop residues or organic matter into the soil and improper crop cycle planning also undermine soil fertility over time (Ministry of Agriculture 2006).

Industrial activities further aggravate soil degradation through contamination. Improper disposal of industrial waste, coupled with the use of hazardous chemicals and certain pesticides, can render soils unsuitable for agriculture or other beneficial uses. For example, heavy metals from industrial effluents accumulate in the soil, disrupting its natural composition and harming plant and microbial life (FAO 2015).

Salinization is another critical issue, often resulting from over-irrigation and poor water management. The excessive accumulation of salts in the soil alters its structure and impedes plant growth, posing a severe challenge in arid and semi-arid regions where irrigation is a necessity (Pimentel and Burgess 2013). Additionally, activities such as landfilling and mining can significantly disrupt soil structure and composition. Mining operations, in particular, often leave behind barren landscapes where vegetation struggles to thrive (Lal 2001). Urbanization further accelerates soil degradation through extensive land development and infrastructure projects. The construction of roads, buildings and other structures compacts soil and disrupts its natural processes. This urban sprawl not only reduces the area of arable land but also creates impermeable surfaces that increase runoff and erosion (FAO 2015).

Finally, abandoned or degraded agricultural lands reflect the long-term impacts of unsustainable farming practices. Continuous monoculture cropping, excessive reliance on chemical fertilizers and pesticide misuse degrade soil quality over time, reducing its ability to support productive agriculture. The lack of organic carbon inputs and failure to replenish essential nutrients further deplete soil health, making restoration efforts more challenging (Ministry of Agriculture 2006).

Thus, soil degradation is a multifaceted issue influenced by a complex interplay of natural and human-induced factors. Addressing this challenge requires integrated land management strategies, sustainable agricultural practices and stringent regulations on industrial and urban development to conserve soil health and ensure long-term ecological and agricultural sustainability. Efforts should focus on improving soil nutrient management, promoting afforestation, enhancing irrigation efficiency and mitigating the impacts of urbanization and industrialization. The need for such measures is particularly urgent in regions like India, where soil degradation poses a direct threat to food security and environmental health (FAO 2015; Pimentel and Burgess 2013).

Soil Reclamation Methods

Soil contamination is a critical environmental challenge that necessitates the implementation of robust remediation techniques to restore soil quality and prevent detrimental impacts on ecosystems and human health. Contaminants such as heavy metals, organic pollutants and toxic anions compromise soil productivity and pose long-term risks. Over the years, various remediation strategies have been developed to address this issue, each with distinct benefits and limitations. Selecting the most appropriate technique involves careful consideration of factors such as the type and concentration of contaminants, site-specific conditions, environmental implications and economic feasibility (Singh *et al.* 2013). Frequently, an integrated approach combining multiple methods is employed to achieve the desired outcomes effectively (Bhatnagar *et al.* 2011).

Physical Methods

Physical remediation strategies involve mechanical or structural interventions to contain or mitigate the impact of soil contamination. A prominent method is surface insulation, which entails covering the contaminated soil with a protective layer to prevent the spread of pollutants via wind or water erosion. Materials used for this barrier include synthetic fibers, clay, or concrete. By creating a physical shield, surface insulation limits contaminant mobility and reduces their interaction with surrounding environments. While effective for containment, this method does not eliminate contaminants and requires continuous monitoring and maintenance to ensure long-term efficacy (Qi *et al.* 2019).

Chemical Methods

Chemical remediation focuses on utilizing chemical agents to extract, stabilize, or neutralize contaminants within the soil matrix. One widely used technique is soil washing, also known as the hydraulic method. This method employs chemical solutions to each contaminant from the soil, effectively removing substances like heavy metals, radionuclides and organic pollutants. The choice of leaching agent depends on the nature of the pollutants, ranging from water for mild contamination to strong inorganic acids for persistent pollutants. Although soil washing can achieve high decontamination levels, it generates secondary waste, such as contaminated washing solutions, necessitating proper disposal or further treatment (FRTR 2020; Khan *et al*. 2018).

Biological Methods

Biological approaches leverage the natural capabilities of plants and microorganisms to degrade, immobilize, or remove contaminants, offering environmentally sustainable and cost-effective solutions for soil remediation.

Phytoremediation utilizes plants to stabilize or extract pollutants from the soil. In phytostabilization, plants immobilize contaminants within the soil by adsorbing them onto root surfaces and precipitating them in the rhizosphere. This approach prevents the migration of pollutants, particularly in erosion-prone areas. Another variant, phytoextraction, involves the uptake of contaminants by plant roots and their accumulation in aerial parts of the plant, which are subsequently harvested for safe disposal or treatment (Ali *et al*. 2013).

Bioremediation employs microorganisms to degrade organic and inorganic contaminants into less harmful substances. Techniques such as biofiltration utilize microbial communities housed in filters or bioreactors to break down pollutants in soil or water. Typical microorganisms include bacteria (e.g., *Streptomyces* spp. and pseudomonads), fungi (e.g., *Aspergillus* and *Penicillium*), and protozoa, which work synergistically to degrade a wide range of contaminants (Chowdhury *et al*. 2017). Similarly, air sparging, a subsurface remediation method, involves injecting pressurized air into contaminated groundwater to volatilize hydrocarbons, facilitating their extraction and removal (EPA, 2017).

Composting, another biological technique, involves mixing contaminated soil with organic matter to enhance microbial degradation of pollutants. Aerobic microorganisms break down organic contaminants, converting them into simpler compounds such as carbon dioxide and water. The composting process also enriches soil organic matter, improving its fertility and structure (Tremblay *et al*. 2019).

Phycoremediation represents an innovative approach to soil and water reclamation that leverages the abilities of algae to absorb and metabolize contaminants such as heavy metals, organic pollutants and nutrients. Algae like *Chlorella vulgaris*, *Nostoc* sp., and *Scenedesmus* are particularly effective due to their rapid growth, adaptability to diverse environments and ability to utilize solar energy for metabolic processes (Singh and Ahluwalia 2013). Key advantages of phycoremediation include environmental sustainability, as it aligns with natural biological processes and cost-effectiveness, as it requires minimal operational expenses compared to conventional methods (Bhatnagar *et al*. 2011). Additionally, phycoremediation avoids introducing harmful chemicals into the environment, making it a non-toxic alternative for soil reclamation (Bhola *et al*. 2014). The adaptability of algae allows them to thrive in a variety of contaminated sites and their use promotes biodiversity by creating new habitats for other organisms (Gupta *et al*. 2013). Moreover, phycoremediation reduces the risk of secondary pollution, a common issue in chemical-based treatments. It is an energy-efficient approach, as algae rely on photosynthesis, eliminating the need for external energy inputs. The versatility of this method enables its application to a wide array of pollutants, including heavy metals, nutrients and organic compounds, making it a promising solution for both urban and industrial contamination (Razzak *et al*. 2013; Mata *et al*. 2012). Several successful case studies worldwide have demonstrated the efficacy of phycoremediation in restoring contaminated soils and water bodies. In Tamil Nadu, researchers utilized *Chlorella vulgaris* to remediate heavy metal-contaminated soil near tannery industries, finding that the algae effectively reduced concentrations of chromium (Cr), lead (Pb) and cadmium (Cd) by over 70% within four weeks, highlighting its potential for industrial pollution management (Annamalai *et al*. 2018). Similarly, in Brazil, where mining activities have significantly contributed to soil contamination with toxic heavy metals such as mercury (Hg) and arsenic (As), a study on *Scenedesmus obliquus* demonstrated its ability to accumulate and detoxify these metals while simultaneously improving soil microbial diversity and fertility, emphasizing the potential of algal-based treatments in rehabilitating abandoned mining lands for agricultural use (Pereira *et al*. 2018). Meanwhile, in Australia, excessive irrigation has led to severe soil salinity issues, reducing farmland productivity. To address this challenge, researchers explored the application of *Dunaliella salina*, a salt-tolerant microalga, for salinity mitigation, revealing that it successfully absorbed excess salts, improved soil structure, and restored the fertility of previously unproductive lands, offering a promising solution for sustainable agriculture in salt-affected regions (Wang *et al*. 2019).

While physical and chemical methods provide effective containment and removal, biological approaches, particularly phycoremediation, offer sustainable, cost-effective and environmentally friendly solutions. Future research should focus on enhancing the efficiency of these methods, integrating innovative technologies, and addressing challenges related to scalability and long-term implementation. Collaborative efforts among scientists, policymakers, and local communities will be crucial in promoting sustainable soil management and restoring contaminated lands to their productive potential.

Strategies of Cyanobacteria in Soil Reclamation

Cyanobacteria, a group of photosynthetic prokaryotes, play an instrumental role in soil reclamation and phytoremediation through a range of ecological and biochemical mechanisms. Their ability to interact with and transform soil properties makes them indispensable in sustainable land management and rehabilitation practices. By enhancing soil fertility, improving structural stability and aiding in nutrient cycling, cyanobacteria offer a versatile and eco-friendly approach to addressing soil degradation and contamination issues (Singh 1961; Kaushik and Subhashini 1985).

Nitrogen Fixation for Enhanced Fertility

One of the key contributions of cyanobacteria to soil health is their ability to fix atmospheric nitrogen, a critical nutrient for plant growth. This unique capability, facilitated by specialized cells known as heterocysts, converts inert atmospheric nitrogen (N_2) into bioavailable forms like ammonium (NH_4^+), enriching nitrogen-deficient soils. This process not only supports plant growth but also reduces the dependency on synthetic nitrogen fertilizers, aligning with sustainable agricultural practices. For instance, species such as *Anabaena* and *Nostoc* are widely recognized for their nitrogen-fixing capabilities, particularly in marginal and degraded soils (Singh 1961; Kumar *et al*. 2020).

Soil Structure Enhancement

Cyanobacteria also contribute significantly to the physical improvement of soils. Their filamentous structures form networks that bind soil particles, enhancing aggregation and promoting better soil structure. This process improves soil porosity, increases water retention, and reduces susceptibility to erosion, particularly in sandy or loose soils. Such structural stabilization is vital in arid regions prone to desertification and erosion (Kaushik and Subhashini 1985; Pandhal*et al*. 2008). The role of cyanobacteria in binding soil particles also helps create microhabitats that support diverse microbial communities, further enhancing soil health.

Organic Acid Secretion and Mineral Weathering

Cyanobacteria play an essential role in mobilizing nutrients through the secretion of organic acids during metabolic processes. These organic acids facilitate the weathering of minerals, breaking them down into bioavailable forms that plants can readily absorb. This mechanism not only enhances nutrient accessibility but also contributes to the development of soil profiles in degraded lands. For example, the release of compounds like citric and oxalic acids by certain cyanobacterial species aids in dissolving phosphates and other essential minerals, thereby enriching the soil nutrient pool (Singh 1961; Mishra and Rai 2015).

Biofilm Formation and Soil Stabilization

Another critical strategy employed by cyanobacteria is biofilm formation. These biofilms, formed on the soil surface, create a protective layer that shields the underlying soil from erosion caused by wind and water. Biofilms also reduce the loss of organic matter and nutrients, which is particularly beneficial in degraded or nutrient-poor soils. This stabilization effect is vital in arid environments where soil erosion is a significant concern. Cyanobacteria such as *Scytonema* and *Nostoc* have been observed to form resilient biofilms that effectively prevent soil degradation (Pandhal*et al*. 2008; Yadav *et al*. 2022).

Alkaline Substance Production and pH Regulation

In acidic soils, where high hydrogen ion concentration limits plant growth, cyanobacteria contribute by producing alkaline substances. These substances help neutralize soil acidity, creating a more hospitable environment for plant roots and associated microorganisms. This pH regulation capability is particularly valuable in rehabilitating acidic soils in agricultural and mining-affected areas (Singh 1961; Priyadarshanee *et al*. 2020).

Microbial Interactions and Soil Biodiversity

Cyanobacteria establish symbiotic and mutualistic relationships with other microorganisms, enhancing microbial diversity and soil fertility. These interactions foster a balanced ecosystem where various microbial species contribute to nutrient cycling, decomposition and organic matter accumulation. For instance, cyanobacteria often associate with nitrogen-fixing bacteria and mycorrhizal fungi, amplifying their collective benefits for soil and plant health (Singh, 1961; Gupta *et al*. 2013).

Carbon Sequestration and Climate Mitigation

Through photosynthesis, cyanobacteria absorb atmospheric carbon dioxide (CO_2) and convert it into organic carbon compounds, contributing to soil organic matter and carbon sequestration. This process not only mitigates climate change by reducing greenhouse gas levels but also enhances soil carbon content, which is vital for maintaining soil fertility and water retention (Singh, 1961; Mishra and Rai, 2015). Cyanobacterial contributions to carbon dynamics underscore their role in promoting sustainable land-use practices.

Multifunctional Roles and Biofertilizer Potential

Cyanobacteria also produce bioactive compounds, including plant hormones like auxins and gibberellins, which stimulate plant growth and suppress pests and pathogens. Their biomass can be harvested and processed into biofertilizers, providing a sustainable alternative to synthetic fertilizers. Cyanobacteria-derived biofertilizers have shown potential to enhance soil microbial activity, improve nutrient availability, and promote plant development, thereby reducing reliance on chemical inputs (Kaushik and Subhashini1985; Bhola *et al*. 2014).

Recent research highlights the potential of cyanobacterial biocrusts in improving soil fertility and plant productivity. For example, Yadav *et al*. (2022) conducted experiments using *Nostoccalcicolea* and *Scytonema*sp. inoculated into different soil types, including coarse sand, fine sand, and loamy soil. Their findings revealed that cyanobacterial consortia significantly enhanced fertility in sandy soils and promoted superior plant growth in loamy soils compared to individual strains. This study underscores the viability of using cyanobacterial biocrusts as a cost-effective and eco-friendly strategy for land rehabilitation and soil health improvement.Cyanobacteria offer a suite of ecological and biochemical strategies for soil reclamation, ranging from nitrogen fixation and structural stabilization to nutrient cycling and carbon sequestration. Their multifunctional roles make them invaluable in addressing soil degradation and contamination while supporting sustainable agricultural practices. Future research should focus on optimizing cyanobacterial applications for large-scale soil restoration projects and integrating their use into holistic land management strategies.

Conclusion and prospects

Technological advancements hold transformative potential for modern agriculture, offering innovative solutions to critical challenges such as soil degradation and declining soil fertility. Among these advancements, phycoremediation stands out as an environmentally friendly and sustainable approach to restoring soil health. This technique harnesses the natural abilities of microalgae and cyanobacteria to detoxify and rejuvenate contaminated or degraded soils, providing an effective alternative to chemical-intensive methods. The successful application of phycoremediation in various regions globally highlights its potential in addressing soil contamination, improving soil fertility and promoting sustainable agricultural practices. From heavy metal remediation in industrially polluted soils to restoring nutrient balance in degraded farmlands, microalgae have demonstrated their adaptability and efficiency in soil reclamation. However, large-scale implementation requires further research on optimizing algal strains, improving cultivation techniques and integrating this method into existing agricultural frameworks. To maximize the benefits of phycoremediation, a concerted effort is required from all stakeholders. Policymakers must prioritize research funding, create supportive policies, and facilitate large-scale implementation through incentives and subsidies for sustainable agricultural practices. Farmers should be encouraged to adopt microalgae-based remediation techniques by providing them with accessible training programs and financial assistance. Meanwhile, researchers must continue exploring innovative ways to enhance the efficiency of microalgae in diverse environmental conditions, ensuring their practical application in real-world scenarios. By integrating eco-friendly approaches like phycoremediation into mainstream agricultural practices, we can mitigate soil degradation, enhance long-term soil productivity, and ensure food security. This holistic approach not only supports environmental conservation but also empowers farming communities by reducing dependence on chemical fertilizers and enhancing soil resilience against climate change. Collaboration among governments, agricultural institutions and local communities will be key to making microalgae-based soil remediation a widespread and impactful reality. The future of agriculture lies in sustainable innovations and phycoremediation represents a promising step toward achieving a balance between productivity and environmental stewardship.

"In this handful of soil is your future. Take care of it; it will take care of you. Destroy it, and it will destroy you" (a quote from Veda).

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Fameline K. Marak

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1. The NEHU Journal solicits original pieces of research in the form of articles and book reviews. The word limit for research articles and book reviews would be 5000 words and 1500 words respectively.
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4. The main paper should have Introduction, sub-sections and Conclusion at the end, followed by End Notes and References. All headings and subheadings should be marked in bold and subheadings should be italicised. In the case of contributions other than the full articles, the abstract and key words are not required.
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The Journal Style Sheet

- **Book**

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- **Journal Article**

  Mayoux, L. 1999. ‘Questioning Virtuous Spirals: Microfinance and Women’s Empowerment in Africa’, Journal of International Development, 11(7): 957-984.

  McCaffrey, D.F., Ridgeway, G. and Morral, A.R. 2004. ‘Propensity Score Estimation with Booted Regression for Evaluating Causal Effects in Observational Studies’, Psychological Methods, 9(4): 403-425.
- **Government and Institutional Documents**

  B L Center for Development Research and Action (BLCDRA). 2005. Micro finance and Empowerment of Scheduled Caste Women: An Impact Study of Self-help Groups in Uttar Pradesh and Uttaranchal. Government of India, New Delhi: 56.
- **Conference and Seminar Papers**

  Scandizzo, P., Gaiha, R. and Imai, K. 2005. ‘Option Values, Switches and Wages - An Analysis of the Employment Guarantee Scheme in India’, presented at the Conference on Social Protection for Chronic Poverty Risk, Needs, and Rights: Protecting What? How?, organized by IDPM, University of Manchester, 23-24, February 2005.

- **Online sources**

  Islam, A. 2009. 'Medium and Long-term Participation in Microfinance: An Evaluation Using a Large Panel Dataset from Bangladesh'. Department of Economics, Monash University, Australia:12. https://editorialexpress.com/cgi-in/conference/download.cgi? db, accessed on 12/12/2012.

- **For Book Reviews**: All book reviews should begin with the basic details as given below:

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