About the Book

The intersection of Microbiology and Biotechnology represents one of the most dynamic and impactful areas of modern science. Microbes, though microscopic, wield immense power in shaping our environment, health, and industry. In recent decades, our understanding of microorganisms has expanded exponentially, leading to innovative applications that address global challenges and improve the quality of life.

"Microbes and Biotechnology: Real World Application" is conceived as a comprehensive exploration of the practical uses of microbes in biotechnology. This book aims to bridge the gap between foundational microbial science and its applications, illustrating how the manipulation of microorganisms can lead to significant advancements across diverse sectors. By focusing on real-world applications, this volume serves as both a reference and an inspiration for professionals in the field, offering insights into the ways in which microbial technologies are transforming industries.

Biotechnology, driven by microbial innovation, is pivotal in areas such as healthcare, agriculture, environmental management, and industrial processes. The chapters in this book delve into key applications, ranging from the development of novel therapeutics to advancements in environmental bioremediation. Each chapter is authored by leading experts, providing in-depth analyses, case studies, and the latest developments in microbial biotechnology.

This book is designed to cater to a wide audience, including researchers, practitioners, policymakers, and students. For the researcher, it offers a rich source of information on current trends and future directions. For the practitioner, it provides practical guidance and examples of successful applications. For policymakers, it highlights the critical role of microbial biotechnology in addressing global challenges. And for students, it serves as an accessible introduction to the field, fostering a deeper understanding of the potential of microbes.

As we move forward in an era where sustainability and innovation are paramount, the role of microbes in biotechnology will undoubtedly continue to grow. This book stands as a testament to their transformative power, underscoring the importance of continued research and development in this field.

We present this book with the conviction that it will contribute meaningfully to the advancement of science and industry, and we invite readers to engage with its contents as a gateway to the remarkable potential of microbes.

Thank you for joining us on this enlightening journey through the fascinating world of Biotechnological interventions.

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MICROBES AND BIOTECHNOLOGY

Real-World Applications

Dr. Pritha Pal

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Chapter - 1

Uncovering Microbiome Secrets: Metagenomics in Advancing Sustainable Agriculture

Deeti Das and Aritri Laha

Abstract

Microorganisms are involved in a variety of metabolic processes and thrive in a wide range of habitats. Numerous microbes are difficult to culture using conventional microbiological techniques. This constraint is addressed by a newly developed non-culture strategy, known as metagenomics, which looks at entire microbial populations in environmental samples and yields a plethora of genetic information. Improving agricultural production requires an understanding of the complex interactions that exist between microorganisms, soil, and plants, especially in populations associated with plant roots. Even though some microbes are difficult to grow in a lab, metagenomic tools provide information on their identity and useful characteristics, opening doors for sustainable agriculture by creating ecologically conscious biopesticides and fertilisers.

Keywords: Metagenomics, omic approach, shotgun sequencing, bioinformatics, sustainable agriculture

Introduction

The intricate connections among agriculture progress and ecological sustainability rely on a web of factors like green tech, biodiversity, health, culture, and politics. (Goel *et al*., 2017). Furthermore, estimates indicate that by 2050, there will be 10 billion people on the planet, underscoring the need to increase agricultural yields in order to maintain agricultural productivity and provide food security (Levy and Lubell, 2018).Promoting a favourable environment is essential for the profitable cultivation of food in urban farming systems like bioponic schemes. Urban farming addresses urban sustainability, including equitable food distribution, waste management, and food security (Russo *et al*., 2017). Large-scale production of perishable vegetables boosts profitability in urban farming, which has developed into a sustainable enterprise. This is accomplished by utilising targeted marketing techniques

like growing plants using organic manure, expanding crop varieties, incorporating tourism and other non-agricultural services, establishing lively living spaces, providing culinary services, assisting in the restoration of ecosystems, and raising public awareness of ecological concerns (Pölling *et al*., 2017). Microorganisms in the soil ecology have essential functions, yet much remains unknown about the functions of unculturable microorganisms. The study of genetic material from natural resources is known as Metagenomics, particularly in agroecosystems, enables a deeper understanding of microbial diversity. This approach, also known as ecogenomics, holds promise for therapeutic, biotechnological, and agricultural advancements (Abram, 2015; Garrido-Oter *et al*., 2018). According to (Martínez-Porchas and Vargas-Albores., 2017), metagenomics has made it possible to investigate microbial populations in particular microniches, offering new perspectives on the traits of bacteria, archaea, and eukaryotes in soil and soilless agriculture.

Omics Approach for Sustainable Soil Analysis

Next-generation sequencing, like amplicon and shotgun metagenomics (Table 1) holds promise in addressing challenges in profiling microbial communities. Shotgun metagenomics, replacing traditional methods, is widely applied for studying genes and microbiological variations in ecological sample (Zhang *et al*., 2021).

Table 1: Benefits and Drawbacks of Amplicon and Shotgun Sequencing

Sequencing techniques	Advantages	Disadvantages	References
Amplicon metagenomic sequencing	a) Quick prep, low DNA, flexible experiments, cheap, fast results. b) Targets genetic variants, common in phylogeny and taxonomy. c) Probes capture specific regions, precise resequencing. d) Multiplexing for high amplicon coverage. e) Identifies variants, rare mutations in tumors and germline DNA.	i) PCR primers may bias by binding to non-conserved microbe regions. ii) Short reads, errors, and biases hinder reproducibility and quantification in sequencing. iii) Horizontal gene transfer and species definition issues limit marker genes. iv) Amplicon identification limited to genus level.	(Sabale *et al*., 2019)

Shotgun metagenomic sequencing	a) PCR avoids bias, efficient for taxonomic classification. b) Enhances microbial diversity detail, better resolution, increased accuracy. c) No specific gene targeting or amplification. d) Metagenomic data enables diverse analyses, including antibiotics and metabolism profiling.	i) Bias may arise from DNA prep, sample complexity, and sequencing platforms.	(Vargas-Albores *et al*., 2019), (Sabale *et al*., 2019)

MiSeq by Illumina sequenced plant root and bioponic system organic matter, exposing a variety of microbiological groups (Wongkiew *et al*., 2022). According to (Goodwin *et al*., 2016) the third-generation sequencing method offers rapid read completion within 4-6 hours, producing incredibly lengthy reads of thousands of bases. It's cost-effective, connects to a computer without specialized training, and provides high taxonomic resolution (Winand *et al*., 2019). Preprocess data using UCHIME, MG-RAST and RDP tools after sequencing (Bolger *et al*., 2014). New bioinformatics tools have been developed, and existing ones have been enhanced to yield more effective results (Table 2).

Table 2: Recently Upgraded Tools for Metagenomic Analysis.

Tools	Functions	References
MetaPhlAn 4	Metagenomic profiling.	(Blanco-Míguez *et al*., 2023)
MEGAN6 and MeganServer	Analyse and visualize metagenomic data.	(Gautam *et al*., 2023)
HUMAnN 3	Effective functional profiling.	(Beghini *et al*., 2021)
StrainPhlAn 3/PanPhlAn 3	Strain profiling via genetic variants.	(Beghini *et al*., 2021)
PhyloPhlAn 3	Assigning taxonomy through phylogeny.	(Beghini *et al*., 2021)

Microbial Agricultural Phylogenomic Study

Phylogenomics compares whole genomes to reveal links between organisms through evolutionary reconstructions. The resulting species tree, which rewrites evolutionary history and offers information about biological

processes, is crucial for a variety of investigations (Figure 1) (Emms and Kelly, 2019).

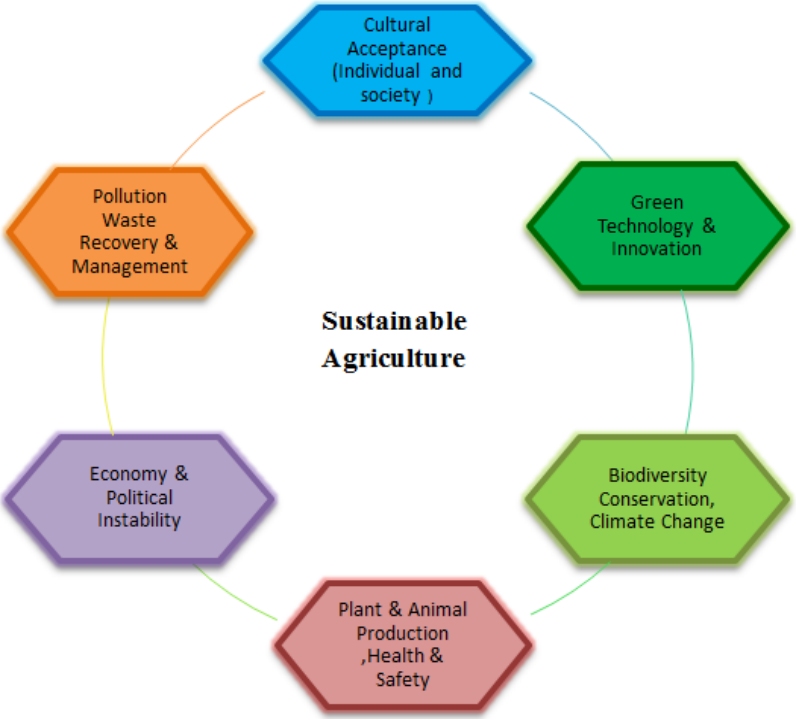

Fig 1: Incorporating sustainable farming particles into society

In order to determine homologous relationships between genes-coding genes and derive gene collections or orthologous groupings, phylogenomic methods generally include comparison pairwise sequences across complete genomes (Rabiee *et al.*, 2019).Large-scale microbial populations present a number of obstacles for phylogenomic study of genes, including incorrect results, redundancy, misidentification of gene factors, and assembly mistakes. It is best to prioritise visible shifts in evolution over genetic traits when addressing closeness difficulties (Young and Gillung, 2020; Earle *et al.*, 2016).

Investigating Advantageous Microorganisms Involved in Biogeochemical Cycles

Metagenomics has unveiled novel genomes shaping biogeochemical cycles. Kuenenia stuttgartiens' anaerobic ammonium-fixing ability is detailed. Shotgun and MS-based proteomics identified a crucial iron oxidation cytochrome (Bevivino and Dalmastri, 2017).

The Potential Uses of Metagenomic Research

Metagenomics holds undiscovered potential applications, and we delve into future perspectives on this field (Figure 2).

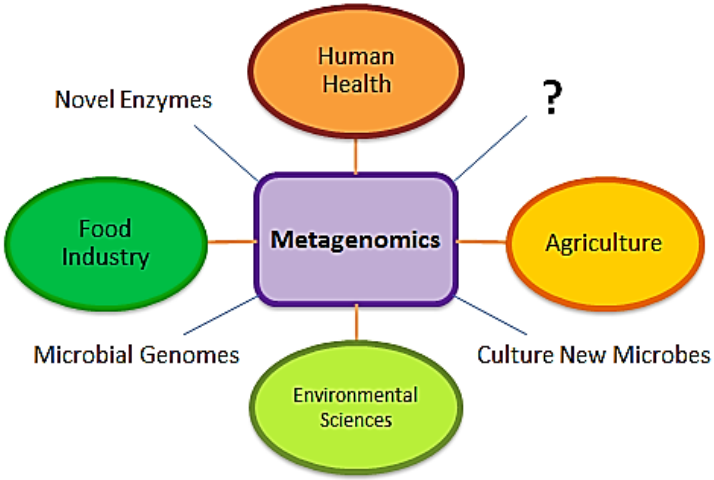

Fig 2: Contributions of metagenomics to various field. The question mark represents the undiscovered aspects of metagenomics.

Metagenomic utilisation in sustainable farming

Metagenomics reveals PGPR traits, enhancing understanding of soil-plant connections in agriculture (Gupta *et al*., 2018). Using the nifH gene, the metagenomic approach successfully identified diazotrophs in the soil of the red kidney bean rhizosphere. (Sabale *et al*., 2019). To support resilient agriculture, new genes that promote plant development must be developed for bioinoculants, taking into account the potential of microorganisms (Gupta *et al*., 2018). According to (Goel *et al*., 2017) metagenomics stresses functioning over mere listing and promotes sustainable agriculture by routinely evaluating and analysing the microbes that promote plant growth. Soil microbiologists use metagenomic techniques, like sequence-driven and function-driven methods, to overcome challenges, enabling evaluation of agricultural products efficiently (Garrido-Oter *et al*., 2018) (Figure 3). With the help of differential analysis that reveals microbial biomarkers under particular circumstances, microbiome networks highlight symbiotic interactions and keystone taxa. Urban agriculture can benefit from improved bioponic systems that integrate metagenomics and efficiency assessment in a sustainable manner (Bao *et al*., 2021).

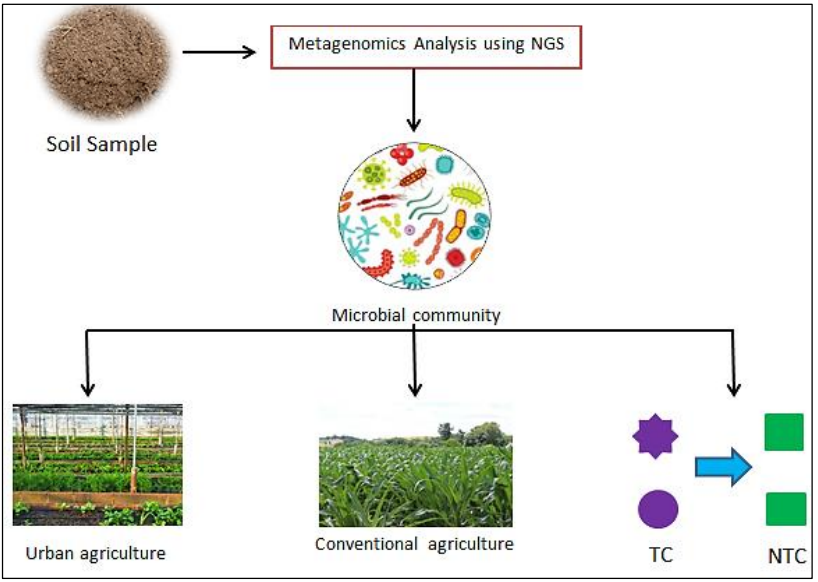

Fig 3: Soil microbial genetic diversity and their applications in sustainable agriculture. Microbes obtained and identified from rhizosphere soil can be harnessed to enhance plant growth and productivity in both urban and conventional agriculture. Some of such microbes may also play a role in converting toxic contaminants to their respective non-toxic forms. NGS, next generation sequencing; TC, toxic contaminants; NTC, non-toxic contaminants

Conclusion and Future prospects

The microbes play an important part in the growth of the surroundings and the well-being of plants. Still, a large part of the microbial populations in the rhizosphere is a mystery. By combining traditional techniques with metagenomics, it is possible to gain understanding of soil microbial communities, which can help with agricultural sustainability by identifying previously unexplored microbes and their uses, increasing the production of crops, and tackling ecological problems.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Deeti Das - Data collection and Writing-Original draft preparation; Aritri Laha. Reviewing and Supervision.

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References

1. Goel, R., Suyal, D. C., Dash, B., and Soni, R. (2017). "Soil metagenomics: A tool for sustainable agriculture," in Mining of Microbial Wealth and Metagenomics, eds V. C. Kalia, Y. Shouche, H. J. Purohit, and P. Rahi (New York, NY: Springer), 217–225.
2. Levy, M. A., and Lubell, M. N. (2018). Innovation, cooperation, and the structure of three regional sustainable agriculture networks in California. Reg. Environ.Change 18, 1235–1246. doi: 10.1007/s10113-017-1258-6
3. Russo, A., Escobedo, F. J., Cirella, G. T., and Zerbe, S. (2017). Edible green infrastructure: an approach and review of provisioning ecosystem services and disservices in urban environments. Agric. Ecosyst. Environ. 242, 53–66. doi: 10.1016/j.agee.2017.03.026
4. Pölling, B., Sroka, W., and Mergenthaler, M. (2017). Success of urban farming's city-adjustments and business models-Findings from a survey among farmers in Ruhr Metropolis, Germany. Land Use Policy 69, 372–385. doi: 10.1016/j.landusepol.2017.09.034
5. Abram, F. (2015). Systems-based approaches to unravel multi-species microbial community functioning. Comput. Struct. Biotechnol. J. 13, 24–32. doi: 10.1016/j.csbj.2014.11.009
6. Garrido-Oter, R., Nakano, R. T., Dombrowski, N., Ma, K. W., Team, T. A., Mchardy, A. C., *et al*. (2018). Modular traits of the rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. Cell Host Microbe 24, 155–167.e5. doi: 10.1016/j.chom.2018.06.006
7. Martínez-Porchas, M., and Vargas-Albores, F. (2017). Microbial metagenomics in aquaculture: a potential tool for a deeper insight into the activity. Rev. Aquacult.9, 42–56. doi: 10.1111/raq.12102
8. Zhang L, Chen F, Zeng Z, Xu M, Sun F, Yang, L., *et al*. (2021). Advances in metagenomics and its application in environmental microorganisms. Front. Microbiol. 12, 766364–766364. doi: 10.3389/fmicb.2021.766364
9. Sabale SN, Suryawanshi PP, and Krishnaraj P. 2019. Soil Metagenomics: Concepts and Applications. Metagenomics-Basics, Methods and Applications.London: IntechOpen.

10. Vargas-Albores, F., Martínez-Córdova, L. R., Martínez-Porchas, M., Calderón, K., and Lago-Lestón, A. (2019). Functional metagenomics: a tool to gain knowledge for agronomic and veterinary sciences. Biotechnol. Genet. Eng. Rev. 35, 69–91. doi: 10.1080/02648725.2018.1513230

11. Wongkiew, S., Polprasert, C., Koottatep, T., Limpiyakorn, T., Surendra, K., and Khanal, S. K. (2022). Chicken manure-based bioponics: effects of acetic acid supplementation on nitrogen and phosphorus recoveries and microbial communities. J. Waste Manag. 137, 264–274. doi: 10.1016/j.wasman.2021.11.023

12. Goodwin, S., Mcpherson, J. D., and Mccombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. Nat. Rev. Genet. 17, 333.doi: 10.1038/nrg.2016.49

13. Winand, R., Bogaerts, B., Hoffman, S., Lefevre, L., Delvoye, M., Van Braekel, J., *et al*. (2019). Targeting the 16s rRNA gene for bacterial identification in complex mixed samples: comparative evaluation of second (illumina) and third (oxford nanopore technologies) generation sequencing technologies. Int. J. Mol. Sci. 21, 298. doi: 10.3390/ijms21010298

14. Bolger, A. M., Lohse, M., and Usade, L. B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. doi: 10.1093/bioinformatics/btu170

15. Emms, D. M., and Kelly, S. (2019). OrthoFinder: phylogenetic orthology inference for comparative genomics. Genome Biol. 20, 1–14.doi: 10.1186/s13059-019-1832-y

16. Rabiee, M., Sayyari, E., and Mirarab, S. (2019). Multi-allele species reconstruction using astral. Mol. Phylogenet. Evol. 130, 286–296.doi: 10.1016/j.ympev.2018.10.033

17. Young, A. D., and Gillung, J. P. (2020). Phylogenomics-principles, opportunities and pitfalls of big-data phylogenetics. Syst. Entomol. 45, 225–247. doi: 10.1111/syen.12406

18. Earle, S. G., Wu, C.-H., Charlesworth, J., Stoesser, N., Gordon, N. C., Walker, T. M., *et al*. (2016). Identifying lineage effects when controlling for population structure improves power in bacterial association studies. Nat. Microbiol. 1, 1–8.doi: 10.1038/nmicrobiol.2016.41

19. Blanco-Míguez, A.; Beghini, F.; Cumbo, F.; McIver, L.J.; Thompson, K.N.; Zolfo, M.; Manghi, P.; Dubois, L.; Huang, K.D.; Thomas,A.M.; *et*

al. Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4. Nat.Biotechnol. 2023. [CrossRef]

20. Beghini, F.; McIver, L.J.; Blanco-Míguez, A.; Dubois, L.; Asnicar, F.; Maharjan, S.; Mailyan, A.; Manghi, P.; Scholz, M.; Thomas,A.M.; *et al*. Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3. eLife2021, 10, e65088. [CrossRef]
21. Gautam, A.; Zeng, W.; Huson, D.H. MeganServer: Facilitating interactive access to metagenomic data on a server. Bioinformatics 2023, 39, btad105. [CrossRef] [PubMed]
22. Gupta, N., Vats, S., and Bhargava, P. (2018). "Sustainable agriculture: role of metagenomics and metabolomics in exploring the soil microbiota," in Silico Approach for Sustainable Agriculture, eds D. K. Choudhary, M. Kumar, R. Prasad, and V. Kumar (New York, NY: Springer), 183–199
23. Bao, Y., Feng, Y., Qiu, C., Zhang, J., Wang, Y., and Lin, X. (2021). Organic matter- and temperature-driven deterministic assembly processes govern bacterial community composition and functionality during manure composting. J. Waste Manag. 131, 31–40. doi: 10.1016/j.wasman.2021.05.033

Chapter - 2
Delving Into the Importance of Bioactive Peptide from Marine Algae & Their Potential Health Impacts

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Chapter - 2

Delving Into the Importance of Bioactive Peptide from Marine Algae & Their Potential Health Impacts

Abu Jishan, Semanti Ghosh, Subhasis Sarkar, Suranjana Sarkar and Bidisha Ghosh

Abstract

Marine organisms forms rich source of health and of aesthetic value due to their vast applications in nutraceutical, cosmetics and in pharmaceutical industries. The diverse group of marine microbes & derived bioactive peptides obtained from oceanic hub differs in terms of their diversity, structure and functional aspect from their terrestrial counterparts that resulted mainly through long span evolutionary leap. These bioactive compounds can thus be seen as a magical elixir that have attracted considerable attention due to their diverse biological actions spanning antimicrobial, anticancer, anti-inflammatory and antioxidant properties. Different techniques of extraction are been applied to isolate these peptides from different species of marine algae, such as the convenient techniques of fermentation, chemical extraction, and other noted processes like enzymatic hydrolysis. Moreover the knowledge of several bioactive compounds from marine habitats are obtained from metagenomics. The potential health benefits and industrial uses of bioactive peptides that are been isolated, refined, and identified so far from marine algae are highlighted in this review. This has resulted in a renewed focus on researching the marine environment, specifically concerning marine microorganisms, with the aim of discovering novel chemical entities that may function as sources of lead compounds.

Keywords: Antimicrobial, anticancer, anti-inflammatory bioactive peptides, marine organisms, metagenomics

1. Introduction

Bioactive peptides, which typically consist of 2–20 amino acid residues, have drawn a lot of interest because of their biological properties and advantageous effects on health. Bioactive peptides can be released in three general ways: solvent extraction, enzymatic hydrolysis, and microbial fermentation (Fan *et al* 2014). Numerous bioactivities, including anticancer,

antioxidant, anti-inflammatory, immunomodulatory, antiatherosclerosis, antihypertensive, and antimicrobial peptides, were investigated (Cunha, S. A. *et al* 2022). It is a reality that marine species are becoming increasingly important as sources of novel chemicals. Given that half of all life on Earth are marine, the ocean provides a vast reservoir for new chemical compounds (Aneiros, A. *et al* 2004).These chemicals' chemical structures differ significantly from those derived from microbial and terrestrial systems. They can be found in cyclic and linear peptides with natural or synthetic amino acids, as well as depsipetides (peptides in which one or more amide linkages are substituted by ester bonds). These compounds can be derived from marine microbes with which the organism has a symbiotic relationship, or they can be created by the organism itself (Lazcano-Pérez, F., *et al*. 2012). Marine-derived bioactive peptides have the potential to kill cancer cells through a variety of ways, including blocking angiogenesis, altering the tubulin-microtubule equilibrium, or inducing apoptosis. (Pangestuti, R., & Kim, *et al*. (2017).A significant number of marine anticancer chemicals are anticipated to enter the drug discovery pipeline, with 16 of the 20 now undergoing clinical trials coming from microbial origins. This has led to a resurgence of interest in studying the marine environment, particularly in relation to marine microbes, in an effort to find new chemical entities that could serve as sources of lead compounds (Romano, G., Costantini, M., *et al*. 2017).

1.1 Classes of marine algae

Based on their form, marine algae are classified into two groups: macroalgae, which include green, brown, and red algae, and microalgae, which include diatoms, dinoflagellates, and Cyanophyta (Wu, J., Gu *et al*. 2021).Known by most as macroalgae, seaweed is a broad category of about 10,000 species. Up to 94% of the biomass of fresh marine algae can be made up of water (Ashraf, A., Din, *et al*.2023).Data show that red seaweed species have greater protein concentrations (12.5-35.2%), followed by green (9.6-23.3%) and brown (4.5-16.8%). However, a large number of red seaweed species contain substantial protein content that is on par with that of fish, eggs, grains, and soybeans. In light of this, it may be possible to extract proteins from seaweeds for functional uses (Echave, J., *et al*. 2022).Microalgae are a very diversified class of photosynthetic organisms that use light and inorganic materials to produce biomass that is high in fats, proteins, and other valuable compounds like omega-3 fatty acids and carotenoids (Barkia, I., *et al*. 2019). Red algal peptides are divided into several types based on their therapeutic effects, including immunomodulatory, antidiabetic, prebiotic, antioxidant, and anti-obesity. Red algae Eucheuma denticulatum is well-known for having high

levels of astaxanthin, β-carotene, vitamin B, vitamin E, polyunsaturated fatty acid, and polyphenols (Wani, H. M. U. D., *et al*. 2023). Tiny, unicellular algae belonging to the Haptophyceae family are found throughout the oceans and can make up a significant fraction of marine phytoplankton (Kobayashi, J., *et al*.1993).

2. Production of bioactive peptide

Certain food samples might already contain the bioactive peptides. They can also be produced technologically (during the hydrolyzate preparation process) or by processing (during the digestion process, when the parent protein is liberated). Products already contain ribosomal or non-ribosomal peptides (Pavlicevic, M., *et al*. 2020).One of the methods most frequently employed to produce marine peptides is solvent extraction. Diverse marine peptides can be extracted using a wide range of solvents or solvent combinations, depending on the polarity and peptide solubilizing ability. Most notably, it has frequently been demonstrated that adding acid to a solvent increases the effectiveness of marine peptide extraction (Sridhar, K., *et al*. 2021).Once optimized, the enzymatic hydrolysis method can provide high yields of high-quality bioactive peptides. It is also straightforward and quick to inactivate. Peptides' unique structural characteristics, such as their amino acid content, sequence, chain length, hydrophobicity, and net charge, are primarily responsible for their biological actions (Mora, L., *et al*. 2023).For the enzyme to carry out its functions, it is vital that it first binds to the substrate and then continue with enzymatic catalysis. To do this, the enzyme contains certain active sites with residues that both catalyze the interaction with the substrate and create short-term bonds with it. Catalytic sites and binding sites are created in this manner. The enzyme-substrate complex is often composed of hydrophobic, hydrogen, or Van der Waals contacts. Lastly, protein hydrolysis can be guaranteed when the enzyme-substrate combination is in a particular shape (Cruz-Casas, D. E., *et al*. 2021).since the end products of these reactions don't contain any hazardous compounds or leftover organic solvents. Furthermore, it is simple to regulate the hydrolysis conditions. For ideal hydrolysis, a few crucial variables, like pH and temperature, must be monitored and adjusted during digestion (Marciniak, A., Suwal, *et al*. 2018).In general, bioactive peptides made using food-grade enzymes through enzymatic hydrolysis are regarded as GRAS (Generally Recognized as Safe). However, the industry is looking for alternate techniques because to the high cost of enzymes and limited peptide yield (caused by the selectivity of the cleavage sites by an enzyme) (Ulug, S. K., *et al*. 2021).In order to produce BAPs, the reaction conditions are changed to tip the equilibrium in favor of

peptide bond formation. Conversely, the transpeptidation mechanism takes place when a peptide bond breaks and an active acyl-enzyme intermediate is created. A new peptide bond is formed by this intermediate in the presence of a nucleophile, which is a peptide or amino acid that is blocked in the a-carboxyl group(Chauhan, V., & Kanwar,*et al*. 2020).Specific molecular weight bioactive peptides are released by proteolytic enzymes. Low molecular weight peptides would be produced, nevertheless, if hydrolysis were to continue. To acquire an optimum degree of hydrolysis, it's critical to keep the ratio of enzyme to substrate in check. Desalting, freeze-drying, membrane ultrafiltration, crossflow membrane filtration, or column chromatography are the methods used to recover the peptides. Gel filtration is an excellent way to separate low molecular weight peptides according to their size (Patil, S. M., Sujay, S., *et al*.2020).

3. Role of the different extracted bioactive peptide

3.1 Anticancer potential of bioactive peptides

Microbes' natural products are a rich source of bioactive compounds that can be used as therapeutic leads, mainly in the treatment of cancer. One of the most important compounds created from nature are peptides (Sukmarini, L.*et al*. 2021).One of the potential organisms that could be the richest source of known and novel bioactive compounds is thought to be marine blue-green algae. These compounds can either kill cancer cells by inducing apoptosis or affect cell signaling by activating members of the protein kinase-c family of signaling enzymes (Sithranga Boopathy, *et al.2010*).Bioactive peptides have the ability to penetrate the membrane of malignant cells through processes known as necrosis or apoptosis, which can result in cell death. The peptides cause cell lysis in necrosis by targeting the negatively charged molecules on the cancer cell membrane; in apoptosis, they break the mitochondrial membrane. An great illustration of the lethal action of bioactive peptides is the initiation of pathways leading to extrinsic apoptotic activity and the suppression of angiogenesis. Most bioactive peptides, which are specifically antitumor peptides, are thought to be particularly active when combined with the old-style chemotherapy because of their propensity to function in concert, but in reality, they are chemotherapeutic agents (Ahmed, I., Asgher, M., *et al*. 2022).(8)A cyclic depsipeptide molecule called Sansalvamide was identified from a variety of marine-dwelling fungi. It demonstrated cytotoxicity against a wide range of cell lines, including those from breast, prostate, colon, pancreatic, and sarcoma melanoma, suggesting that it may have anticancer properties. The precise mechanism of action of these molecules is yet unknown, however recent research in mammalian cell lines revealed a

connection between client cancer protein and heat shock protein (Rauf, A., Khalil, *et al*. 2022).By using a sequence from the interaction region, peptides can be engineered to block these interactions. Because of this, BPs are simple to make and their sequence is easily changed through the use of chemical synthesis or molecular biology methods (González-Montoya, *et al*. 2017). Certain peptides, including BEPT II and BEPT II-1 from Bullacta exarata, have also shown an apoptotic effect on prostate cancer cells (Cicero, A. F., Fogacci, *et al*. 2017).

3.2 Antioxidant potential of bioactive peptides

In all living things, oxidation is a necessary response because it produces free radicals and other reactive oxygen species (ROS), which are crucial for signal transduction (Wang, X., *et al*. 2017) Dietary antioxidants such carotenoids, polyphenols, and vitamins E and C are thought to be effective in preventing disorders linked to oxidative stress (Hayes, M., & Flower, *et al*. 2013). To slow down peroxidation processes, synthetic antioxidants including propyl gallate (PG), tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) have been employed. However, because of the possible health risks associated with these artificial antioxidants, usage needs to be closely regulated (Ngo, D. H., *et al* 2013). Free radicals can attack nearby molecules by taking away electrons, which sets off a chain reaction in which the newly created molecule attacks more molecules. This happens when free radicals are generated in excess or if they are produced but are not eliminated (Lee, J. K., *et al*. 2012). Since histidine, tyrosine, and methionine have been shown to be effective radical scavengers, peptides containing these amino acids are assumed to have increased radical scavenging and consequently antioxidant properties (Hayes, M.*et al*.2013). A peptide's ability to function as an antioxidant may be enhanced by hydrophobic amino acids like glycine (G), isoleucine (I), and/or leucine (L) located near the N-terminus. Moreover, higher antioxidant activity has been linked to branched chain amino acids including valine, leucine, and isoleucine (Bashir, K. M. I.*et al*. 2020).The hydrophobicity, size, type, and location of amino acids within peptides can all affect a peptide's antioxidant function. Antioxidant peptides are useful components that can be added to food products to regulate the oxidation of protein and fat. These peptides regulate oxidation processes and may be useful in the management and treatment of illnesses in humans (Nikoo, M., *et al*.2016).Using a variety of methods, including the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging assay, the hydroxyl/superoxide radicals (OH/O_2-) scavenging

assay, the ferric reducing antioxidant power (FRAP), and the lipid peroxidation inhibition capacity assay, relative polysaccharides and pigment compounds related to seaweed have been verified as effective antioxidants (Hamidi, M., Kozani, *et al*. 2019).

3.3 Antimicrobial nature of the marine algal products

Antimicrobial peptides (AMPs) are crucial components of innate immunity and are frequently the first line of defense against invasive infections (Salas, C. E., *et al*. 2015). A significant portion of this field's research over the past ten years has been concentrated on the bioprospecting of novel antimicrobial compounds that can exhibit a broad spectrum of activity against a variety of microorganisms, including gram-positive and gram-negative bacteria, yeasts, fungi, viruses, protozoa, and parasites like nematodes, in light of the diverse functions that AMPs exert (Otero-Gonzáiez, *et al*. 2010). Antimicrobial peptides, which are usually 20–40 amino acids long, are used by a variety of organisms as an infection-prevention strategy. The majority can quickly eradicate a variety of microorganisms. Big antimicrobial proteins (>100 amino acids) frequently target certain microbial peptides by interfering with the structure or function of microbial cell membranes, or they are lytic, nutrient-binding proteins. Antimicrobial peptides (AMP) have a dual function as endogenous antibiotics and also play a role in wound healing, inflammation, and the control of the adaptive immune system (Kang, H. K., *et al*. 2015).The potential of marine antimicrobial peptides is equivalent to that of terrestrial AMPs. The two main characteristics of an AMP are its amphipathicity and cationicity, which indicate a broad range of activity. Amphipathicity allows the peptide to pass across the cytoplasmic membrane, while cationicity ensures that the peptide and lipid interact. It is clear that the antibacterial activity of many of these is limited to smaller areas of the original peptide. Penaeidin is composed of two unique domains: an N-terminal area rich in Pro-Arg and a C-terminal region containing Cys. Research has indicated that the Pro-Arg rich region is not enough to sustain full activity on its own (Sperstad, S. V., *et al*.2011).Research has indicated that antimicrobial peptides have the potential to disrupt regular cellular processes, including gene transcription, apoptosis, and cytokine release, thereby augmenting the host's innate and adaptive immunity. Because antimicrobial peptides have a wide range of targets and diverse modes of action, they significantly lower the risk of usage by preventing bacteria from developing drug resistance. Antibiotics are predicted to be replaced by a new class of antimicrobial medications (Wu, R., *et al*. 2021).

3.4 Anti-inflammatory nature of marine compounds

Marine-derived nutrients and bioactive compounds have more promise as functional food ingredients since they can have a variety of positive impacts in addition to pharmaceutical and other health advantages, such as anti-inflammatory effects (Elbandy, M. *et al*. 2022). An effective immunological response to foreign stimuli is inflammation, which eventually leads to the return of normal function. An essential mediator of the inflammatory response are macrophages. Pro-inflammatory enzymes like inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and pro-inflammatory cytokines like tumor necrosis factor-α (TNF-α) and interleukins (IL-1β and IL-6), which are crucial mediators of the inflammatory response, are produced by activated macrophages.(Lee, H. A., Kim, I. H., *et al*.2015). Terpenoid pacifenol was extracted from seaweeds belonging to the marine alga Laurencia claviformis. Its anti-inflammatory and anti-allergy properties can be used to contrast the phlogistic processes linked to the aetiology of numerous inflammatory and allergic disorders. It achieves this by inhibiting the important enzyme phospholipase A2 and subsequently modulating the cyclooxigenase pathway. A terpenoid called epitaondiol was identified from Stypopodium flabelliforme seaweeds. By modifying the cyclooxigenase pathway and inhibiting the generation of eicosanoids (LTB4 and TXB2), it also shown notable anti-inflammatory benefits. This is accomplished by inhibiting phospholipase A2, a crucial enzyme that is involved in the synthesis of lipid mediators and the release of arachidonic acid. Compared to indomethacine, it exhibits stronger anti-inflammatory properties. (D'Orazio, N., G. *et al*. 2012).

4. Conclusion

The possibility of a number of marine species as a source of bioactive chemicals has been investigated. This review delves into the high potential of marine-derived bioactive peptides, which are derived from a variety of marine algae. These peptides have been shown to have a wide range of biological activities, including anti-inflammatory, anti-cancerous, anti-oxidant, and antimicrobial properties, among many other health-related benefits and therapeutic potential in the treatment and/or prevention of numerous diseases. Fascinatingly, amino acids and bioactive peptides might function as substitutes for small-molecule medications. Furthermore, these have demonstrated a significant benefit over traditional medications in terms of their high bioavailability and bio-specificity to the targets. These elements have also been discussed in relation to their contemporary pharmaceutical requirements, as well as the consequences and individual functional

bioactivities. Nevertheless, a lot more work needs to be done before bioactive peptides may be effectively used for human nutrition and health.

Reference

1. Fan X, Bai L, Zhu L, Yang L, & Zhang X. Marine algae-derived bioactive peptides for human nutrition and health. *Journal of Agricultural and Food Chemistry*, 2014; *62*(38), 9211-9222.
2. Cunha SA, & Pintado ME. Bioactive peptides derived from marine sources: Biological and functional properties. *Trends in Food Science & Technology*, 2022; *119*, 348-370.
3. Aneiros A, & Garateix A. Bioactive peptides from marine sources: pharmacological properties and isolation procedures. *Journal of Chromatography B*, 2004; *803*(1), 41-53.
4. Lazcano-Pérez F, A Roman-Gonzalez S, Sánchez-Puig N, & Arreguin-Espinosa R. Bioactive peptides from marine organisms: a short overview. *Protein and Peptide Letters*, 2012; *19*(7), 700-707.
5. Pangestuti R, & Kim SK. Bioactive peptide of marine origin for the prevention and treatment of non-communicable diseases. *Marine Drugs*, 2017; *15*(3), 67.
6. Romano G, Costantini M, Sansone C, Lauritano C, Ruocco N, & Ianora A. Marine microorganisms as a promising and sustainable source of bioactive molecules. *Marine Environmental Research*, 2017; *128*, 58-69.
7. Wu J, Gu X, Yang D, Xu S, Wang S, Chen X, & Wang Z. Bioactive substances and potentiality of marine microalgae. *Food Science & Nutrition*, 2021; *9*(9), 5279-5292.
8. Ashraf, A., Din, A. S. U., Hussain, M., Khan, I., Hassan, F., Ahmad, A., & Lou, Z. (2023). Characterization, therapeutic applications, structures, and futures aspects of marine bioactive peptides. *International Journal of Environment, Agriculture and Biotechnology*, *8*, 6.
9. Echave, J., Otero, P., Garcia-Oliveira, P., Munekata, P. E., Pateiro, M., Lorenzo, J. M. & Prieto, M. A. (2022). Seaweed-derived proteins and peptides: promising marine bioactives. *Antioxidants*, *11*(1), 176.
10. Barkia, I., Al-Haj, L., Abdul Hamid, A., Zakaria, M., Saari, N., & Zadjali, F. (2019). Indigenous marine diatoms as novel sources of bioactive peptides with antihypertensive and antioxidant properties. *International Journal of Food Science & Technology*, *54*(5), 1514-1522.

11. Wani, H. M. U. D., Chen, C. W., Huang, C. Y., Singhania, R. R., Sung, Y. J., Dong, C. D., & Patel, A. K. (2023). Development of Bioactive Peptides Derived from Red Algae for Dermal Care Applications: Recent Advances. *Sustainability*, *15*(11), 8506.

12. Kobayashi, J., & Ishibashi, M. (1993). Bioactive metabolites of symbiotic marine microorganisms. *Chemical Reviews*, *93*(5), 1753-1769.

13. Sukmarini, L. (2021). Natural Bioactive Cyclopeptides from Microbes as Promising Anticancer Drug Leads: A Mini-review. *Indonesian Journal of Pharmacy/Majalah Farmasi Indonesia*, *32*(3).

14. Sithranga Boopathy, N., & Kathiresan, K. J. J. O. (2010). Anticancer drugs from marine flora: An overview. *Journal of oncology*, *2010*.

15. Ahmed I, Asgher M, Sher F, Hussain SM, Nazish, N., Joshi, N., & Iqbal HM. (2022). Exploring marine as a rich source of bioactive peptides: Challenges and opportunities from marine pharmacology. *Marine drugs*, *20*(3), 208.

16. Rauf, A., Khalil, A. A., Khan, M., Anwar, S., Alamri, A., Alqarni, A. M., & Wan, C. (2022). Can be marine bioactive peptides (MBAs) lead the future of foodomics for human health?. *Critical Reviews in Food Science and Nutrition*, *62*(25), 7072-7116.

17. González-Montoya, M., Cano-Sampedro, E., & Mora-Escobedo, R. (2017). Bioactive peptides from le-gumes as anticancer therapeutic agents. *Int J Cancer Clin Res*, *4*, 81.

18. Cicero A.F, Fogacci F, & Colletti A. (2017). Potential role of bioactive peptides in prevention and treatment of chronic diseases: A narrative review. *British journal of pharmacology*, *174*(11), 1378-1394.

19. Wang X, Yu H, Xing R, & Li P. 2017. Characterization, preparation, and purification of marine bioactive peptides. *Bio Med research international*, *2017*

20. Pavlicevic, M., Maestri, E., & Marmiroli, M. (2020). Marine bioactive peptides-an overview of generation, structure and application with a focus on food sources. *Marine drugs*, *18*(8), 424.

21. Sridhar, K., Inbaraj, B. S., & Chen, B. H. (2021). Recent developments on production, purification and biological activity of marine peptides. *Food Research International*, *147*, 110468.

22. Mora, L., & Toldrá, F. (2023). Advanced enzymatic hydrolysis of food proteins for the production of bioactive peptides. *Current Opinion in Food Science*, *49*, 100973.

23. Cruz-Casas, D. E., Aguilar, C. N., Ascacio-Valdés, J. A., Rodríguez-Herrera, R., Chávez-González, M. L., & Flores-Gallegos, A. C. (2021). Enzymatic hydrolysis and microbial fermentation: The most favorable biotechnological methods for the release of bioactive peptides. *Food Chemistry: Molecular Sciences*, *3*, 100047.

24. Marciniak, A., Suwal, S., Naderi, N., Pouliot, Y., & Doyen, A. (2018). Enhancing enzymatic hydrolysis of food proteins and production of bioactive peptides using high hydrostatic pressure technology. *Trends in Food Science & Technology*, *80*, 187-198.

25. Ulug SK, Jahandideh F, & Wz J. Novel technologies for the production of bioactive peptides. *Trends in food science & technology*, 2021;*108*, 27-39.

26. Chauhan V, & Kanwar SS. 2020. Bioactive peptides: synthesis, functions and biotechnological applications. In *Biotechnological production of bioactive compounds* (pp. 107-137). Elsevier.

27. Patil SM, Sujay S, Tejaswini, M., Sushma, P. P., Prithvi, S., & Ramu, R. (2020). Bioactive peptides: Its production and potential role on health. *Innov. Food Sci. Emerg. Technol*, *7*, 167-182

28. Hayes M, & Flower D. Bioactive peptides from marine processing byproducts. *Bioactive compounds from marine foods: Plant and animal sources*, 2013; 57-71.

29. Ngo DH, Vo TS, & Kim SK. Biological activities of marine bioactive peptides. *Marine Proteins and Peptides: Biological Activities and Applications*, 2013; 509-521.

30. Lee, J. K., Jeon, J. K., Kim, S. K., & Byun, H. G. (2012). Characterization of bioactive peptides obtained from marine invertebrates. *Advances in food and nutrition research*, *65*, 47-72.

31. Hamidi, M., Kozani, P. S., Kozani, P. S., Pierre, G., Michaud, P., & Delattre, C. (2019). Marine bacteria versus microalgae: who is the best for biotechnological production of bioactive compounds with antioxidant properties and other biological applications?. *Marine drugs*, *18*(1), 28.

32. Hayes, M. (2013). Biological activities of proteins and marine-derived peptides from byproducts and seaweeds. *Marine proteins and peptides: biological activities and applications*, 139-165.

33. Bashir KMI, Sohn, J. H., Kim, J. S., & Choi, J. S. (2020). Identification and characterization of novel antioxidant peptides from mackerel

(Scomber japonicus) muscle protein hydrolysates. *Food Chemistry*, *323*, 126809.

34. Nikoo, M., Rabiei, S., Rezaei, M., & Khezri, M. (2016). Antioxidant peptides from marine sources: Identification, purification and mechanism of their action-Review. *Journal of Aquatic Physiology and Biotechnology*, *4*(3), 99-122.

35. Salas, C. E., Badillo-Corona, J. A., Ramírez-Sotelo, G., & Oliver-Salvador, C. (2015). Biologically active and antimicrobial peptides from plants. BioMed research international, 2015.

36. Otero-Gonzáiez, A. J., Magalhaes, B. S., Garcia-Villarino, M., López-Abarrategui, C., Sousa, D. A., Dias, S. C., & Franco, O. L. (2010). Antimicrobial peptides from marine invertebrates as a new frontier for microbial infection control. *The FASEB journal*, *24*(5), 1320-1334.

37. Kang, H. K., Seo, C. H., & Park, Y. (2015). Marine peptides and their anti-infective activities. *Marine drugs*, *13*(1), 618-654.

38. Sperstad, S. V., Haug, T., Blencke, H. M., Styrvold, O. B., Li, C., & Stensvåg, K. (2011). Antimicrobial peptides from marine invertebrates: challenges and perspectives in marine antimicrobial peptide discovery. *Biotechnology advances*, *29*(5), 519-530.

39. Wu R, Patocka J, Nepovimova E, Oleksak P, Valis M, Wu W, & Kuca K. 2021. Marine invertebrate peptides: Antimicrobial peptides. *Frontiers in microbiology*, *12*, 785085.

40. D'Orazio, N., Gammone, M. A., Gemello, E., De Girolamo, M., Cusenza, S., & Riccioni, G. (2012). Marine bioactives: Pharmacological properties and potential applications against inflammatory diseases. *Marine drugs*, *10*(4), 812-833.

41. Elbandy M. Anti-inflammatory effects of marine bioactive compounds and their potential as functional food ingredients in the prevention and treatment of neuroinflammatory disorders. *Molecules*, 2022; *28*(1), 2.

42. Lee, H. A., Kim, I. H., & Nam, T. J. (2015). Bioactive peptide from Pyropia yezoensis and its anti-inflammatory activities. *International Journal of Molecular Medicine*, *36*(6), 1701-1706.

Chapter - 3
Gestational Diabetes Mellitus: A Global Challenge for Maternal and Fetal Health

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Chapter - 3

Gestational Diabetes Mellitus: A Global Challenge for Maternal and Fetal Health

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Abstract

A major public health concern, gestational diabetes mellitus (GDM) affects 1.9–3.6% of pregnancies. Women with GDM who are pregnant have a higher chance of having type 2 diabetes after giving birth. In recent studies there are several evidence present, which saying there was a potential link between gut microbiota and diabetes management. Proteobacteria as a phylum and many genera, such as Escherichia, Orchobacterium, Cronobacter, Shigella, Salmonella, Enterobacter, Klebsiella, Kosakonia, Vibrio, and Gamma-Proteobacterium, constituted the majority of the GDM group. On the other hand, the healthy group had phylum-level Firmicutes dominance, with greater abundance in genera like Eubacterium, Roseburia, Lachnospiraceae, Clostridium, and Clostridiales. Along with the alteration of gut-microbiota, also there was alteration of both amniotic fluid and vaginal microbiota was discovered from recent studies GDM compared to non-GDM pregnant women. While no significant variations were found in the vaginal microbiota, the amniotic fluid communities exhibited differences in species-level beta-diversity. Microbial functions involved host metabolic pathways, with no significant group differences but distinct site-specific patterns. Differences in composition were noted, and certain species of bacteria (Escherichia, Orchobacterium, Cronobacter, Shigella, Salmonella, Enterobacter, Kosakonia, Vibrio, and Gamma-Proteobacterium in Bacteroidetes, and Ruminococcus, Clostridium, Clostridiales, Lachnospiraceae, Roseburia, Weisella, and Eubacterium in Firmicutes) correlated with insulin signalling and lipopolysaccharide biosynthesis pathways. This systematic review will help in deep understanding the intricate relationships between gestational conditions, microbiota, and pregnancy outcomes, offering insights for future clinical considerations and interventions.

Keywords: Gut microbiota, microbiome, gestational diabetes mellitus, diabetes, metagenome

Introduction

Indeed, the development of poor glucose tolerance during pregnancy in women without a history of diabetes is a hallmark of gestational diabetes mellitus (GDM). It increases the likelihood that the mother and the child may experience difficulties throughout pregnancy and labour. Furthermore, women with a history of GDM are at a higher risk of developing type 2 diabetes (T2D) later in life. In the context of type 2 diabetes, the significance of gut bacteria has garnered increasing attention in recent studies. The varied population of microbes living in the digestive system is referred to as the gut microbiota. Research has indicated that changes in the makeup and functionality of gut microbiota may impact metabolic pathways and have a role in the emergence of diseases such as type 2 diabetes(Minarti *et al*., 2020) Numerous factors, including lifestyle choices, diagnostic standards, screening techniques, and the population under study, affect the prevalence of gestational diabetes mellitus(Zheng *et al*., 2022). Different groups experience different dynamics of the gut microbiota (GM) during pregnancy, which are impacted by a range of factors such as living environment and diet.This study is to investigate the primary variables influencing the changes in gastrointestinal microecology as well as to monitor and assess the changes in the structure and function of the GM in women from the first to the third trimester of pregnancy (Li *et al*., 2023). Between 1996 and 2010, the prevalence of GDM rose from 2.7% to 5.6%. When the most recent International Association of Diabetes in Pregnancy Study Group (IADPSG) guidelines were compared to the World Health Organisation (WHO) 1999 criteria, the prevalence of GDM increased by 2.4 times. According to the revised standards, the 15 centres that took part in the hyperglycemia and bad pregnancy outcome (HAPO) research had GDM prevalence ranging from 9% to 26% (Zheng *et al*., 2022).

Gestational Diabetes Mellitus (GDM) in Pregnant Women

One kind of diabetes known as gestational diabetes mellitus, or GDM, is brought on by pregnancy. Its characteristic is elevated blood sugar levels that appear or are first noticed during pregnancy. GDM poses a risk to the unborn child as well as the mother if treatment is not received. A type of diabetes known as gestational diabetes mellitus (GDM) usually appears between weeks 24 and 28 of pregnancy. Blood sugar levels rise as a result of the body's inability to manufacture enough insulin to satisfy the increasing demands. A number of risk factors, such as being overweight, belonging to a certain ethnic group, and having a family history of diabetes, raise the likelihood of developing GDM. Both the mother and the unborn child are at danger due to

this disease. The mother's high blood pressure and increased chance of caesarean section are among the complications. Uncontrolled GDM can lead to macrosomia, which increases the difficulty of delivery and puts the unborn child at risk for low blood sugar and respiratory distress (Minarti *et al*., 2020). Gestational diabetes mellitus (GDM) increases the risk of complications during pregnancy, including dystocia and a higher requirement for caesarean sections. Long-term risks include preeclampsia, gestational hypertension, postpartum type 2 diabetes, and cardiovascular disease are linked to it. Maternal hyperglycemia in GDM affects delivery and the baby's health in addition to causing foetal macrosomia and abnormalities. Obesity, metabolic syndrome, and type 2 diabetes are more common in children. In order to lessen societal costs, it is imperative that GDM be addressed for both short- and long-term health. This includes effective screening, diagnosis, and management (Zheng *et al*., 2022).

Research has indicated that genetic modifications may have a significant impact on the onset and progression of GDM. The GDM condition is linked to the GM imbalance, as evidenced by the much lower a-diversity of the GM in GDM patients and the increased content of Parobacteria and Klebsiella, compared to the larger abundances of Bifidobacterium and Eubacterium in healthy pregnant women (Li *et al*., 2023). During a normal pregnancy, women undergo a variety of hormone-related, immune-mediated, and metabolic alterations, including a decrease in sensitivity to insulin and a boost in insulin resistance (IR).To offset these alterations, the ability to produce insulin rises. Nonetheless, some expectant mothers experience difficulty increasing insulin production, which leads to the development of GDM (Zheng *et al*., 2022).

The gut microbiota has a crucial role in controlling the inflammatory response and insulin resistance in GDM pregnancies, according to a few studies. It is well known that diet may change the microbiota's makeup quite quickly-within a few days. According to recent studies, different nutrients affect metabolic results differently based on the specific microbial pattern. This is an intriguing and important research that raises the possibility that, rather than being generally healthy, a diet could be more appropriate for some individuals or situations. Additionally, it fervently advocates for a customised approach to human nutrition. (Ponzo *et al*., 2019).

Microbiome Alteration and Impact on Health

The influence of microbe dysbiosis on maternal and unborn health is highlighted by the metagenomic study regarding gestational diabetes mellitus, which reveals notable changes in the gut and vaginal microbiota. A

comprehensive study was made easier by bioinformatics techniques, which also offered insights into possible bacterial biomarkers for risk estimation. Gaining insight into these microbiome alterations is essential to creating focused treatments, tackling the worldwide issue of diabetes during gestation, and enhancing outcomes for mothers and babies. The term "gut microbiota" refers to all microorganisms that live in the intestinal tract of humans. Residential microorganisms and their host have a relationship that is mutually beneficial because they can derive energy from foods that humans are unable to absorb and create bioactive substances like short-chain fatty acids (SCFAs), which have been showed to have beneficial impacts on the host's metabolism. The gut microbiota regulates several human processes, making it a sizable virtual endocrine-metabolic organ (Ponzo *et al*., 2019). The pathogenesis of GBS illness in GDM is reflected in a diet-induced gestational diabetes model. This is due to changes in the makeup of the vaginal microbiota during pregnancy, differential transcriptional adaptability of GBS, and abnormal maternal immunity. Taking into account the different immunological, microbiological, and metabolic characteristics in gestational diabetes as opposed to non-diabetic pregnancy (Mercado-Evans *et al*., 2024). The study aimed to do an ongoing observational study to evaluate the rapid changes in the microbiome that occur in maternal GDM women. Following their diagnosis of GDM, all patients received an educational dietary intervention, as per the guidelines; nevertheless, there is variation in the adherence of women to these recommendations. Thus, the objectives of our research were to determine whether the composition of the gut microbiota varied within patients from the second to the third trimester of pregnancy; whether patients who adhered to dietary guidelines better than those who did not; whether alterations in microbiota composition were linked to variations in nutrient intakes, anthropometric data, and laboratory variables; and finally, whether particular microbiota oligotypes were implicated in these associations (Ferrocino *et al*., 2018).

Conclusion

In conclusion, metagenomic analysis of the gut and vaginal microbiota in research on gestational diabetes mellitus, or GDM, and its effects on the wellness of the mother and fetus has provided significant understanding into the intricate relationship between the microbial composition of the stomach and gestational diabetes. A thorough knowledge of the changes in microbiota throughout pregnancy and their possible role in the development of GDM has been made possible by the use of bioinformatics techniques. The results of this study show that, in comparison to healthy controls, women with gestational

diabetes had notable changes in their gut and vaginal flora. These modifications point to a possible link between the emergence of GDM and microbial dysbiosis. Targeted therapies to modify the microbiome and maybe reduce the risk of gestational diabetes may be made possible by identifying certain microbial taxa that are either abundant or deficient in GDM patients. The study also emphasizes how crucial it is to take into account the vaginal and intestinal microbiota when figuring out the whole microbial landscape during pregnancy. The way these microbial communities interact may have a systemic impact on immune response and metabolism in mothers, which might affect how quickly GDM progresses and how it affects the health of the fetus. This study's application of bioinformatics has made it easier to comprehend complicated metagenomic data, leading to a more sophisticated knowledge of the microbial alterations connected to GDM. Future research opportunities, such as discovering microbial biomarkers for GDM risk assessment and investigating potential therapeutic treatments targeting the microbiome, have been made possible by the incorporation of computational approaches. The study's overall findings highlight the worldwide health threat that gestational diabetes mellitus poses and the necessity of a comprehensive strategy that takes the microbiome into account as a key component of maternal and fetal health. Subsequent research endeavors ought to scrutinize the correlation between microbial dysbiosis and gestational diabetes (GDM) with greater rigor, as this would facilitate the creation of innovative strategies for the management and prevention of the illness and improve outcomes for mothers and their offspring.

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References

1. Farhat S, Hemmatabadi M, Ejtahed HS, Shirzad N, & Larijani B. 2022. Microbiome alterations in women with gestational diabetes mellitus and their offspring: A systematic review. Frontiers in Endocrinology, 13, 1060488. https://doi.org/10.3389/fendo.2022.1060488

2. Ferrocino I, Ponzo V, Gambino R, Zarovska A, Leone F, Monzeglio C, *et al.* (2018). Changes in the gut microbiota composition during pregnancy in patients with gestational diabetes mellitus (GDM). Scientific Reports, 8(1), Article 1. https://doi.org/10.1038/s41598-018-30735-9

3. Hu P, Chen X, Chu X, Fan M, Ye Y, Wang Y, Han M, Yang X, *et al.* 2021. Association of gut microbiota during early pregnancy with risk of incident gestational diabetes mellitus. The Journal of Clinical Endocrinology and Metabolism, 106(10), e4128–e4141. https://doi.org/10.1210/clinem/dgab346

4. Kuang YS, Lu JH, Li SH, Li JH, Yuan *et al.* Connections between the human gut microbiome and gestational diabetes mellitus. GigaScience, 2017; 6(8), 1-12. https://doi.org/10.1093/gigascience/gix058

5. Li M, Zhang G, Cui L, Zhang L, Zhou Q, Mu C, Chi R, Zhang N, & Ma G. 2023. Dynamic changes in gut microbiota during pregnancy among Chinese women and influencing factors: A prospective cohort study. Frontiers in Microbiology, 14. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2023.1114228

6. Marleen, S., Kodithuwakku, W., Nandasena, R., Mohideen, S., Allotey, J., Fernández-García, S., Gaetano-Gil, A., Ruiz-Calvo, G., Aquilina, J., Khalil, A., Bhide, P., Zamora, J., & Thangaratinam, S. (2024). Maternal and perinatal outcomes in twin pregnancies following assisted reproduction: A systematic review and meta-analysis involving 802,462 pregnancies. Human Reproduction Update, 30(3), 309–322. https://doi.org/10.1093/humupd/dmae002

7. Mercado-Evans V, Mejia ME, Zulk JJ, Ottinger S, Hameed ZA, Serchejian C, *et al.* 2024. Gestational diabetes augments group B Streptococcus infection by disrupting maternal immunity and the vaginal microbiota. Nature Communications, 15(1), 1035. https://doi.org/10.1038/s41467-024-45336-6

8. Minarti, M., Triananinsi, N., Nurqalbi, N., Sumarni, S., & Kamaruddin, M. (2020). Metagenomic diversity of gut microbiota of gestational diabetes mellitus of pregnant women. Biomedika, 13(1), Article 1. https://doi.org/10.31001/biomedika.v13i1.747

9. Ponzo, V., Fedele, D., Goitre, I., Leone, F., Lezo, A., Monzeglio, C., Finocchiaro, C., Ghigo, E., & Bo, S. (2019). Diet-gut microbiota interactions and gestational diabetes mellitus (GDM). Nutrients, 11(2), Article 2. https://doi.org/10.3390/nu11020330

10. Zheng W, Huang Y, Li D, Hu D, Jin C, Sadykova A, Cai W, Liao C, & Pan S. Amniotic fluid and vaginal microbiota in pregnant women with gestational diabetes mellitus by metagenomics. Medicine in Microecology, 2022; 15, 100074. https://doi.org/10.1016/j.medmic.2022.100074

Chapter - 4
Synthetic Seed Technology: Current Views

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Chapter - 4

Synthetic Seed Technology: Current Views

Sakshi Shaw and Priyankar Pal

Abstract

Artificially encapsulated somatic embryos, shoots, or any other vegetative propagules suitable for seed production are known as synthetic seeds. They serve as a substitute for conventional seeds in the propagation of plants. Plant micro propagules, such as somatic embryos, shoot buds/shoot tips, calli, nodal segments, embryogenic masses, protocorms, and protocorm-like bodies, are encapsulated with particular coating materials to create synthetic seeds. With this technology, plant cells are isolated from their parent plant, grown in tissue culture to form embryos, covered in a protective gel that contains nutrients and growth hormones, and the artificial seeds are subsequently planted. The encapsulated plant tissues receive protection and nourishment from the outer coating matrix. The best protective covering currently on the market is calcium alginate gel because it promotes capsule formation and gives alginate beads enough firmness to withstand mechanical damage to propagules. These instruments offer procedures for the in vitro and in vivo production of artificial seeds intended for transformation into plantlets. Elite agricultural and endangered medicinal plant species are difficult to reproduce from natural seeds or conventional methods. This technology can help conserve and multiply these species. Many economically significant plant species, including vegetable crops, forage legumes, industrially significant crops, cereals, spices, plantation crops, fruit crops, ornamental plants, orchids, medicinal plants, and forest trees that yield wood, were the subjects of the development of synthetic seed technology.

Keywords: Synthetic seeds, in vitro, in vivo plant propagation.

Introduction

Synthetic seeds are artificially encapsulated plant propagation material. This material could be somatic embryos, shoot buds, cell aggregates, or any other tissue that we can use as a seed for propagation. Synthetic seed technology primarily involves encapsulating somatic embryos in a protective

coating. These seeds have the potential to develop into a whole plant in vitro as well as under ex vitro conditions. They retain this potential even after storage (Bajaj, 1995). The first synthetic seeds were produced by Kitto and Janick in 1982 using carrot. Nowadays, synthetic seed technology is one of the most important tools to breeders and scientists of plant tissue culture. It has offered powerful advantages for large scale mass propagation of elite plant species (Pierik, 1984).

The aim and scope for switching towards artificial seed technology was for the fact that the cost-effective mass propagation of elite plant genotypes will be promoted. There would also be a channel for new transgenic plants produced through biotechnological techniques to be transferred directly to the greenhouse or field. The artificial seed technology has been applied to a number of plant species belonging to angiosperms (Nongdam, 2016). Present review aimed to give a brief description of methodology involved in synseed preparation, types of synthetic seeds, species in which this technique has been developed successfully.

History

It is difficult to pinpoint where the concept about an artificial seed first emerged. Certainly, individuals responsible for the initial somatic embryo production may have thought of this use (Reinert, 1959; Steward *et al*., 1958). F. C. Steward (USA) and J. Reinert almost simultaneously discovered somatic embryogenesis in carrots in 1958 (Germany). Distinguished plant physiologist F. C. Steward works at Cornell New York University. However, the idea of employing somatic embryos as a viable propagation mechanism for seed-sown crops did not start to be put out until the early 1970s. Using somatic embryos, (Drew, 1980) actively developed techniques for commercial crop propagation. He recommended using a fluid drilling device to transfer carrot somatic embryos; however, he was only able to grow three units from carrot embryos in a medium devoid of carbohydrates. Through this approach, he was unable to produce numerous plants. He discovered the extremely sluggish rate of growth of plantlets obtained from culture, which was a critical issue. Dumplings of carrot embryos, roots and Cellus were coated with oligoethylene by (Kitto & Janick, 1985). Some embryos made it through the coating and desiccation steps. Since somatic embryogenesis is a relatively new field of study in rice, there is a lot of potential for the widespread dissemination of better, elite hybrid (Brar & khush, 1994).

Technology

The primary barrier to the development of synthetic seeds was that somatic embryos lacked crucial auxiliary tissues like endosperm and

protective coverings, which made them difficult to handle and store (Redenbaugh, 1993). Additionally, they are typically thought to lack a silent resting phase and be immune to dehydration. In order to use somatic embryos like a group for clonal plants, the main objective behind synthetic seed development was to create somatic embryos that more nearly mimic seed embryos in storage and handling properties. Germplasm preservation and propagation. Encapsulation technology has advanced as the first significant step in the manufacturing of synthetic seeds in order to achieve this aim. Later, it was believed that the synthetic seeds with encapsulation should also include growth nutrients, microbes that promote plant growth (such mycorrhizae), as well as other biological elements required for the best possible transition from embryo to plant. Another crucial element in the manufacture of synthetic seeds is the selecting of coating material. Two kinds of somatic embryos are known as desiccated and hydrated seeds based on the techniques developed thus far. The somatic embryos used to create the dehydrated synthetic seeds are either left naked or encased in polyethylene glycol before being dried. Desiccation can be accomplished quickly by releasing the pier dishes and letting them on the work bench overnight to dry, or slowly over the course of one or two weeks progressively employing chambers with decreasing relative humidity. Only species of plants whose somatic embryos are desiccation-tolerant are capable of producing these kinds of synthetic seeds. On the other hand, plants that have somatic embryos that are resistant to desiccation and are susceptible to it generate hydrated synthetic seeds. By placing somatic embryos in hydrogel capsules, synthetic seeds that have been hydrated are created.

Synthetic seed production:

Production of synthetic seeds by encapsulating somatic embryos has been reported in few species. One prerequisite for the application of synthetic seed technology in micropropagation is the production of high-quality, vigorous somatic embryos that can produce plants with frequencies comparable to natural seeds. Inability to recover such embryos is often a major limitation in the development of synthetic seeds. Synthetic seed technology requires the inexpensive production oflarge numbers of high quality somatic embryos with synchronous maturation. The overall quality of the somatic embryos is critical for achieving high conversion frequencies. Encapsulation and coating systems, though important for delivery of somatic embryos, are not the limiting factors for development of synthetic seeds. At present, the characteristic lack of developmental synchrony in embryogenic systems stymies multi-step procedures for guiding somatic embryos through maturation. The lack of synchrony of somatic embryos is, arguably, the single

most important hurdle to be overcome before advances leading to widespread commercialization of synthetic seeds can occur. Synchronized embryoid development is required for the efficient production of synthetic seed.

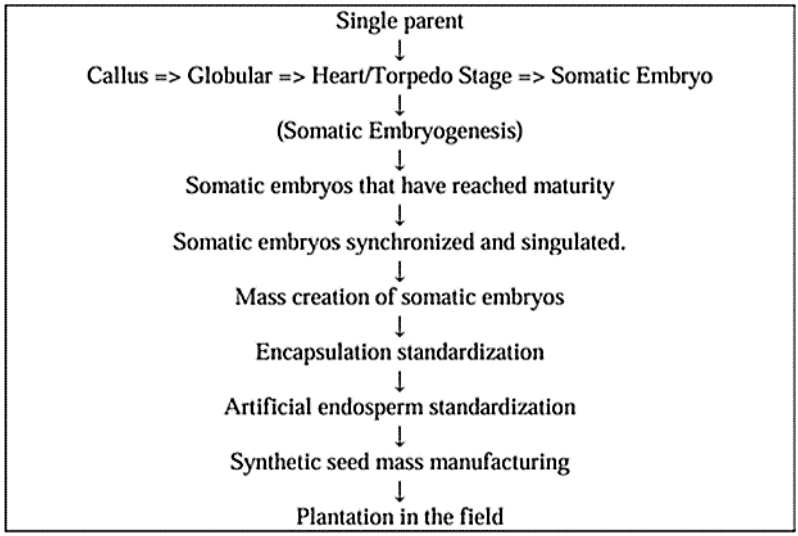

Fig: Production of synthetic seed.

Types of gelling agents used for encapsulation

Several gels like agar, alginate, carboxy methyl cellulose, carrageenan, gelrite, guargum, sodium pectate, tragacanth gum, etc. were tested for synthetic seed production, out of which alginate encapsulation was found to be more suitable and practicable for synthetic seed production. Alginate hydrogel is frequently selected as a matrix for synthetic seed because of its moderate viscosity and low spinnability of solution, low toxicity for somatic embryos and quick gellation, low cost and bio-compatibility characteristics. The use of agar as gel matrix was deliberately avoided as it is considered inferior to alginate with respect to long term storage. Alginate was chosen because it enhances capsule formation and also the rigidity of alginate beads provides better protection (than agar) to the encased somatic embryos against mechanical injury.

Fig: Synthetic seed production by alginate encapsulation.

Types of synthetic seeds

i) **Desiccated:** The desiccated synthetic seeds are produced from somatic embryos either naked or encapsulated in polyoxyethylene glycol (Polyoxr) followed by their desiccation. Desiccation can be achieved either slowly over a period of one or two weeks sequentially using chambers of decreasing relative humidity, or rapidly by unsealing the petri-dishes and leaving them on the bench overnight to dry. Such types of synseeds are produced only in plant species whose somatic embryos are desiccation tolerant.

ii) **Hydrated:** The hydrated synthetic seeds are produced in those plant species where the somatic embryos are recalcitrant and sensitive to desiccation. Hydrated synthetic seeds are produced by encapsulating the somatic embryos in hydrogel capsules.

Structure of synthetic seed:

A typical synthetic seed contains three components namely; explant material or plant propagules (somatic embryos or bud, shoot or any other active meristematic tissue), somatic embryos are bipolar structures with apical and basal meristematic regions that can produce shoots and roots, respectively. The second component, the matrix, is a gelling material that encases plant propagules and includes nutrients, plant growth regulators, anti-pathogenic compounds, bio-controllers, and bio-fertilizers similar to endosperm in the ordinary seed. The third component, the seed shells, are artificial seed coats made from a complicated mixture of alginate and gelatin that is utilized to build the encapsulation coat system that resembles the outer covering of conventional seed that is known as testa.

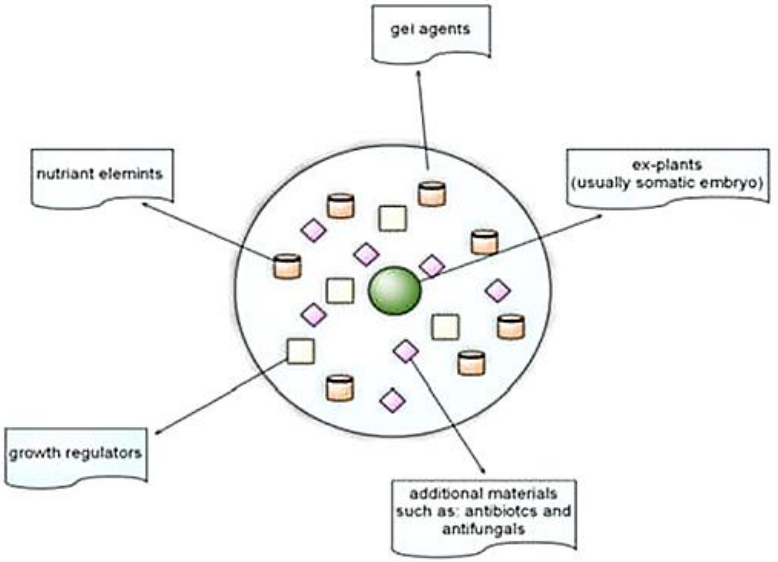

Fig: Structure of synthetic seed.

Application

Synthetic seeds are employed in biotechnology to cultivate a variety of important plant species, and they are considered as a promising method for propagating plants that don not produce viable seeds, such as orchids and some medicinal plants. They have various advantages, including inexpensive manufacturing costs, convenience of handling and the possibility of long-term storage. They also possess the ability to produce superior and clonal plants in the same way that real seeds do, preserving rare plant species and thus increasing biodiversity, producing uniform plantlets, continuous mass propagation in less time and season independent production of identical plantlets, and more consistent and synchronized harvesting critical crops. Furthermore, this strategy allows for ex situ conservation of a gene bank of elite material as well as easy transit of germplasm between countries. It enables the storage of plants that are genetically heterozygous or have a single outstanding combination of genes that would be impossible to sustain using traditional seed production procedures. It can also be utilized to mass-produce cost-effective hybrid seeds in autogamous species (such as barley, wheat, and oats) using artificial seed multiplication.

Cereals

The application of synthetic seed technology to the cereals started from the year 1989. Most of investigations were carried out to increase their yield

and vigor. Artificial seeds are playing a major role in increasing the genetically transformed plant material and haploid plant production. George and Eapen (1995) reported the encapsulation of somatic embryos in *Eleusine coracana*. Suprasanna *et al*. (1996) showed that the encapsulation of somatic embryos and conversion into plantlets of Oryza sativa. Suprasanna *et al*. (2002) studied the viability of encapsulated embryos derived from five year old long term culture of *Oryza sativa*. They also studied the conversion of synthetic seeds into seedlings in hybrid rice and reported that the application of self-breaking gel beads technology increased the germination (52%) and conversion (47%) of synthetic seeds. Roy and Mandal (2008) reported the development of synthetic seeds involving androgenic and pro-embryos in elite *Oryza sativa*. Model systems for synchronous somatic embryo production combined with encapsulation to form synthetic seeds were studied in *Zea mays*.

Vegetables

Production of artificial seeds for different vegetables, were started at different time from different part of the plant. The production of synthetic seeds was by the encapsulation of multiple carrot somatic embryos (Kitto and Janick, 1982). Encapsulation of different explants (somatic embryos, plantlets, cell aggregates from hairy roots), conservation of root regeneration potential of cell aggregates in coated capsules even after stored at 25°C up to 60 days and plant regeneration from them were observed. Encapsulation of somatic embryos, nodal segments, shoot tips and cell suspension cultures and estimation of yield and canopy of field cultivated plants derived from synthetic seeds of *Solanum tuberosum* (Nyende *et al*., 2005) were investigated.

Ornamental plants

In ornamental plants and orchids, the synthetic seeds have very much commercial importance, because of their minute seed size and presence of reduced endosperm in seeds (Lambardi *et al*., 2006). Ruffoni *et al*. (1994) produced synthetic seeds of somatic embryos in two ornamental species (*Eustoma grandiflorum* and *Genista monosperma*). Piccioni and Standardi (1995) produced synthetic seeds of shoot tips in Betula pendula and bulbs in *Lilium longiflorum* (birch).

Spices and plantation crops

The germination capacity and survival rate of artificial *Coriandrum sativum* seeds were 82% and 83% respectively (Chen *et al*. 1991). Artificial seeds of *Coriandrum sativum* were produced by using somatic embryos derived from hypocotyl explants (Jayabalan, 2000). Production of disease-free encapsulated shoot buds of *Zingiber officinale* and their conversion into

plantlets was reported by Sharma *et al*. (1994). Cold storage of shoot cultures and alginate encapsulation of shoot tips of *Camellia japonica*, *Citrus reticulata* and propagation of tea (*C. sinensis*) by shoot proliferation of alginate encapsulated nodal explants stored at 4°C were reported (Mondal *et al*. 2002). Induction of somatic embryogenesis, production of synthetic seeds and 70% of germination in *Elaeis guineensis* was studied by Mariani *et al*. (2008).

Medicinal plants

Naturally most of the important medicinal plants are rare, endangered and endemic category. It is due to the low fruit and seed formation, poor germination capacity of seeds and due to the many other environmental conditions such as habitat modification, urbanization, climatic change and pollution etc. So, it is important to propagate and conserve these plant species. The production of synthetic seeds by encapsulating somatic embryos and vegetative propagules is rapidly becoming an applied technique with potential for mass propagation of medicinal plant species.

Limitation

The development of highly valued micopropagules on a large scale, at a low cost per culture unit, that are appropriate for encapsulation in sodium alginate medium, is the primary prerequisite for an effective artificial seed production process. The micropropagation method is still one of the main barriers to the advancement of artificial seed technology, even if the design of such systems has been accomplished in several species of plants, such as cauliflower (H. Z. Rihan *et al*., 2012a, 2017b). Despite the use of somatic embryos for artificial seed generation in a variety of plant species (H. Rihan *et al*., 2013) has been widely documented, there are still some significant problems that must be resolved to increase the effectiveness of these methods. The benefits of artificial seed technology are offset by drawbacks like storage restrictions brought on by dormancy deficiency, synchronic defect in somatic embryo development, improper maturation, low rates of conversion into plantlets, production restrictions on viable mature somatic embryos, and decreased viability and plant recovery when artificial seeds are stored at low temperatures (Andrzejewska-Golec & Makowczyńska, 2011). The idea of employing non-embryogenic propagules as artificial seed generation was examined in several species of plants and found to be a promising option as a propagation approach in species that are resistant to somatic embryogenesis. However, there were some challenges along the road, including getting non-embryogenic artificial seeds to take one rooting step (Hung & Trueman,

2011). One of the primary drawbacks of using this technology practically is thought to be the challenges involved in directly planting fake seeds in non-sterile soil or commercial substrates like compost, vermiculite, etc.

Advantages of synthetic seeds:

- This method is for large-scale productions.
- It maintains genetic uniformity for a high number of generations. Most plant tissue culture methods fail to maintain genetic uniformity for longer durations.
- According to the literature, the costs of producing a plant using this technology is low.
- It facilitates the rapid multiplication of plants.
- One of the biggest merits of this method is the direct delivery of plant parts (protected with viable coating) to the field.
- These seeds have the potential for short and medium-term storage without losing viability.
- As compared to plantlets, it is easy to handle and transport synthetic seeds.

Disadvantages of synthetic seeds

- Somatic embryos have low survival rates for most plant species, which also limits the value of synthetic seeds.
- There are not many protocols available to produce propagules from different plant parts using plant tissue culture methods. Hence less useful material is available for producing synthetic seeds.
- In some cases, inefficient maturation of somatic embryos leads to poor germination and hence poor growth and development.
- According to scientists, somatic embryos from some plant species are not capable of germinating out of the capsule or coating. Hence, they are not able to form normal plants rapidly.
- The concentration of coating material is also a limiting factor for producing synthetic seeds. It should have nutrient supplementing materials for facilitating germination and growth.
- When the shape of synthetic seeds is not matching the farm machinery then it is hard to use them for transplantation. Hence, seeds should be transplantable.
- One of the major problems these seeds face is quick drying out of

capsules. We need to store them in a humid environment and coat them with hydrophobic materials to prevent drying.

Conclusion

Artificial seeds were produced successfully from encapsulated plant propagules in different plant species. Procedures were optimized and proper plantlets were obtained. This technique has great advantages such as: a cost-effective delivery system, minimization of the cost of plantlets, simple methodology with high potential for mass production, a promising technique for the direct use of artificial seedlings in vivo, and a high storage capacity. The advances of this technique depend on the plant species in the first step. However, despite the advantages of artificial seeds, further research is required in order to improve root formation of non-embryogenic artificial seeds. More investigations are needed to improve the capacity of artificial seed cultivation in commercial substrates and under non-sterilized conditions. This could be improved by the use of suitable types and concentrations of anti-diseases and antibiotics, and further detailed research is needed for improvement in the artificial seed cryopreservation capacity in some plant species.

Reference

1. Bajaj YPS. 1995. Somatic Embryogenesis and Its Applications for Crop Improvement. Somatic Embryogenesis and Synthetic Seed I, 105–125.
2. Bhojwani SS. and Razdan M. K. (1983). Plant Tissue Culture: Theory and Practice. Elsevier publications. Faisal, M., & Alatar, A. A. (Eds.).(2019).
3. Synthetic Seeds. DOI: 10.1007/978-3-030-24631-0 Hussein S, Ibrahim R., Ling Pick Kiong, A. (2006).
4. Somatic Embryogenesis: An Alternative Method for in vitro Micropropagation. Iranian Journal of Biotechnology, 4(3), 156-161. Zimmerman Lynn J. (1993).
5. Somatic Embryogenesis: A Model for Early Development in Higher Plants. The Plant Cell, 5, 1411-1423.
6. Bewley, J. D. and Black, M. 1985. Seeds: Physiology of Development and Germination, Plenum Press, New York, 367.
7. Saiprasad G.V.S. Artificial Seeds and their applications. Resonance. 2001; 6:39-47.
8. Nongdam, P. 2016. Development of synthetic seed technology in plants and its applications: A Review. Int. J. Curr .Sci. 19(4): E 86-101.

9. Gray DJ, and Purohit A. Critical Review. Plant Science. 1991; 10: 33-61.
10. Roy A, and Mandal B. Development of synthetic seeds involving androgenic and proembryos in elite indica. Indian J. Biotech. 2008; 7(4):515-519.
11. Steward, F.C., Mapes, M.O., and K. Mears. 1958. Growth and organized development of cultures cells. Il. Organization in cultures grown from freely suspended cells. Am. J. Bot. 45: 705–708.
12. Bajaj YPS. Somatic Embryogenesis and Synthetic Seed. In: Biotechnology in Agriculture and Forestry. Vol. 30. Springer-Verlag, Berlin. 1995.
13. Cyr DR. Seed Substitutes from the Laboratory. In: Seed Technology and its Biological Basis. Black, M. and Bewley, J.D. (Eds.). Sheffield Acad. Press, Sheffield, 2000; pp: 326-372.
14. Kitto SK, and Janick, J. Polyox as an artificial seed coat for asexual embryos. Hort. Sci. 1982; 17: 488-490.
15. Redenbaugh, K., Nichol, J., Kossler, M. E, and Paasch, B. D. 1984. Encapsulation of somatic embryos for artificial seed production. In Vitro Cell Dev. Biol. Plant. 20: 256-257.
16. Sahoo, S. L., Rout, J. R., and Satyajit, K. S. 2012. Synthetic Seed. In: Plant Tissue Culture: Toptipotency to Transgenic, Ed; Sharma H. P., Dogra J. V. V., Misra A. N. Agrobios, India.
17. Magray MM, Wani KP, Chatto MA. & Ummyiah HM. Synthetic Seed technology. Int. J. Curr. Microbiol. App. Sci. 2017; 6(11): 662-674.
18. Cartes, P. R., Castellanos, H. B., Ríos, D. L., Sáez, K. C., and Spierccolli, S. H. 2009. Encapsulated somatic embryos and zygotic embryos for obtaining artificial seeds of rauli-beech [Nothofagus alpina (Poepp. & Endl.) Oerst.]. Chil. J Agric. Res. 69:112-118.
19. Bhojwani, S.S. and Razdan, M.K. (2004) Plant tissue culture: Theory and practice. Revised Edition, Elsevier Publication, Amsterdam.
20. K Redenbaugh, (ed.), Synthetic seeds, CRC Press, Boca Raton, 1993.
21. J A Fujii, D T Slade, K Redenbaugh, and K A Walker, Artificial seeds for plant propagation, Trends in Biotechnology, 5, 335-339,1987.
22. Kinoshita, The production and use of artificial seed, Research Journal of Food and Agriculture, 15 (3), 6-11, 1992.
23. T Senaratna, Artificial seeds, Biotechnology Adf) Qnces-, 10, 379-392, 1992.

Chapter - 5
Detrimental Effect of Maternal Smoking during Gestation on Offspring

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Chapter - 5

Detrimental Effect of Maternal Smoking during Gestation on Offspring

Priti Nandi and Rupesh Dutta Banik

Abstract

In the 20th century, smoking cigarettes was a trend in both sexes. According to WHO, around 1.3 billion people smoke cigarettes where 36.7% of males and 7.8% of females. One of the biggest concerns for public health remains to be maternal smoking during pregnancy. Approximately 9.5% of pregnant women smoke in 2020–21. The prevalence of tobacco smoking is increasing in middle-class and low-income countries, and it is typically associated with lower income levels and lower educational attainment. Maternal smoking is associated with significant risks of short- and long-term sickness and death for both the mother and the child, despite being one of the most harmful risk factors during pregnancy. Among the many harmful substances found in cigarettes, nicotine is one of the main ingredients. Smoking before and after pregnancy has been related to several unfavorable pregnancy outcomes. Through a variety of distinct pathways, nicotine poses a significant risk to growing fetuses, and the risks worsen later in the gestational period during exposure. Smoking cigarettes during pregnancy throws the oxidant and antioxidant systems out of balance, which also negatively affects the mother's and the fetus's genetic and cellular health and increases the risk of several disorders in the newborn child including cleft lip, low birth weight, premature lung development, preterm delivery, asthma and most importantly leukemia also. There was a strong correlation between the rate of premature births and the women's nicotine consumption. This review article concludes by discussing the detrimental impacts of mother smoking on the prenatal and neonatal stages of development and their subsequent results.

Keywords: Maternal smoking, pregnant women, nicotine, leukemia, prenatal and neonatal development

Introduction

We live in a lifestyle where smoking cigarettes is the norm. Worldwide, the number of women who smoke and use tobacco products is rising, while

the number of males who smoke is gradually declining. Disease is becoming a double-edged sword in India, with non-communicable diseases (NCDs) bearing an increasing share of the cost. One of the main behavioral risk factors for NCDs, tobacco smoking is linked to several chronic illnesses, such as cancer, lung conditions, and cardiovascular disorders (Mittal 2019). According to data released by the World Health Organization (WHO), tobacco use accounts for one out of every ten fatalities globally and results in 8.7 million deaths annually, reported in 2023. Approximately 80 percent of the 1.3 billion are tobacco smokers, in 2020. Women are now more likely than ever to smoke cigarettes, which is a global public health risk (Jafari *et al*. 2021). When compared to other expectant mothers, women who smoked during their pregnancy had higher rates of nicotine addiction, a partner who also smoked, and a lower level of socioeconomic status. These factors are linked to additional negative outcomes for both the mother and the infant (Nakamura *et al*. 2021). Maternal smoking during pregnancy causes several types of disease such as low birth weight, preterm birth, sudden unexpected infant death, premature lung aging, miscarriage, reduced fetal size, asthma, orofacial clefts (cleft lip or cleft palate), craniosynostosis (fused skull bones that may affect brain growth), cardiovascular defect, limb reduction defects, higher rates of prenatal and neonatal deaths, neurodevelopment disorder, etc. (Jafari *et al*. 2021, Anderson *et al*. 2019, Chattrapiban *et al*. 2020, Mittal 2019). The act of cigarette smoking has a profoundly detrimental effect on the cellular and genetic makeup of both the mother and the developing baby (Diabelková *et al*. 2022). About 4000 chemicals, including nitrosamines, heavy metals like lead, cadmium (Cd), and cobalt, alkaloids (nicotine), nitrosamine, acetaldehyde, benzene, polycyclic aromatic hydrocarbons (PAH), and aromatic amines are among the many types of chemicals known to be toxic found in cigarette smoke. These chemicals have different characteristics and effects on human health (Dechanet *et al*. 2011). This review study discusses the detrimental impacts that smoking by a mother during pregnancy can have on both the fetus and the unborn child. Pregnant women should be urged to quit smoking, or at least cut back, due to the high prevalence of morbidity among premature newborns and the significant financial burden on the healthcare system (Günther *et al*. 2021).

Materials and Methods

A global search of published research papers and articles of review on the detrimental effect of maternal smoking on fetuses turned up pertinent information for this review study. Other searches included PubMed, Google Scholar, PubMed Central, and released research publications. Suggested exposure claims were not taken into consideration, and only publicly

accessible data were used. Using information from reliable sources serves as one of these participation requirements for the topic. English was the only language included in the study.

Result and Discussion

Smoking prevalence in women: Women are likely to pay attention to smoking cigarettes. Due to pressure, lots of mother smoke during pregnancy. Currently, 10.1% of women smoked cigarettes, in 2021. Nowadays maternal smoking has increased. 7.1% of pregnant women smoke, compared to 9.4% of prenatal smokers (Kondracki 2019). In the three months prior to becoming pregnant, 11.5% of moms smoked, and 8.9% did so throughout their gestation in 2011 (**Figure 1**) (Anderson *et al*. 2019). After knowing pregnancy many mother smokers are reduced smoking in the third trimester compared to other trimesters. Numerous businesses have altered the societal definition of women's smoking, lifted cultural restrictions to lessen the pressure that comes with smoking, and increased the percentage of women who smoke (Jafari *et al*. 2021).

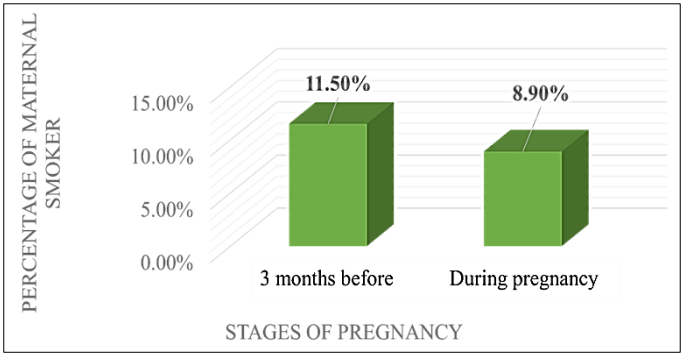

Fig 1: A column chart of rate of maternal smoking (Anderson *et al*. 2019).

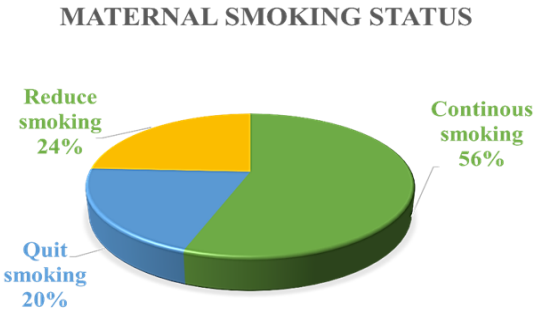

Fig 2: A pie chart of the percentage of smoking stages in pregnant women (Anderson *et al*. 2019).

Mainly pregnant women quit smoking after the third trimester, but the amount is much less. After the third trimester, 55% of pregnant women are still smoking continuously **(Figure 2)** (Anderson *et al*. 2019). Several factors force forcing to be involved in cigarettes such as teen pregnancy, less educative, living with another smoker, low income, unmarried, and depressive mental health. Those factors increase the chance of smoking during pregnancy (McEvoy & Spindel 2017). In low- and middle-income nations, tobacco smoking is on the rise and is typically associated with these demographics' low levels of education and income. The mean age at which tobacco usage began was 17.8 years, while 25.8% of females began using tobacco before the age of 15 (Mittal 2019). The most probable group of ages of women to smoke while pregnant was 20–24, followed by 15–19 and 25–29-year-olds as well (**Table 1**). The lowest percentage of pregnant women who smoked was seen in those over 45 (2%) and under 15 (2.5%) (*American Academy of Family Physicians, 2018).*

Table 1: Percentag of pregnant women smoked during their pregnancy according to their ages *(*American Academy of Family Physicians, 2018).

Age of pregnant women	Percentage of pregnant women smoked (%)
<15	2.5
15-19	8.5
20-24	10.7
25-29	8.2
>29	2

Chemical composition and impacts of tobacco smoke: Tobacco smoke contains a complicated mixture of compounds that includes nicotine, phenol, carbon monoxide, hydrogen cyanide, benzene, formaldehyde, and tobacco-specific nitrosamines (TSNAs) (**Table 2**) (Engstrom *et al*. 2003). Women in our nation, particularly those with lower socioeconomic standing and limited literacy, also choose hand-rolled "bidis". In addition to making quitting smoking more challenging, menthol cigarettes raise the risk and severity of dependency on nicotine among new smokers (Mittal 2019). Those toxic components have carcinogenic effects, reproductive damage, and genotoxic (Agraval & Chu 2022).

Table 2: Components of cigarettes and their effects (Agraval & Chu 2022).

Components of cigarette	Effects
Nicotine	Genotoxic effect, reduce rapid eye movement (REM)
Polycyclic aromatic hydrocarbon (PAHs)	Reproductive system damage, mutagenic and carcinogenic effect

Benzene	Carcinogenic effect, reproductive damage, bone marrow damage, anemia
Acrolein	Carcinogenic and mutagenic effect
N-nitrosamines	Reproductive damage and carcinogenic effect

Effects of maternal smoking on prenatal and neonatal period: One of the main risk factors that may be changed is smoking, which causes many early deaths and high expenditures to healthcare systems (Diabelková *et al*. 2022). During the first trimester, the placenta undergoes morphological changes, the thickness of the basement membrane, and a rise in tissue. Additionally, reduced vascularization leads to a reduction in nutritional supply (Zacharasiewicz 2016). The primary factor reducing the rate of fetal growth in smokers is intrauterine hypoxia, or the absence of oxygen reaching the bodily tissues within the uterus. The hypoxia may be brought on by smoking-related variables such as elevated blood levels of carbon monoxide, reduced circulation, and decreased respiratory enzymes (Diabelková *et al*. 2022). Reactive oxygen species (ROS) and free radicals are two of them that have the ability to oxidatively damage DNA, proteins, enzymes, and lipids found in cellular membranes. An aberrant placental capillary network and its activities may be facilitated by an excess of reactive oxygen species (ROS), which would compromise the growing fetus's ability to receive nutrients and oxygen (Pizent *et al*. 2020). The weight difference between the offspring of non-smoking mothers and those whose mothers smoked during their pregnancies was determined to be around 170–377 g (Abbott & Winzer-Serhan 2012) (**Figure 3**). There is a higher risk of premature delivery before <37 weeks gestation, according to most research evaluating prenatal cigarette smoking and gestation in birth (Froggatt *et al*. 2020).

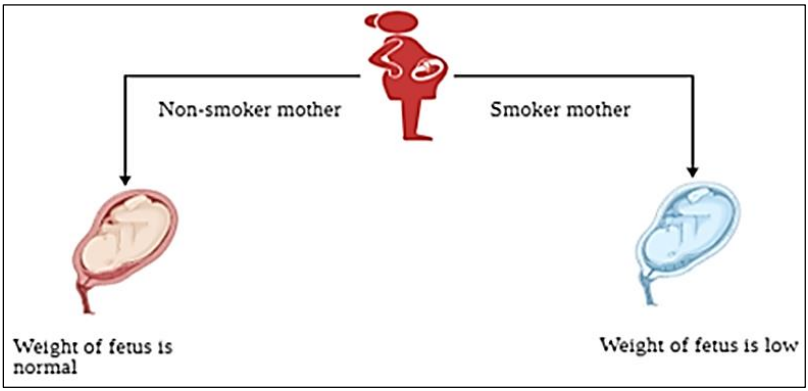

Fig 3: Comparison of fetus birth-weight (Abbott & Winzer-Serhan 2012).

Exposing to CO is correlated with low birth weight because CO binding to hemoglobin decreases blood flow, which in turn causes growth restriction (Froggatt *et al*. 2020). In addition to degenerative effects, cigarette smoke causes the placenta to age prematurely. The latter have thicker sub-trophoblastic foundation membranes, less vascularization, and a lot of collagens in the chorionic villi (Günther *et al*. 2021). Nicotine exposure during pregnancy is known to raise the incidence of orofacial clefting; its impact on the likelihood of other significant congenital abnormalities is less evident (Holbrook 2016). Additionally, smoking may modify the endometrium, which may complicate implantation. It has been determined that 55% of infants with Down syndrome who have cryptorchidism were born to pregnant mothers who smoked (Mittal 2019). Pregnant women who smoked throughout their pregnancies typically have children with weakened immune systems and decreased lung function, which increases their risk of respiratory illnesses (McEvoy & Spindel 2017). Impairment in children's neural development is connected with mother tobacco usage during pregnancy with a greater chance of attention-deficit hyperactivity disorder (ADHD), behavioral and disciplinary disorders, as well as challenges with memory, academic, and learning success (Nakamura *et al*. 2021).

Possibility of leukemia: Additionally, there was a slight but imprecise correlation between the mother's smoking habits during gestation and an increased incidence of acute myeloid leukemia (AML) (Frederiksen *et al*. 2020). Although the majority of research has found null relationships, epidemiological findings regarding the relationship between mother smoking and children's AML and acute lymphoid leukemia (ALL) are not always consistent (Frederiksen *et al*. 2020). In this study, 8 genes (CDKN2A, IKZF1, PAX5, ETV6, BTG1, PAR1 region, RB1, and EBF1) were rapidly deleted which causes ALL, because of maternal smoking during gestation and breastfeeding. If a mother smokes 5 cigarettes per day that leads increase in the deletion rate of 8 genes (de Smith *et al*. 2017).

Different mechanisms on the effect of offspring: Numerous studies have demonstrated the profoundly detrimental impacts of cigarette smoking on the cellular and genetic makeup of both the mother and the child. Studies show that mothers of offspring who smoked during pregnancy had methylation alterations in a collection of genes [AHRR, Cytochrome P450 1A1 (CYP1A1) and GFI1] at birth. These genes appear to be crucial for the aryl hydrocarbon signalling system, which facilitates the removal and detoxification of tobacco smoke's harmful components (Mund *et al*. 2013). The metabolism and purification of harmful compounds from tobacco smoke

are facilitated by the enzymes GSTT1 and CYP1A1. The glutathione transferase gene is subject to structural polymorphisms or widespread deletions that raise the fetus's susceptibility to smoking by the mother and asthma risk (McEvoy & Spindel 2017). Compared to non-smoker mothers, children of smoker mothers had elevated placental CYP1A1 activity and substantial hypomethylation of placental DNA. Research suggests that the methylation of DNA based on the placenta serves as a useful surrogate for brain tissue. This is especially true for the placenta-regulated pathways of serotonin and glucocorticoids, which are critical for brain development and understanding (Nakamura *et al*. 2021). Nicotine is an alkaloid compound present in cigarette smoke, which is toxic to our bodies. Nicotine inhibits the growth and development of the lung, with abnormal lung development in the fetus. As a result of the larger alveoli, there may be fewer alveolar–bronchiolar attachment sites, which might lead to decreased alveolarization and decreased lung function (Maritz & Harding 2011). A reduction in forced respiration was seen in children exposed to prenatal nicotine during gestation day 14 and post-natal day 7, which was mediated via the α7 nAChR receptor (Nicotine acetylcholine receptor) (Zacharasiewicz 2016) (**Figure 4).** α7 nAChR receptor found in the placenta, fetal membrane, and umbilical cord by intense staining method (Alwazzan *et al*. 2020).

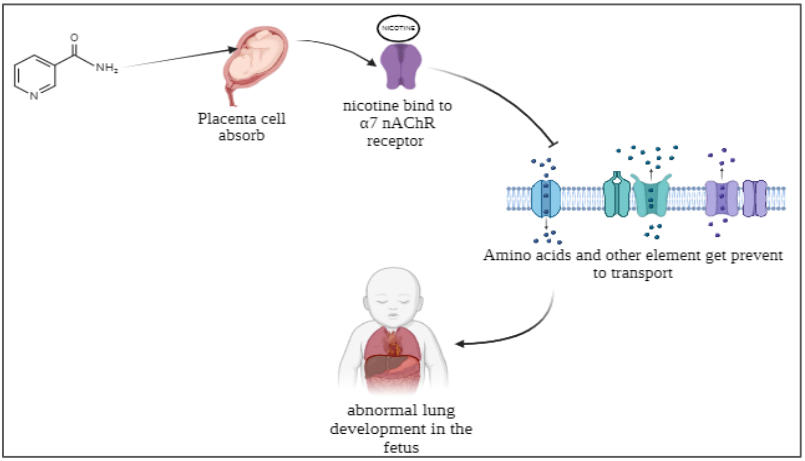

Fig 4: Mechanism of abnormal lung development in the fetus (Zacharasiewicz 2016).

Nicotine readily and rapidly passes through the placenta to enter the fetal circulation after being absorbed by the mother. By inhibiting cholinergic receptors, nicotine operates in the placenta to hinder the transfer of amino acids. Acetylcholine facilitates the route of amino acids, and inhibiting these receptors essentially stops it **(Figure 4).** Furthermore, the transfer of other

chemicals, such as amino acids, would be restricted by the hypoxia brought on by breathing in carbon monoxide while smoking cigarettes. The growth limitation linked to prenatal nicotine exposure may also be caused by a decreased availability of amino acids along with other essential substrates (Holbrook 2016).

Hemoglobulin has a higher affinity towards the Carbon monoxide (CO) instead of Oxygen (O_2). While maternal smoking during pregnancy leads to a rise in CO concentration in pregnant women. Therefore, hemoglobulin binds with CO to resist the blood flow and growth of the fetus, in the prenatal period (Froggatt *et al*. 2020) (**Figure 5**).

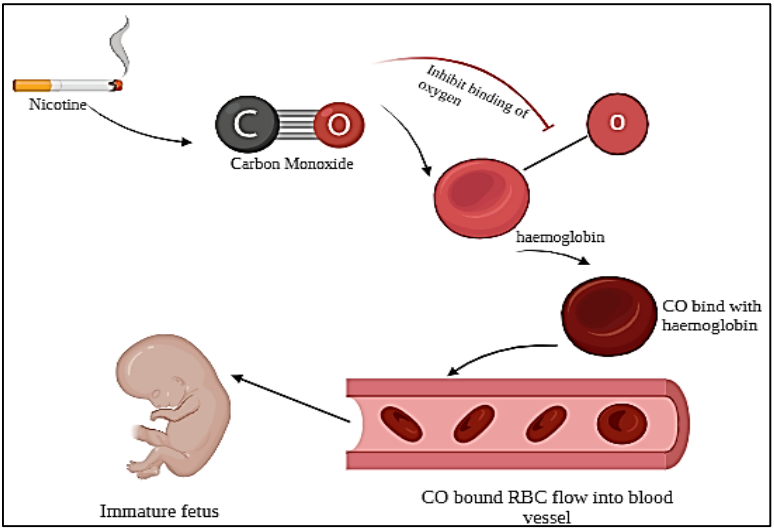

Fig 5: Immature fetus development mediated by accumulation of CO (Froggatt *et al*. 2020).

Table 3: Tabular form of case study of maternal smoking effect on their offspring based on countries.

S. No.	Country name	Smoking dose (Cig/day)	Effects on offspring	Reference
1.	United State (USA)	1-9	Fetal mortality in-utero minimum 20 week during gestation.	Salihu *et al*.,2008
2.	United Kingdom (UK)	Not mentioned	Fetal death occurring after 20 weeks of gestation	Sutan *et al*.,2010
3.	Sweden	1-9, more than 10	Following 28 weeks of pregnancy late stillbirth was shown.	Högberg & Cnattingius,2007

4.	Canada	Not mention	Stillbirth occurs.	Miller *et al*., 2010
5.	Denmark	1-9, more than 10	Preterm death of fetus and stillbirth occur.	Wisborg *et al*., 2001
6.	Australia	Not mention	Death of fetus observed more than 20 weeks of pregnancy, increase birth weight.	Moshin *et al*., 2005
7.	Japan	1-9, more than 20	Cryptorchidism, low birth weight, hypospadias occurs.	Kurahashi *et al*., 2005
8.	Brazil	1-9,10-19, more than 20	Cleft lip and cleft palate.	Leite & Koifman, 2009
9.	China	1-9	Hypoxia, heart defect, neonatal asphyxia.	Liu *et al*., 2009
10.	India	More than 10	Increased strabismus and miscarriage.	Mittal, 2019

Conclusion

In developed nations, pregnant women most frequently misuse nicotine, which comes from smoking during pregnancy. Maternal smoking has numerous effects on the fetus as well as the newborn baby. According to longitudinal research, there is minimal to nonexistent improvement from these negative impacts, and most of them, which include adverse medical consequences, low academic success, and behavioral disturbances, will be with the kid for the remainder of their lives. Despite the multitude of risks linked with it, pregnant women in an educated society frequently engage in smoking. Many research shows that when gestation goes on, nicotine exposure causes increasingly serious side effects; the most damage is done in the 3rd trimester. Therefore, this study acts as an educational tool to increase people's kindness in the hope of a better tomorrow.

Future scope: This offers an opportunity of time when expectant mothers can get help to end their pregnancies, benefiting both the gestation's prospects and the infant's long-term health. Future initiatives aimed at preventing and quitting tobacco use should prioritize improving the outcomes of pregnancies in poor middle-income nations.

Conflict of interest: There are no conflicting interests associated with the study.

Author contributions: Acquisition and interpretation of data is done by Priti Nandi. Conception, design and revising of the article are done by Rupesh Dutta Banik.

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References

1. Abbott LC, & Winzer-Serhan, U. H. (2012). Smoking during pregnancy: lessons learned from epidemiological studies and experimental studies using animal models. *Critical reviews in toxicology*, *42*(4), 279-303, DOI: 10.3109/10408444.2012.658506
2. Agraval, H., & Chu, H. W. (2022). Lung Organoids in Smoking Research: Current Advances and Future Promises. *Biomolecules*, *12*(10), 1463, DOI: 10.3390/biom12101463
3. Alwazzan, A., Mehboob, R., Gilani, S. A., Hassan, A., Perveen, S., Tanvi, I., Waseem, H., Ehsan, K., Ahmad, F. J., & Akram, J. (20202). Immunohistochemical Expression of the Alpha Nicotinic Acetylcholine Receptor 7 in the Human Normal, Diabetic, and Preeclamptic Placenta and Products of Conception. *Frontiers in Physiology*, *11*, 607239, DOI: 10.3389/fphys.2020.607239
4. *American Academy of Family Physicians*. (2018, March 6). Despite Known Dangers, Women Still Smoke During Pregnancy. https://www.aafp.org/news/health-of-the public/20180306maternalsmoking.html.
5. Anderson, T. M., Lavista Ferres, J. M., Ren, S. Y., Moon, R. Y., Goldstein, R. D., Ramirez, J. M., & Mitchell, E. A. (2019). Maternal smoking before and during pregnancy and the risk of sudden unexpected infant death. *Pediatrics*, *143*(4), DOI: 10.1542/peds.2018-3325
6. Chattrapiban, T., Smit, H. A., Wijga, A. H., Brunekreef, B., Vonk, J. M., Gehring, U., & van Rossem, L. (2020). The joint effect of maternal smoking during pregnancy and maternal pre-pregnancy overweight on infants' term birth weight. *BMC pregnancy and childbirth*, *20*(1), 1-10, DOI: 10.1186/s12884-020-2816-3
7. de Smith, A. J., Kaur, M., Gonseth, S., Endicott, A., Selvin, S., Zhang, L., Roy, R., Shao, X., Hasan, H. M., Kang, A. Y., Walsh, K. M., Dahl, G. V., McKean-Cowdin, R., Metayer, C., & Wiemels, J. L. (2017). Correlates of prenatal and early-life tobacco smoke exposure and frequency of common gene deletions in childhood acute lymphoblastic leukemia. *Cancer research*, *77*(7), 1674-1683, DOI:10.1158/0008-5472.CAN-16-2571

8. Dechanet C, Anahory T, Mathieu Daude JC, Quantin X, Reyftmann L, Hamamah S, Hedon B, & Déchaud H. Effects of cigarette smoking on reproduction. *Human reproduction update*, 2011; *17*(1), 76-95, DOI: 10.1093/humupd/dmq033

9. Diabelková J, Rimárová K, Urdzík P, Dorko E, Houžvičková A, Andraščíková Š., Kaňuková, L., Kluková, D., Drabiščák, E., Konrádyová, N., & Škrečková, G. (2022). Influence of maternal smoking during pregnancy on birth outcomes. *Cent Eur J Public Health*, *30*(S1), S32-S36, DOI:*10.21101/cejph.a6811*

10. Engstrom, P. F., Clapper, M. L., & Schnoll, R. A. (2003). Physiochemical composition of tobacco smoke. *Holland-Frei cancer medicine*, *6*.

11. Frederiksen, L. E., Erdmann, F., Wesseling, C., Winther, J. F., & Mora, A. M. (2020). Parental tobacco smoking and risk of childhood leukemia in Costa Rica: a population-based case-control study. *Environmental Research*, *180*, 108827, DOI: 10.1016/j.envres.2019.108827

12. Froggatt S, Reissland N, & Covey J. The effects of prenatal cigarette and e-cigarette exposure on infant neurobehaviour: a comparison to a control group. *EClinicalMedicine*, 2020.*28*, DOI: 10.1016/j.eclinm.2020.100602

13. Günther, V., Alkatout, I., Stein, A., Maass, N., Strauss, A., & Voigt, M. (2021). Impact of smoking and fetal gender on preterm delivery. *Journal of Developmental Origins of Health and Disease*, *12*(4), 632-637, DOI: 10.1017/S2040174420000999

14. Högberg, L., & Cnattingius, S. (2007). The influence of maternal smoking habits on the risk of subsequent stillbirth: is there a causal relation?. *BJOG: An International Journal of Obstetrics & Gynaecology*, *114*(6), 699-704, DOI: 10.1111/j.1471-0528.2007.01340.x

15. Holbrook, B. D. (2016). The effects of nicotine on human fetal development. *Birth Defects Research Part C: Embryo Today: Reviews*, *108*(2), 181-192, DOI: 10.1002/bdrc.21128

16. Jafari, A., Rajabi, A., Gholian-Aval, M., Peyman, N., Mahdizadeh, M., & Tehrani, H. (2021). National, regional, and global prevalence of cigarette smoking among women/females in the general population: a systematic review and meta-analysis. *Environmental health and preventive medicine*, *26*(1), 1-13, DOI: 10.1186/s12199-020-00924-y

17. Kondracki, A. J. (2019). Prevalence and patterns of cigarette smoking before and during early and late pregnancy according to maternal characteristics: the first national data based on the 2003 birth certificate

revision, United States, 2016. *Reproductive Health*, *16*(1), 1-11, DOI: 10.1186/s12978-019-0807-5

18. Kurahashi, N., Sata, F., Kasai, S., Shibata, T., Moriya, K., Yamada, H., Kakizaki, H., Minakami, H., Nonomura, K., & Kishi, R. (2005). Maternal genetic polymorphisms in CYP1A1, GSTM1 and GSTT1 and the risk of hypospadias. *Molecular human reproduction*, *11*(2), 93-98, DOI: 10.1093/molehr/gah134

19. Leite, I. C. G., & Koifman, S. (2009). Oral clefts, consanguinity, parental tobacco and alcohol use: a case-control study in Rio de Janeiro, Brazil. *Brazilian oral research*, *23*, 31-37, DOI: 10.1590/S1806-83242009000100006

20. Liu, S., Liu, J., Tang, J., Ji, J., Chen, J., & Liu, C. (2009). Environmental risk factors for congenital heart disease in the Shandong Peninsula, China: a hospital-based case–control study. *Journal of epidemiology*, *19*(3), 122-130, DOI: 10.2188/jea.JE20080039

21. Maritz, G. S., & Harding, R. (2011). Life-long programming implications of exposure to tobacco smoking and nicotine before and soon after birth: evidence for altered lung development. *International journal of environmental research and public health*, *8*(3), 875-898, DOI: 10.3390/ijerph8030875

22. McEvoy, C. T., & Spindel, E. R. (2017). Pulmonary effects of maternal smoking on the fetus and child: effects on lung development, respiratory morbidities, and lifelong lung health. *Paediatric respiratory reviews*, *21*, 27-33, DOI: 10.1016/j.prrv.2016.08.005

23. Miller, E. C., Cao, H., Wen, S. W., Yang, Q., Lafleche, J., & Walker, M. (2010). The risk of adverse pregnancy outcomes is increased in preeclamptic women who smoke compared with nonpreeclamptic women who do not smoke. *American journal of obstetrics and gynecology*, *203*(4), 334-e1, DOI: 10.1016/j.ajog.2010.05.020

24. Mittal, S. (2019). Smoking and tobacco use: Ill effects on reproductive, maternal, newborn, child health, and adolescent (rmncha) program—a review. *Annals of the National Academy of Medical Sciences (India)*, *55*(02), 065-073, DOI: 10.1055/s-0039-1694071

25. Mund, M., Louwen, F., Klingelhoefer, D., & Gerber, A. (2013). Smoking and pregnancy-a review on the first major environmental risk factor of the unborn. *International journal of environmental research and public health*, *10*(12), 6485-6499, DOI: 10.3390/ijerph10126485

26. Nakamura, A., François, O., & Lepeule, J. (2021). Epigenetic alterations of maternal tobacco smoking during pregnancy: a narrative review. *International Journal of Environmental Research and Public Health*, *18*(10), 5083, DOI: 10.3390/ijerph18105083

27. Pizent A, Lazarus M, Kovačić J, Tariba Lovaković B, Brčić Karačonji I, Živković Semren T, 2020. Cigarette smoking during pregnancy: Effects on antioxidant enzymes, metallothionein and trace elements in mother-newborn pairs. *Biomolecules*, *10*(6), 892, DOI: 10.3390/biom10060892

28. Salihu, H. M., Aliyu, M. H., & Kirby, R. S. (2005). In utero nicotine exposure and fetal growth inhibition among twins. *American journal of perinatology*, 421-427, DOI: 10.1055/s-2005-915219

29. Sutan R, Campbell D, Prescott GJ, & Smith WCS. 2010. The risk factors for unexplained antepartum stillbirths in Scotland, 1994 to 2003. *Journal of Perinatology*, *30*(5), 311-318, DOI: 10.1038/ jp.2009.158

30. Wisborg, K., Kesmodel, U., Henriksen, T. B., Olsen, S. F., & Secher, N. J. (2001). Exposure to tobacco smoke in utero and the risk of stillbirth and death in the first year of life. *American journal of epidemiology*, *154*(4), 322-327, DOI: 10.1093/aje/154.4.322

31. Zacharasiewicz, A. (2016). Maternal smoking in pregnancy and its influence on childhood asthma. *ERJ open research*, *2*(3), DOI: 10.1183/ 23120541.00042-2016

Chapter - 6
A Review on Role of Long Non Coding RNA in Dengue Fever

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Chapter - 6

A Review on Role of Long Non Coding RNA in Dengue Fever

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Abstract

Dengue fever, a basic overall human concern, the transcendent in tropical and subtropical scenes. For sure with expansive ask about, the nuclear instruments that underlines the pathogenesis of dengue fever aren't totally appreciated. Vital regulators of value articulation and cell structures, Long non Coding RNAs (lnc RNAs), are brought as basic up in various contaminations, counting viral illnesses. Later contemplates have found that dengue illness have infection natural are changed by lnc RNAs. The expansion expected of right currently get it of lnc RNAs. Lnc RNAs managerial limits in dengue disease replication, safe response equilibrium, and pathogenesis were analyzed in this study. In extension, the potential for lnc RNAs to be illustrative and helpful focuses for dengue fever is featured. Understanding into the piece of lnc RNAs in dengue sickness might give unused procedures for organization and control. The results of this disease run comprehensively from genital desease. It was crucial to get it the DENV's ability to start the outflow of different lnc RNAs in vascular endothelial cells to uncover the pathology of dengue. Lnc RNAs impelled by DENV-2 sickness in hepatic cells uncovered that lnc RNA might act as a cutting edge definite marker. Help asserted these disclosures and watched updated degrees of miR-150 in serious dengue patients. Notwithstanding the way that the differential articulation of lnc RNA was connected with *Wolbachia wMelPop* tainting in *A. aegypti*. The *Ae. aegypti* lnc RNA characteristics shown a barely lower GC substance. Progressing interdisciplinary examination of lnc RNA-interceded guideline in DF are vital for upgrading our comprehension and moving disclosures into clinical application.

Keywords: Dengue fever, long non coding RNA, dengue infection, pathogenesis, treatment

Introduction

Dengue infection (DENV) was a solitary positive-strand RNA infection had a place with Flaviviridae family, and spread by *Aedes* mosquitoes. DENV serotypes (DENV-1-4), all circled around the world, remembering for Asia, Africa, and the Americas (Guzman *et al*., 2010). The yearly reports of DENV contaminations were assessed 100 to 400 million (WHO, 2020). Long non Coding RNAs (lnc RNA), they were RNA atoms without protein coding potential, were in excess of 200 nucleotides long. Lnc RNAs were generally present in different species, including creatures (Barlow and Bartolomei, 2014), plants (Yu *et al*., 2019), bugs (Lopez-Ezquerra *et al*., 2017; Zhang *et al*., 2020), prokaryotes (Lejars *et al*., 2019) and furthermore in infections (Chen *et al*., 2018; Liu *et al*., 2020).

1. Interpretation examinations of m6A control to safe non-coding RNA

Notwithstanding the way that the transcriptome ask about of dengue sickness was wonderful advanced by the inconceivable undertakings that made, various inquiries remained explained. Especially, the part played by the host's safe response in dengue tainting was suggested basic; nevertheless, there was a far reaching cleft in get it the enthusiastic safe climate in the midst of the development of the dengue pollution disease. The execution of mass transcriptome and epitranscriptome examinations in dengue pollution was accounted for, and a couple of biomarkers were recognized because of these considers (Gokhale *et al*. 2020, Robinson *et al*. 2019, Ubol *et al*. 2008). Regardless, a thorough data described the progression of a definite decisive test for dengue pollution. It was normal that with the advancement of single cell sequencing data (Papalexi, Satija; 2018), the vigorous safe natural frameworks that were firmly connected with the development of dengue tainting was revealed.

2. Vast profile of lnc RNA articulation

In outrageous cases, dengue disease, a created extending concern, can possibly bring about passing. The response to this tainting was mind boggling and portrayed by different cytokines being made (de Kruif *et al*.; 2008). Among the MAGE homology space (MHD)-containing protein superfamily (Zhao *et al*.; 2012). The prospect of these proteins as potential proposed because of the striking (Bertrand *et al*.; 2004). All through the mind, MAGED1 was viewed as significantly imparted (Bertrand *et al*.; 2004). A previous believe was recognized a raised articulation level of MAGED1 patients (da Silva *et al*.; 2013). Shockingly, in dengue patients, counting serious DHF patients was watched. The association of MAGED1 was well

known (Põld *et al*.; 1999). Hence, in patients with outrageous DHF, DENV sickness expanded apoptosis (da Silva *et al*.; 2013).

3. ERG-Related lnc RNA (ERGAL) Advances the Robustness and Perception of Vascular Endothelial Hindrance In the midst of Dengue Viral Pollution through Cooperation With miR-183-5p

In view of a far off better comprehension of the basic pathogenic parts, it was a way far off stayed foggy. Wang *et al*. (2017) natty gritty that the deferentially conveyed lnc RNAs impelled by DENV-2 sickness in hepatic cells revealed that lnc RNA might act as a cutting edge expressive marker and supportive objective for DENV-prompted liver mischief. Moreover, the outflow of lnc RNA-NEAT1 was diminished in periphery blood of patients with serious dengue, which is connected with the aggregate of serious dengue fever, suggesting that it would be strong to get it the development of DENV-actuated sicknesses by checking the declaration of NEAT1 and IFI27 in periphery blood (Pandey *et al*., 2017).

4. Control of Have Regular Immunity by Non Coding RNAs In the midst of Dengue Disease

Defilement Chen and associates watched that while miR-150 was significantly activated in DHF patients, levels of SOCS1 were diminished in the equivalent in this manner indicated toward the comparing communication among SOCS1 and miR-150. One more ponder help attested these revelations and watched redesigned degrees of miR-150 in serious dengue patients (Hapugaswatta *et al*., 2020). SOCS1 was help perceived in patients with extraordinary dengue sicknesses (Hoang *et al*., 2010).

5. *Wolbachia* Uses lnc RNAs to Impel the Counter Dengue Cost Pathway and Change Responsive Oxygen Species Stretch in *Aedes aegypti* Through a Serious Endogenous RNA Coordinate

Utilizing a computational pipeline, a great many lnc RNAs were recognized in light of RNA-seq data of *A. aegypti* from open information bases (Azlan *et al*., 2019). Despite the way that the differential articulation of lnc RNA was connected with *Wolbachia wMelPop* pollution in *A. aegypti*, not many productive examinations were described *Wolbachia* directed lnc RNAs in *A. aegypti* (Etebari *et al*., 2016).

6. Unmistakable evidence of *Aedes aegypti* Long Intergenic Non-coding RNAs and their Relationship with *Wolbachia* and Dengue VirusInfection

In amount to, 3,482 putative lnc RNAs in 1,114 *Ae. aegypti* genome structures were recognized. The *Ae. aegypti* lnc RNA characteristics shown a

barely lower GC substance (mean: 40.1%) in contrast with 47.8% in their protein-coding quality plans. The lower GC substance or AT progress was a common trait of lnc RNAs and our revelations were viable with expected lnc RNAs in different species (Etebari *et al.* ; 2015, Broadbent *et al.* ; 2011, Clark *et al.* ; 2012).

Conclusion

All in all, it has been highlighted that the multifaceted administrative jobs in Dengue Fever (DF) pathogenesis are played by lengthy non-coding RNAs (lnc RNAs), stressing their significance as expected demonstrative biomarkers and restorative targets. Quite, a significant development has been seen as of late in the comprehension of the contribution of lnc RNA in DF, illuminating their diverse jobs in overseeing viral replication, resistant reaction, and sickness movement. It was found that changed safe reactions have been connected to disregulated articulation of lnc RNAs, heightening sickness seriousness in specific occasions. Also, encouraging possibilities for early location and anticipation of DF are presented by the recognizable proof of lnc RNAs as expected demonstrative markers. Analytic exactness could be upgraded and clinical direction could be educated by using lnc RNA profiles in understanding examples, consequently working on the results for patients. Besides, the potential for DF the executives of focusing on lnc RNAs restoratively holds tremendous commitment. Control of viral replication, moderation of resistant disregulation, and weakening of illness seriousness could be accomplished through clever methodologies presented by systems pointed toward adjusting lnc RNA articulation levels or movement. Regardless of these progressions, a few impediments stay that need tending to. Further examination is expected to clarify the specific systems fundamental the guideline of DF pathogenesis interceded by lnc RNAs, as well as approving the symptomatic and helpful utility of lnc RNAs in clinical conditions. The test of making compelling conveyance frameworks for lnc RNA-based therapeutics is a huge obstacle that needs consideration. All in all, the developing job of lnc RNAs in DF pathogenesis addresses a quickly changing field of study with wide-arriving at suggestions for overseeing and controlling the illness. Progressing interdisciplinary exploration endeavors equipped towards unwinding the intricacies of lnc RNA-interceded guideline in DF are vital for improving our comprehension and moving disclosures into clinical application. We may at last have the option to work on the finding, treatment, and avoidance of DF by saddling the administrative capability of lnc RNAs, subsequently facilitating the weight of this worldwide danger.

Reference

1. Azlan A., Obeidat S. M., Yunus M. A., Azzam, G.; (2019); Systematic Identification and Characterization of Aedes Aegypti Long Noncoding RNAs (lncRNAs); Sci. Rep.; 9 (1); 12147; doi: https://doi.org/10.1038/s41598-019-47506-9

2. Barlow D. P., Bartolomei M. S.; (2014); Genomic Imprinting in Mammals; Cold Spring Harb. Perspect. Biol.; 6 (2); a018382; doi: https://doi.org/10.1101/cshperspect.a018382

3. Bertrand M., Huijbers I., Chomez P., Backer O. D.; 2004; Comparative expression analysis of the MAGED genes during embryogenesis and brain development; Dev Dyn; 230(2); 325-334; doi: https://doi.org/10.1002/dvdy.20026

4. Broadbent K. M., Park D., Wolf A. R., Tyne D. V., Sims J. S., Ribacke U., Volkman S., Duraisingh M., Wirth D., Sabeti P. C., Rinn J. L.; 2011; A global transcriptional analysis of Plasmodium falciparum malaria reveals a novel family of telomere-associated lncRNAs; Genome Biol.; 12(6); R56; doi: https://doi.org/10.1186/gb-2011-12-6-r56

5. Chen L., Zhou Y., Li H.; (2018); LncRNA, miRNA and lncRNA-miRNA Interaction in Viral Infection; Virus Res.; 257; 25–32; doi: https://doi.org/10.1016/j.virusres.2018.08.018

6. Clark M. B., Johnston R. L., Inostroza-Ponta M., Fox A. H., Fortini E., Moscato P., Dinger M. E., Mattick J. S.; 2012; Genome-wide analysis of long noncoding RNA stability; Genome Res.; 22(5); 885–898; doi: https://doi.org/10.1101/gr.131037.111

7. da Silva M. M. C., Gil L. H. V. G., Marques Júnior E. T. A., Calzavara-Silva C. E.; 2013; Potential biomarkers for the clinical prognosis of severe dengue; Mem Inst Oswaldo Cruz; 108(6); 755–762; doi: https://doi.org/10.1590/0074-0276108062013012

8. de Kruif M. D., Setiati T. E., Mairuhu A. T. A., Koraka P., Aberson H. A., Spek C. A., Osterhaus A. D. M. E., Reitsma P. H., Brandjes D. P. M., Soemantri A., van Gorp E. C. M.; 2008; Differential Gene Expression Changes in Children with Severe Dengue Virus Infections; PLOS Neglected Tropical Diseases; e215; doi: https://doi.org/10.1371/journal.pntd.0000215

9. Etebari K., Asad S., Zhang G., Asgari S.; (2016); Identification of Aedes Aegypti Long Intergenic Non-Coding RNAs and Their Association With Wolbachia and Dengue Virus Infection; PloS Negl. Trop. Dis.; 10 (10); e0005069; doi: https://doi.org/10.1371/journal.pntd.0005069

10. Etebari K., Furlong M. J., Asgari S.; (2015); Genome wide discovery of long intergenic non-coding RNAs in Diamondback moth (Plutella xylostella) and their expression in insecticide resistant strains; Sci Rep.; 5; 14642; doi: https://doi.org/10.1038/srep14642

11. Gokhale N. S., McIntyre A. B. R., Mattocks M. D., Holley C. L., Lazear H. M., Mason C. E., Horner S. M.; 2020; Altered m6A Modification of Specific Cellular Transcripts Affects Flaviviridae Infection; Molecular Cell; 77(3); 542-555; DOI: https://doi.org/10.1016/j.molcel.2019.11.007

12. Guzman M. G., Halstead S. B., Artsob H., Buchy P., Farrar J., Gubler D. J., Hunsperger E., Kroeger A., Margolis H. S., Martínez E., Nathan M. B., Pelegrino J. L., Simmons C., Yoksan S., Peeling R. W.; 2010 ; Dengue: a continuing global threat; Nat. Rev. Microbiol.; 8(12); S7-S16; doi: https://doi.org/10.1038/nrmicro2460

13. Hapugaswatta H., Amarasena P., Premaratna R., Seneviratne K., Jayathilaka, N.; (2020); Differential expression of microRNA, miR-150 and enhancer of zeste homolog 2 (EZH2) in peripheral blood cells as early prognostic markers of severe forms of dengue; J. Bio Med. Sci.; 27 (1); 25; doi: https://doi.org/10.1186/s12929-020-0620-z

14. Hoang L. T., Lynn D. J., Henn M., Birren B. W., Lennon N. J., Le P. T., Duong K. T. H., Nguyen T. T. H., Mai L. N., Farrar J. J., Hibberd M. L., Simmons C. P.; 2010; The early whole-blood transcriptional signature of dengue virus and features associated with progression to dengue shock syndrome in Vietnamese children and young adults; J. Virol.; 84(24); 12982-94; doi: https://doi.org/10.1128/JVI.01224-10

15. Lejars M., Kobayashi A., Hajnsdorf E.; (2019); Physiological Roles of Antisense RNAs in Prokaryotes; Biochimie; 164; 3–16; doi: https://doi.org/10.1016/j.biochi.2019.04.015

16. Liu W., Wang Z., Liu L., Yang Z., Liu S., Ma Z., Liu Y., Ma Y., Zhang L., Zhang X., Jiang M. , Cao X.; (2020); LncRNA Malat1 Inhibition of TDP43 Cleavage Suppresses IRF3-Initiated Antiviral Innate Immunity; Proc. Natl. Acad. Sci.; 117(38); 23695–23706. doi: https://doi.org/10.1073/pnas.2003932117

17. Lopez-Ezquerra A., Harrison M. C., Bornberg-Bauer E.; (2017); Comparative Analysis of lincRNA in Insect Species; BMC Evol. Biol.; 17 (1); 155; doi: https://doi.org/10.1186/s12862-017-0985-0

18. Pandey A. D., Goswami S., Shukla S., Das S., Ghosal S., Pal M., Bandyopadhyay B., Ramachandran V., Basu N., Sood V., Pandey P.,

Chakrabarti J., Vrati S., Banerjee A.; 2017; Correlation of altered expression of a long non-coding RNA, NEAT1, in peripheral blood mononuclear cells with dengue disease progression; J. Infect.; 75(6); 541-554; doi: https://doi.org/10.1016/j.jinf.2017.09.016

19. Papalexi E., Satija R.; 2018; Single-cell RNA sequencing to explore immune cell heterogeneity; Nat Rev Immunol; 18(1); 35-45; doi: https://doi.org/10.1038/nri.2017.76

20. Põld M., Zhou J., Chen G. L., Hall J. M., Vescio R. A., Berenson J. R.; 1999; Identification of a new, unorthodox member of the MAGE gene family; Genomics; 59(2); 161-167; doi: https://doi.org/10.1006/geno.1999.5870

21. Robinson M., Sweeney T. E., arouch-Bentov R., Sahoo M. K., Kalesinskas L., Vallania F., Sanz A. M., Ortiz-Lasso E., Albornoz L. L., Rosso F., Montoya J. G., Pinsky B. A., Khatri P., Einav S.; 2019; A 20-Gene Set Predictive of Progression to Severe Dengue; Cell Rep; 26(5); 1104–1111; doi: https://doi.org/10.1016/j.celrep.2019.01.033

22. Ubol S., Masrinoul P., Chaijaruwanich J., Kalayanarooj S., Charoensirisuthikul T., Kasisith J.; 2008; Differences in global gene expression in peripheral blood mononuclear cells indicate a significant role of the innate responses in progression of dengue fever but not dengue hemorrhagic fever; J Infect Dis; 197(10); 1459-1467; doi: https://doi.org/10.1086/587699

23. Wang X. J., Jiang S. C., Wei H. X., Deng S. Q., He C., Peng H. J.; (2017); The differential expression and possible function of long non coding RNAs in liver cells infected by dengue virus; Am. J. Trop. Med. Hyg.; 97; 1904–1912; doi: https://doi.org/10.4269/ajtmh.17-0307

24. World Health Organization; (2020); Dengue and severe dengue; https://www.who.int/newsroom/fact-sheets/detail/dengue-and-severe-dengue

25. Yu Y., Zhang Y., Chen X., Chen Y.; (2019); Plant Noncoding RNAs: Hidden Players in Development and Stress Responses; Annu. Rev. Cell Dev. Biol.; 35; 407–431; doi: https://doi.org/10.1146/annurev-cellbio-100818-125218

26. Zhang L., Xu W., Gao X., Li W., Qi S., Guo D., Ajayi O. E., Ding S. W., Wu Q.; 2020; lncRNA Sensing of a Viral Suppressor of RNAi Activates Non-canonical Innate Immune Signaling in Drosophila; Cell Host Microbe; 27(1); 115-128; DOI: https://doi.org/10.1016/j.chom.2019.12.006

27. Zhao Q., Caballero O. L., Simpson A. J. G., Strausberg R. L.; 2012; Differential evolution of MAGE genes based on expression pattern and selection pressure; PLoS One; 7(10); e48240; doi: https://doi.org/10.1371/journal.pone.0048240

Chapter - 7
Nanotechnology-based Immunotherapeutic Medication for Cancer Diagnosis and Treatment

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Chapter - 7

Nanotechnology-based Immunotherapeutic Medication for Cancer Diagnosis and Treatment

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Abstract

Cancer is one of the leading causes of mortality and short life spans worldwide. Periodically tumor cell evaluation and high-specificity use of drugs to reduce effects are critical components of offering the best potential treatment for cancer. Other techniques, such as nanotechnologies are currently employed to improve monitoring and reduce the impact of the condition because conventional tumor diagnosis and therapy instruments have a higher probability of general malignancies and resistance. Nanotechnology-based immunotherapeutic medication has been used for many cancer types all over the years in order as a way to decrease infiltration of tumor cells while maintaining beneficial cells at the location of the tumor. Nanotechnology-based immunotherapeutic medication has been used for a number of cancer types through the years to reduce the impact of tumor cells while preserving cells that are healthy at the area of interest. Nanotechnology including liposomes being carbon nanotubes, and polymer micelles have demonstrated important pharmacological and therapeutic benefits in the process of developing cancer medicines, particularly in the detection and management of malignancy. Along the present study, we take into consideration the widely used nanoparticles that are utilized in cancer diagnosis and therapy. The chemical and biological characteristics of these the nanoparticles have been demonstrated as being ideal for the cancer therapy. We went further over the challenges caused by numerous nanotechnologies, which restrict their uses and render it challenging for nanomaterials to be employed in clinical settings with particular types of cancer.

Keywords: Cancer, immunotherapy, liposomes, nanoparticles, polymer-micelles, tumor cell.

1. Introduction

One of the leading causes deaths and an impact on the global healthcare system is cancer. By 2018, it had been estimated that there would be a total of

9,000,000 loss of life due to cancer and 18.2 million instances of new cancers (Bray *et al*., 2018). Unregulated expansion of cells that expands from a unique focus site to other areas throughout the human body and eventually ends in mortality is the characteristic of the disease known as cancer. For all of these reasons, controlling the propagation of the disease and reducing the rate of death require rapid cancer recognition and therapy. Nanotechnology represents one of the techniques that has recently been used widely in research on cancer. The use of nanoparticles in drug delivery, mutation evaluation and detection, biomarker visualization, tailored therapy, and molecular visualization has produced a number of encouraging outcomes in the diagnosis and treatment of cancer. The utilization of nanotechnology has taken place in the development of nanoparticles, including quantum dot particles and gold nanoparticles, all of which have been used for biological cancer diagnostics. Genetic screening using nanotechnologies, such as the creation of biomarkers, may swiftly and readily identify tumors. Medications utilizing the field of nanotechnology which includes the development of the delivery of drugs at the nanoscale, thus may assure exact targeted of malignant tumor with no negative side effects (Huang *et al*., 2019). However, there are a number of medications that may be utilized for treating cancer, their sensitivity generally results in insufficient effects and may result in a number of side effects in along with injuring healthy cells. All of this, numerous studies have been looking at various kinds of nanotechnologies, including polymer compounds, molecules, antibodies, and liposomes. The results indicate that using several of these tiny particles in the development of cancer drugs may balance the requirement for increased efficacy by reducing toxic effects (Ye *et al*., 2018). The article covers the utilization of nanoparticles in cancer prevention and treatment, and focusing its advantages and disadvantages due to its fast growth.

1.1 The Use of Nanotechnology in the Diagnosis of Cancer

Genetic variations may affect how some macromolecules are synthesized, which might end up in uncontrolled division of cells and ultimately cancerous tissues (Chaturvedi *et al*., 2019). Initial detection and prevention of malignant tumor growth and metastasis are the primary objectives of cancer detection as well as therapeutic methods. The adoption of PET (positron emission tomography), MRI, CT, and ultrasonography is noteworthy when compared to other early methods of screening for malignant tumors (Kim *et al*., 2010). Still, these imaging methods are restricted through their limited capacity to provide essential scientific information about different types of cancer and the stages. As a result, it is challenging to achieve a complete analysis of the

disease's state, that is required for selecting the most appropriate method for treatment (Akhter *et al*., 2013).

1.1.1 Application of Nanotechnology in Tumor Imaging

Since many different kinds of nanoparticles being used for molecular photography these days, the deployment of nanoparticles in tumor tracking and identification has garnered a lot of interest in recent years.These advantages-small magnitude, high atomic number, and excellent biocompatibility-have become increasingly popular in the area of cancer research as well as diagnostics within a previous several years. For the purpose of precursory cancer cellular screening and being identified, nanoparticles that can be used in combination with other anti-tumor medications and biomolecules, such as peptides, antibodies, or other chemicals, to label highly specific tumors (Singh *et al*., 2019). Through developing immunological barriers nanoparticles of SPIONs that maybe utilized in MRI and target tumour cell lines, it can be done to recognize metastatic in lung cancer patients (Wan *et al*., 2016). According to recent research, SPIONs are good aerosol components for MRI imaging of lung tumors since they are highly selective and have not been associated with any adverse effects (Jafari *et al*., 2015). Moreover, magnetic powder imaging has proven a high accuracy and selectivity to tumour tissues in tomographic imaging methodology (Stocke *et al*., 2015).

1.1.2 The Utilization of Nanomaterials Tools in Diagnosis of Cancer

In some recent research, tumour imaging at the tissue, cell, and molecular scales can be proven by nanotechnology (Garrigue *et al*., 2018). The temporal as well as spatial approaches developed using nanomaterials that may be applied to accurately monitor living cells and monitor variable cellular processes in tumours will be discussed in this part of the article.

1.1.2.1. Near Infrared (NIR) Quantum Dots

The biggest problem of ultraviolet (UV) radiography is its poor recognition and penetrating ranges. Researchers have created quantum dots that produce light in the 700–1000 nm area of the near infrared spectrum in an attempt to overcome these difficulties. This makes the NIR quantum dots more suitable for in vivo cancer detection in tissues such as the pancreas, gastrointestinal tract, liver, and lymphatic system. The multicoloured quantum dots (QD) imagining capacity utilization in organisms. Meanwhile, it was previously claimed that the development of Ag2Te quantum dots (QDs) rich in silver and which includes a sulfuric source enable the collection of better spatially accurate images through the vast infrared wavelength spectrum (Zhang *et al*.,2020).

1.1.2.2 Nano-shells

Nano-shells are devices usually hydrophobic cores which vary in diameter from 10 to 300 nm and commonly consist of silica encapsulated in a thin gold-coloured metallic casing. These are responsive to optical adjustment nano-shells with an absorption/emission wavelength spanning from ultraviolet to infrared. Plasmon-mediated electrical power is transformed into light through these nano-shells (Ganesh *et al*., 2024). The absence of toxicities from heavy metals in nano-shell imaging makes it attractive.

1.1.2.3 Colloidal Gold Nanoparticle

Among the most promising types of agents for cancer detection is the use of gold nanoparticles. Through their large molecular weight, outstanding biocompatibility, and compact size, gold nanoparticles (AuNPs) are perfect as contrast agents. Research indicate that AuNPs destroy cells in both passive as well as active ways. According to the Permeability Tension Effect (EPR) in cancer cells, the extraction of gold nanoparticles in order to improve imaging regulates the passive focusing method (Fu *et al*., 2018). Moreover, the gold nanoparticles can be mixed with antibodies to be used in biopsies for the identification of pancreatic and cervical cancers. With the technique allowing detection of malignancies in cells that are as small as a few millimetres in diameter, these results have major benefits in early detection (Shrivas *et al*., 2018).

1.2 Use of Nanotechnology Tools in Therapy of Cancer

Current events in the creation of different carriers for efficient drug management are an important factor behind research activities in the field of nanomaterials. Many kinds of vehicles have been developed produced, including carbon nanotubes, liposomes, micelles, dendrimers, quantum dots, and more.

1.2.1 Carbon Nanotubes

Carbon nanotubes (CNTs) are typically categorized into two groups dependent on their diameter and structure: single-walled CNTs (SWNTs) and multiwalled CNTs (MWNTs). Single-walled carbon nanotubes are made up of a single sheet of cylindrical graphene, whereas multiwalled carbon nanotubes are made up of many concentric graphene sheets (Kesharwan *et al*., 2015). The composition, dimension, hardness, metallic properties, thermal & electrical resistance, and extremely low weight properties of CNTs are all associated with their chemical and physical properties. As a result of their various unique physical and chemical characteristics, CNTs are an attractive

option for an extensive variety of biomedical purposes (Bianco *et al*., 2005). CNTs have a capacity to absorb light in the near-infrared (NIR) range, leading the nanotubes to warm up. This process is referred to as a thermal effect, and it has been applied for targeting cancerous cells (Burlaka *et al*., 2010). Folic acid (FA) transporters are overexpressed in tumor cells, and a number of researchers have developed artificial nanocarriers containing linked biomaterials of FA derivatives. Natural nanotubes of carbon are considered to serve as highly efficient mediators for the delivery of various medications into living cells which promote the invasive penetrating of biofilms (Yu *et al*., 2017).

1.2.2 Quantum Dots

The initial quantum dots (QDs), which are tiny, 2–10 nanometer-sized atoms or nanotechnology of semiconductor material, were produced in 1980 by Ekimov and colleagues (Ekimov *et al*., 2005). Since they have their large surface-to-volume ratios, quantum dots have electrical properties which fall in between those of isolated atoms and mass semiconductor. Throughout time, a number of QD-based methods have emerged, including QD immunostaining and improved QD combinations. Together with increased multiplexing capabilities, the redesigned QDs conjugates provide an important boost in time as well as efficiency over single-color research studies. Further research demonstrates that QD immunostaining is superior to traditional immunochemistry studies in terms of precision, accuracy, and reduced noise at lower protein production levels. QD antibodies could serve as a helpful technique to detect different tumor indicators in context with a cancer diagnosis, including cell proteins or other components from various tumor tissues. Applying quantum-dot-based probes, Chen *et al*. succeeded in to effectively identify BC, indicating that, compared to conventional immunohistochemistry, quantum dot immunohistochemistry (IHC) is capable of simultaneous identification as well as the detection of extremely small expression of Human Epidermal Growth Factor Receptor 2 (HER2) (Sung *et al*., 2019).

1.2.3 Dendrimers

A different kind of nanocarrier is dendrimers, that have a round polymeric core with regular branches. The dendrite biomolecules develop more susceptible towards a globular form as their length increases exponentially (Svenson *et al*., 2012). There are two commonly used methods for synthesizing dendrimers, that are the first is the divergent approach, which involves developing the dendrimers from a central core outward and the

second strategy, which produces the dendrimer form the margin inside to the center, is the converging method. Poly (propylene imine), poly (glycerol-co-succinic acid), poly (L-lysine), poly (glycerol), poly(2,2-bis(hydroxymethyl)propionic acid), and melamine dendrimers are among the many compounds that are often used to form dendrimers. These dendrimers display a lot chemical configurations and features, including basicity, capacity to form bonds with hydrogen, charge, etc., that may be modified by promoting the formation of dendrimers or by modifying the groups which make up the outermost layer. This makes it possible for the binding of several pharmaceutical compounds to a single dendrimer molecule, and the nature of these connections controlling the release of these medicinal compounds. Due to its distinct features, different linkage groups, polymer size, charge, and physiologically significant properties like lipid bilayer interactions, cytotoxicity, internalization, blood plasma retention duration, metabolism, and filtration, dendrimers have gained attention as possible nanocarrier candidates. Folic acid conjugated dendrimers have been demonstrated through another study to be able prevent tumor cells that show greater levels of the folic acid receptors (Fatima *et al*., 2022). Genome-polyamides cluster Chromatin-poly (amidoamine) (DNAPAMAM) has demonstrated that dendrimers have the added benefit of connecting to DNA, which makes them extremely efficient in killing tumor cells that express the folate receptor.

1.2.4 Polymeric micelles

The polymeric nanoparticles are the advancements associated with a solid micelle that have a particle size range of 10-1000 nm (PNPs) (Begines *et al*., 2020). PNPs, or are the initial polymer compounds to be characterized for use in drug delivery systems. They are also known by other names, such as polymerization micelles, nanospheres, nano-capsules, or nanoparticles. Micelles consist of both hydrophobic and hydrophilic monomer units and primarily consist of amphiphilic co-polymers. Carrying amphi molecules with charge, such as peptides, proteins, and amino acids, these pieces are flipped to permit interactions in the core and neutralize charges. Micelles can be divided into multiple types according to their structure and bonding. Block copolymer micelles fit into one of these categories, including poly-ion-complex micelles, metal complexation-derived micelles, and hydrophobically formed amphiphilic micelles (Berger *et al*., 2014). PNPs are a suitable carrier for drugs that has excellent endothelial cell permeability and no danger of renal rejection due to their smaller volume and excellent thermal resistance (Jin *et al*., 2020). By injecting PNP cancellations, the hydrophobic macromolecules and medications can reach the core of PNPs as they separate in a water

solution, potentially having a therapeutic impact. Still, the primary challenges in applying PNPs for tumor nanotechnology remain in effectively delivering the drugs to the appropriate region while reducing adverse effects and resistance to drugs. As PNPs offer so many potential advantages for the treatment of patients, they are currently heavily utilized in the development of chemotherapy medications that utilize nanoscale. Blanco *et al*. produced a cancer protection medicine utilizing PEG-PLA micelles that comprised of the insoluble in water chemical β-lapachone, or β-lap. They discovered that the medication developed at the location of the tumor because the PLA micellar core and β-lap combination kept very stable and lasted in the particles in the blood circulation for a long time. The extremely tiny size of tiny particles makes them more useful for transferring drugs compared to various drug transporters, especially for solid tumors, particularly those with inadequate vascularization. Doxil (a liposomal version of doxorubicin) resolves these problems and is less likely to cause heart disease in individuals, which indicates that scientists may be capable to use safer techniques in the future for creating nanoparticles (Sohai *et al*., 2021).

1.2.5 Liposomes

Liposomes are phospholipid-based vesicles, typically around 400 nm in size, with a polar head, non-polar tail, and bilayer membranes composed of cholesterol. As liposomes have a special capacity to dissolve organic compounds that are insoluble in water, it can be employed as a transporter for medicines that are directed towards specific disorders, including cancer. Medicines found inside the membrane of liposomes provide a number of advantages, including defence against degradation, lower nonspecific toxicity, and easy distribution to the target region (Yue *et al*., 2018). When examining the toxicology and effectiveness of free drug transport using various liposomal formulations and cancer prevention medicines, researchers found that liposomes that signal fewer adverse effects at the tumor site that free medications. (Sutradhar *et al*., 2014). Wider liposomes that have a shorter lifespan because the system of mononuclear phagocytes quickly recognizes and destroy them, yet liposomes up to 100 nanometres easily penetrate tumours and stay there for prolonged. Doxil, or liposomal doxorubicin, is the first anticancer nanodrug authorized by the FDA. It is frequently employed in order to cure AIDS patients' kaposi's sarcoma and is produced using PEGylated liposomal formulation (Singh *et al*., 2018). Doxil manufactured and commercialized in India by Sun Pharma Company, which gained approval from the FDA in 2013 for the first generic version of Doxil (Chou *et al*., 2015).

1.4 Comparison of different types of the Nano-carrier

The many different types of nano-carriers may be categorized according to a number of factors, including size, surface characteristics, function, biodegradability, benefits, and drawbacks. Some common types of nano-carriers include Quantum dots, Dendrimers, Polymeric-micelles, Liposome, Carbon nanotube are compared below (Table-1).

Table 1: List of different nano-carriers and their uses

Nano-carrier Types	Size (nm)	Surface charge	Biodegradability	Dis-Advantages	Advantages	Reference
Quantum dots	1-10	Negative	Non-biodegradable	Limited blood flow time, potential reticuloendothelial system elimination, and difficulties with manufacturing scale up	High toxic effects, potential for heavy metal leaching, problems with delivering medicines in the targeted region	(Kumar *et al*.,2022)
Dendrimers	1-10	variable	Non-biodegradable	Limited time for blood circulation, potential damage to the kidneys, and trouble supplying drugs to tumours particularly	High biotoxicity, restricted biodegradability, and difficulties in raising production volume	(Zhao *et al*.,2018)
Polymeric micelles	10-300	variable	Biodegradable	Restricted biocompatibility and difficulties in achieving controlled releases	Restricted blood circulation length, challenges for effective targeting, and the potential for early release of medication	(Ghosh *et al*.,2021)
Liposomes	50-200	Neutral	Biodegradable	Insufficient tumour selection, rapid circulating time, and the risk of medication leaking	Low immunity, versatility in drugs loading and targeting, and good biological compatibility	(Aibani *et al*., 2020)
Carbon Nanotubes	1-100	Negative	Non-biodegradable	Restricted blood circulation, potential reticuloendothelial system authorization, and difficulties in properly supplying medications to cancers	High toxic effects, limited biocompatibility, and challenges to achieving controlled release	(Negri *et al*., 2020)

1.5 Challenges and Future prospective

Multiple advantages of nanomaterials emerge for cancer therapy. Nanoparticles substrates' tiny stature makes it feasible to enter a tumor's vascular through EPR. Also functioning using an oligomer or hydrophilic polymer could offer an extensive circulating half-life and prolong the cancer tissue's exposed duration to anticancer medications; however, the use of

tissue-recognition remains, such as lectins, antibodies, and ligands unique to cancer cells, could help in the identification of tumor cells using nanomaterials tools.

There are numerous challenges to be overcome and promising future possibilities for applying nanotechnology in cancer treatment. However, there remain difficulties standing in the pathway of the development and application of nanomaterials platform for the treatment of cancer; include a shortage of clinical testing criteria, restricted applications and insufficient functionalization of beneficial nanomaterials, and an insufficient knowledge of the metabolism of cancer cells. The present challenges involve the impact of endothelial cell barriers on circulation, cellular digestion of the medicinal agent, medications evacuation from circulation, and tumor variability. In short, improvements in cancer treatment are required. Beyond any doubt, cancer nanotechnology might contribute to a success in the fight to prevent deaths from cancer. A variety of nanotechnologies that have proven beneficial to cancer diagnosis and treatment have been developed. Till that time, plenty of research must be performed to successfully apply cancer nanotechnology in clinics. In summary, our aim became to bring awareness to the primary advantages of nanomaterials as well as its drawbacks especially when it applies to resolving cancer clinical requirements.

Conclusion

Throughout the last few years, the application of nanotechnology across multiple scientific, engineering, and technology fields has increased rapidly. Recently, nanomaterials have become popular in biomedical studies as therapeutic agents or as a drug transport system. Over the years, current advances in nanomaterials have promoted research in the domain of nano-oncology and recognized the area as a feasible tumor therapeutic technique. Researchers can substantially enhance the properties of nanopolymers while developing novel ones by modifying their composition and structure. New substances which function more effectively and are more appropriate to a system of life. In a few years, nano-oncology will become a popular cancer treatment method due to its persistent and extensive research.

References

1. Aibani N, Khan TN, & Callan B. Liposome mimicking polymersomes; A comparative study of the merits of polymersomes in terms of formulation and stability. *International journal of pharmaceutics:* 2020; *X*, *2*, 100040.
2. Alper J. Shining a light on cancer research. *NCI Alliance for Nanotechnology in Cancer USA*, 2005; 1-3.

3. Begines, B., Ortiz, T., Pérez-Aranda, M., Martínez, G., Merinero, M., Argüelles-Arias, F., & Alcudia, A. (2020). Polymeric nanoparticles for drug delivery: Recent developments and future prospects. Nanomaterials, 10(7), 1403.

4. Berger, J. L., Smith, A., Zorn, K. K., Sukumvanich, P., Olawaiye, A. B., Kelley, J., & Krivak, T. C. (2014). Outcomes analysis of an alternative formulation of PEGylated liposomal doxorubicin in recurrent epithelial ovarian carcinoma during the drug shortage era. *OncoTargets and therapy*, 1409-1413.

5. Bianco, A., Kostarelos, K., & Prato, M. (2005). Applications of carbon nanotubes in drug delivery. *Current opinion in chemical biology*, *9*(6), 674-679.

6. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, & Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians, 2018; 68(6), 394-424.

7. Burlaka, A., Lukin, S., Prylutska, S., Remeniak, O., Prylutskyy, Y., Shuba, M., ... & Scharff, P. (2010). Hyperthermic effect of multi-walled carbon nanotubes stimulated with near infrared irradiation for anticancer therapy: in vitro studies. *Experimental oncology*.

8. Chaturvedi, V. K., Singh, A., Singh, V. K., & Singh, M. P. (2019). Cancer nanotechnology: a new revolution for cancer diagnosis and therapy. *Current drug metabolism*, *20*(6), 416-429.

9. Chou H, Lin H, & Liu JM. 2015. A tale of the two PEGylated liposomal doxorubicins. *OncoTargets and therapy*, 1719-1720.

10. Ekimov, A. I., & Onushchenko, A. A. (2005). Quantum size effect in three-dimensional microscopic semiconductor crystals. *SPIE milestone series*, *180*, 3-5.

11. Fatima M, Sheikh, A., Hasan, N., Sahebkar, A., Riadi, Y., & Kesharwani, P. (2022). Folic acid conjugated poly (amidoamine) dendrimer as a smart nanocarriers for tracing, imaging, and treating cancers over-expressing folate receptors. *European Polymer Journal*, *170*, 111156.

12. Fu, N., Hu, Y., Shi, S., Ren, S., Liu, W., Su, S., ... & Wang, L. (2018). Au nanoparticles on two-dimensional MoS 2 nanosheets as a photoanode for efficient photoelectrochemical miRNA detection. *Analyst*, *143*(7), 1705-1712.

13. Ganesh, K. M., Bhaskar, S., Cheerala, V. S. K., Battampara, P., Reddy, R., Neelakantan, S. C., ... & Ramamurthy, S. S. (2024). Review of Gold Nanoparticles in Surface Plasmon-Coupled Emission Technology: Effect of Shape, Hollow Nanostructures, Nano-Assembly, Metal–Dielectric and Heterometallic Nanohybrids. *Nanomaterials*, *14*(1), 111.

14. Garrigue, P., Tang, J., Ding, L., Bouhlel, A., Tintaru, A., Laurini, E., ... & Peng, L. (2018). Self-assembling supramolecular dendrimer nanosystem for PET imaging of tumors. *Proceedings of the National Academy of Sciences*, *115*(45), 11454-11459.

15. Ghosh, B., & Biswas, S. (2021). Polymeric micelles in cancer therapy: State of the art. *Journal of Controlled Release*, *332*, 127-147.

16. Huang, D., Wu, K., Zhang, Y., Ni, Z., Zhu, X., Zhu, C., ... & Hu, J. (2019). Recent advances in tissue plasminogen activator-based nanothrombolysis for ischemic stroke. *Reviews on Advanced Materials Science*, *58*(1), 159-170.

17. Jafari, A., Salouti, M., Shayesteh, S. F., Heidari, Z., Rajabi, A. B., Boustani, K., & Nahardani, A. (2015). Synthesis and characterization of Bombesin-superparamagnetic iron oxide nanoparticles as a targeted contrast agent for imaging of breast cancer using MRI. *Nanotechnology*, *26*(7), 075101.

18. Jin, C., Wang, K., Oppong-Gyebi, A., & Hu, J. (2020). Application of nanotechnology in cancer diagnosis and therapy-a mini-review. *International Journal of Medical Sciences*, *17*(18), 2964.

19. Kesharwani, P., & Iyer, A. K. (2015). Recent advances in dendrimer-based nanovectors for tumor-targeted drug and gene delivery. *Drug discovery today*, *20*(5), 536-547.

20. Kim, D., Jeong, Y. Y., & Jon, S. (2010). A drug-loaded aptamer-gold nanoparticle bioconjugate for combined CT imaging and therapy of prostate cancer. *ACS nano*, *4*(7), 3689–3696.

21. Kumar, A., Singh, K. R., Ghate, M. D., Lalhlenmawia, H., Kumar, D., & Singh, J. (2022). Bioinspired quantum dots for cancer therapy: A mini-review. *Materials Letters*, *313*, 131742.

22. Negri, V., Pacheco-Torres, J., Calle, D., & López-Larrubia, P. (2020). Carbon nanotubes in biomedicine. *Surface-modified Nanobiomaterials for Electrochemical and Biomedicine Applications*, 177-217.

23. Shrivas, K., Nirmalkar, N., Thakur, S. S., Deb, M. K., Shinde, S. S., & Shankar, R. (2018). Sucrose capped gold nanoparticles as a plasmonic

chemical sensor based on non-covalent interactions: Application for selective detection of vitamins B1 and B6 in brown and white rice food samples. *Food chemistry*, *250*, 14-21.

24. Singh, R. (2019). Nanotechnology based therapeutic application in cancer diagnosis and therapy. *3 Biotech*, *9*(11), 415.

25. Singh, S. (2018). Liposome encapsulation of doxorubicin and celecoxib in combination inhibits progression of human skin cancer cells. *International journal of nanomedicine*, *13*(sup1), 11-13.

26. Sohail, M., Sun, Z., Li, Y., Gu, X., & Xu, H. (2021). Research progress in strategies to improve the efficacy and safety of doxorubicin for cancer chemotherapy. *Expert review of anticancer therapy*, *21*(12), 1385-1398.

27. Stocke, N. A., Meenach, S. A., Arnold, S. M., Mansour, H. M., & Hilt, J. Z. (2015). Formulation and characterization of inhalable magnetic nanocomposite microparticles (MnMs) for targeted pulmonary delivery via spray drying. *International journal of pharmaceutics*, *479*(2), 320-328.

28. Sung, S. Y., Su, Y. L., Cheng, W., Hu, P. F., Chiang, C. S., Chen, W. T., & Hu, S. H. (2018). Graphene quantum dots-mediated theranostic penetrative delivery of drug and photolytics in deep tumors by targeted biomimetic nanosponges. *Nano letters*, *19*(1), 69-81.

29. Sutradhar, K. B., & Amin, M. L. (2014). Nanotechnology in cancer drug delivery and selective targeting. *International scholarly research notices*, *2014*.

30. Svenson, S., & Tomalia, D. A. (2012). Dendrimers in biomedical applications—reflections on the field. *Advanced drug delivery reviews*, *64*, 102-115.

31. Wan, X., Song, Y., Song, N., Li, J., Yang, L., Li, Y., & Tan, H. (2016). The preliminary study of immune superparamagnetic iron oxide nanoparticles for the detection of lung cancer in magnetic resonance imaging. *Carbohydrate research*, *419*, 33-40.

32. Wang, J., Sui, M., & Fan, W. (2010). Nanoparticles for tumor targeted therapies and their pharmacokinetics. *Current drug metabolism*, *11*(2), 129-141.

33. Ye, F., Zhao, Y., El-Sayed, R., Muhammed, M., & Hassan, M. (2018). Advances in nanotechnology for cancer biomarkers. *Nano Today*, *18*, 103-123.

34. Yu, X., Gao, D., Gao, L., Lai, J., Zhang, C., Zhao, Y., ... & Liu, Z. (2017). Inhibiting metastasis and preventing tumor relapse by triggering host immunity with tumor-targeted photodynamic therapy using photosensitizer-loaded functional nanographenes. *ACS nano*, *11*(10), 10147-10158.

35. Yue, X., & Dai, Z. (2018). Liposomal nanotechnology for cancer theranostics. *Current medicinal chemistry*, *25*(12), 1397-1408.

36. Zhang, Y., Yang, H., An, X., Wang, Z., Yang, X., Yu, M., ... & Wang, Q. (2020). Controlled synthesis of Ag2Te@ Ag2S Core–Shell quantum dots with enhanced and tunable fluorescence in the second near-infrared window. *Small*, *16*(14), 2001003.

37. Zhao, W., Zhang, R., Xu, S., Cai, J., Zhu, X., Zhu, Y., ... & Luo, J. (2018). Molecularly imprinted polymeric nanoparticles decorated with Au NPs for highly sensitive and selective glucose detection. *Biosensors and Bioelectronics*, *100*, 497-503.

Chapter - 8
Dengue Vaccines and Its New Dimensions: A Short Review

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Chapter - 8

Dengue Vaccines and Its New Dimensions: A Short Review

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Abstract

Now-a-days, Dengue is a remarkable public health matter in different areas of the world. It is a mosquito vector borne disease. It has been reported that per annum nearly 400 million dengue incidents and 22000 deaths occur. The causative organism of this disease is an arbovirus under Flaviviridae family. The Vector of this disease is *Aedes* mosquitoes. The insufficiency of effective antiviral treatments has made the immediate need to design the vaccines to cope up with the dengue spreading. This review article reveals an idea about the development and efficacy of different dengue vaccines to point on the advancements, challenges, and future prospects. Currently researchers are trying to focus on the different vaccines such as viral vectored vaccine, recombinant subunit vaccine, DNA vaccine, inactivated vaccine, live attenuated vaccine, and mentioning their mechanisms of action and immunogenic details. Moreover, different report is already highlighted about the results of clinical trials to know their safety, efficacy, and immunogenicity among the different age groups. Researchers search on the continuous efforts of various research scholars to overcome the various challenges through novel vaccine developments and techniques for increasing the cross-protection against different dengue serotypes. In this context, we try to provide an idea regarding the development of de novo dengue vaccine depending on the epitope-based peptide vaccine designing approach. A potential strategy for vaccine design may provide future guidelines for dengue vaccine research. These guidelines would serve as a foundation for research scholars, academia, industry, and public health stakeholders to develop safe and effective vaccines aimed at combating dengue illness.

Keywords: Dengue vaccines, mosquito-borne, efficacy, immunogenic, epitope, serotype

Introduction

Under Flaviviridae family, Dengue virus (DENV) is a +ssRNA virus [1]. The diameter of mature spherical shaped DENV particles is 50 nm. Mainly

DENV consists of three structural proteins, i.e., Capsid (C), Membrane (M) and Envelope (E) protein layers respectively. In addition with this, a lipid bilayer and a RNA genome also present there [2]. DENV is spread by two mosquitoes *Aedes aegypti* and *Aedes albopictus*. DENV has four different serotypes such as DENV-1, DENV-2, DENV-3, and DENV-4 which co-circulate among masses globally [3, 4].The common symptoms of dengue are anorexia, joint pain, muscle pain, eyelid pain, abrupt fever, stomach pain and headache. Dengue Hemorrhagic Fever (DHF), Dengue Fever (DF), and Dengue Shock Syndrome (DSS) are caused by these four serotypes [5]. Every year 96 million out of 390 million dengue infections show clinical symptoms at any rate of illness intensity [6]. Till now, for treating dengue infection there is no appropriate medication. One general preventive mechanism is vector control but specific preventive mechanism is the dengue vaccine development [7]. Investigations have been directed on five different types of dengue vaccines: viral vectored vaccine, DNA vaccine, inactivated vaccine, recombinant subunit vaccine, live attenuated vaccine [8].These vaccines mainly function by enhancing the immune reactions against the E protein and the non-structural protein-1 of the dengue virus (DENV) [9]. In this review we discuss about various types of vaccines with their serotype inhibiting limitations and also about epitope based peptide vaccine. An appropriate vaccine designing is very critical because DENV alters their genetic make up time to time.

Live attenuated vaccines

They are antigenic substances which are made up of a live pathogen which is transformed to show either virulence or avirulence state. A set of protective antigens have been delivered by this vaccines and long term immunity is seen [10]. Live Attenuated CYD-TDV (Dengvaxia®) vaccines are used only for above 9 years old people [11]. In Thailand, Asia and Latin America, clinical trials reveal that CYD-TDV is unable to work against DENV -2 (Fig.1) [12,13,14]. In April 2018, the WHO Strategic Advisory Panel suggested that only individuals who are dengue-positive should receive the CYD-TDV vaccination due to the high risk of the dengue that comes with vaccinating seronegative subjects [15].Wider humoral and cell-mediated immune response is triggered by the Qdenga® vaccine [16]. But yet it has some limitations. In endemic areas, in the case of children and adolescents aged 4 to 16, Qdenga® show protective effect but vaccination against DENV3 and DENV4 serotypes it did not provide any protection against dengue naïve individuals [17]. Live Attenuated Tetravalent Vaccination (For Ex- TV003/TV005) has been found to be very tolerant to all of the individuals with a mild asymptomatic macular rash development [18]. The re-inoculation effects in offspring of mothers who

had the DENVax vaccination would be affected; AG129 mice were utilized as the model [19]. Trials of these vaccines are represented in Fig.1.

Inactivated virus vaccines

Inactivated Vaccines made up of inactive substance of pathogen like virus, bacteria and can protect the disease causing microorganisms when enter into the body [20]. But this vaccines are less effective to elicit strong immune response for lifetime protection [21].

Recombinant subunit vaccines

Using disassembled viral particles within cell culture or recombinant DNA technique a recombinant subunit vaccine is prepared [22]. But recombinant dengue proteins expression indicates endotoxin contamination and incorrect protein folding issues [23].

Viral vectored vaccines

To produce this vaccine, three kind of viruses like alphavirus, vaccinia virus and adenovirus have been used as vectors for DENV antigens [24]. These vectors have some limitations related to pre-existing immunity [25].

DNA Vaccines

For expressing antigens to boost immune responses, DNA vaccine which is a plasmid can be administered in vivo [26].Due to stability, cost saving, easy to prepare, these vaccines are accurate for huge production but main drawbacks that they lack high immunogenicity [27].

Conclusion and Future perspectives

The major vaccination strategies against the all serotypes of dengue are viral vectored vaccine, DNA vaccine, inactivated vaccine, recombinant subunit vaccine, live attenuated vaccine. Live attenuated vaccine Dengvaxia® or CYD-TDV is ineffective for DENV-2 (Fig.1). Inactivated vaccines and DNA vaccines are less immunogenic. Recombinant subunit vaccines have incorrect protein folding and endotoxin contaminating issues. All these vaccines show their limitations. Standing on this situation, Epitope Based Peptide Vaccine development may take the responsibility to eradicate the above vaccines' limitations because it already reported many advantages. In epitope based vaccines, peptides are economic, time saving, safest and easy to use. By altering peptide's structure, conjugated or multi-epitope structures can be selected and designed against DENV [31, 32].

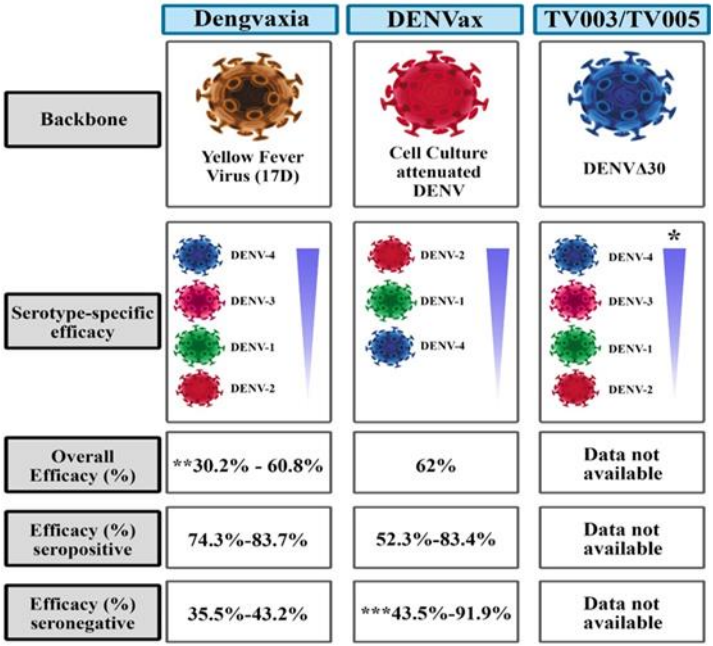

Fig 1: An overview of children's anti-dengue virus (DENV) vaccination efficacy trials conducted in Latin America and Asia. Results from phase III clinical trials for DENVax and Dengvaxia are not entirely consistent. At present phase III clinical trials are being done on TV003/TV005.*Illustrated are the TV003/TV005 seroconversion rates found in phase II clinical trials.**The efficacy recorded in the phase II trial carried out in Thailand is represented by the lower value of the efficacy range. ***DENVax only worked against DENV-2 and DENV-1 in people who were seronegative [28, 29, 30].

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References

1. Clyde K, Kyle JL, & Harris E. Recent advances in deciphering viral and host determinants of dengue virus replication and pathogenesis. *Journal of virology*, 2006; *80*(23), 11418-11431.
2. Dengue WHOI. 2009. Guidelines for diagnosis, Treatment. Prevention and Control. https://www.ncbi.nlm.nih.gov/books/NBK143157.
3. Tripathi NK, & Shrivastava A. Recent developments in recombinant protein-based dengue vaccines. *Frontiers in immunology*, 2018; *9*, 1919.

4. Dasilveira LTC, Tura, B., & Santos, M. (2019). Systematic review of dengue vaccine efficacy. *BMC infectious diseases*, *19*, 1-8.

5. Murrell, S., Wu, S. C., & Butler, M. (2011). Review of dengue virus and the development of a vaccine. *Biotechnology advances*, *29*(2), 239-247.

6. World Health Organization. 2012. Global strategy for dengue prevention and control 2012-2020. https://www.who.int/denguecontrol/9789241504034/en/

7. Deng SQ, Yang X, Wei Y, Chen JT, Wang XJ, & Peng HJ. 2020. A review on dengue vaccine development. *Vaccines*, *8*(1), 63.

8. Yauch LE, & Shresta S. Dengue virus vaccine development. *Advances in virus research*, 2014; *88*, 315-372.

9. Liu Y, Liu J, & Cheng G. 2016. Vaccines and immunization strategies for dengue prevention. *Emerging microbes & infections*, *5*(1), 1-6.

10. Whitehead SS, Blaney JE, Durbin AP, & Murphy BR. 2007. Prospects for a dengue virus vaccine. *Nature Reviews Microbiology*, *5*(7), 518-528.

11. Guy B, Noriega F, Ochiai RL, L'azou M., Delore, V., Skipetrova, A, & Jackson, N. (2017). A recombinant live attenuated tetravalent vaccine for the prevention of dengue. *Expert review of vaccines*, *16*(7), 671-684.

12. Hadinegoro, S. R., Arredondo-García, J. L., Capeding, M. R., Deseda, C., Chotpitayasunondh, T., Dietze, R, & Saville, M. (2015). Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. *New England Journal of Medicine*, *373*(13), 1195-1206.

13. Sabchareon A, Wallace D, Sirivichayakul C, Limkittikul K, Chanthavanich P, Suvannadabba S, & Lang, J. (2012). Protective efficacy of the recombinant, live-attenuated, CYD tetravalent dengue vaccine in Thai schoolchildren: a randomised, controlled phase 2b trial. *The Lancet*, *380*(9853), 1559-1567.

14. Capeding, M. R., Tran, N. H., Hadinegoro, S. R. S., Ismail, H. I. H. M., Chotpitayasunondh, T., Chua, M. N., ... &Bouckenooghe, A. (2014). Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *The Lancet*, *384*(9951), 1358-1365.

15. World Health Organization. (2019). Dengue vaccine: WHO position paper, September 2018-recommendations. *Vaccine*, *37*(35), 4848-4849.

16. EMA (European Medicines Agency). European public assessment report (2022-10-13), https://www.ema.europa.eu/en/documents/assessment-report/qdenga-epar-public-assessmentreport_en.pdf.

17. EMA (European Medicines Agency.). Summary of product characteristics (2022-12-16). https://www.ema.europa.eu/en/documents/product-information/qdenga-epar-product-information_en.pdf.

18. Durbin AP, Karron RA, Sun W, Vaughn DW, Reynolds MJ, Perreault JR, & Whitehead, S. S. (2001). Attenuation and immunogenicity in humans of a live dengue virus type-4 vaccine candidate with a 30 nucleotide deletion in its 3'-untranslated region. *The American journal of tropical medicine and hygiene*, *65*(5), 405-413.

19. Guy, B., Noriega, F., Ochiai, R. L., L'azou, M., Delore, V., Skipetrova, A, & Jackson, N. (2017). A recombinant live attenuated tetravalent vaccine for the prevention of dengue. *Expert review of vaccines*, *16*(7), 671-684.

20. Putnak, R., Barvir, D. A., Burrous, J. M., Dubois, D. R., D'Andrea, V. M., Hoke, C. H., ... &Eckels, K. H. (1996). Development of a purified, inactivated, dengue-2 virus vaccine prototype in Vero cells: immunogenicity and protection in mice and rhesus monkeys. *Journal of Infectious Diseases*, *174*(6), 1176-1184.

21. Wodi AP, & Morelli V. 2021. Chapter 1: Principles of Vaccination. *Epidemiology and Prevention of Vaccine-Preventable Diseases, 14th ed.; Hall, E., Wodi, AP, Hamborsky, J., Morelli, V., Schilllie, S., Eds*.

22. Francis MJ. (2018). Recent advances in vaccine technologies. *Veterinary Clinics: Small Animal Practice*, *48*(2), 231-241.

23. Berlec A, &Štrukelj B. Current state and recent advances in biopharmaceutical production in Escherichia coli, yeasts and mammalian cells. *Journal of industrial microbiology and biotechnology*, 2013; *40*(3-4), 257-274.

24. Yauch LE, & Shresta S. Dengue virus vaccine development. *Advances in virus research*, 2014; *88*, 315-372.

25. Ura T, Okuda K, & Shimada M. Developments in viral vector-based vaccines. *Vaccines*, 2014; *2*(3), 624-641.

26. Kochel T, Wu SJ, Raviprakash K, Hobart P, Hoffman S, Porter K, & Hayes C. 1997. Inoculation of plasmids expressing the dengue-2 envelope gene elicit neutralizing antibodies in mice. *Vaccine*, *15*(5), 547-552.

27. Danko, J. R., Beckett, C. G., & Porter, K. R. (2011). Development of dengue DNA vaccines. *Vaccine*, *29*(42), 7261-7266.

28. Torres-Flores JM, Reyes-Sandoval A, & Salazar MI. Dengue Vaccines: An Update. *BioDrugs: clinical immunotherapeutics, biopharmaceuticals and gene therapy*, 2022; *36*(3), 325-336.

29. Villar L, Dayan GH, Arredondo-García JL, Rivera DM, Cunha R, Deseda C, & Noriega F. 2015. Efficacy of a tetravalent dengue vaccine in children in Latin America. *New England Journal of Medicine*, *372*(2), 113-123.

30. Capeding, M. R., Tran, N. H., Hadinegoro, S. R. S., Ismail, H. I. H. M., Chotpitayasunondh, T., Chua, M. N., ... &Bouckenooghe, A. (2014). Clinical efficacy and safety of a novel tetravalent dengue vaccine in healthy children in Asia: a phase 3, randomised, observer-masked, placebo-controlled trial. *The Lancet*, *384*(9951), 1358-1365.

31. Moisa AA, &Kolesanova EF. Synthetic peptide vaccines. *Insight and control of infectious disease in global scenario*, 2012; 201-228.

32. Sesardic, D. (1993). Synthetic peptide vaccines. *Journal of medical microbiology*, *39*(4), 241-242.

Chapter - 9
Burning Issues: A Comprehensive Study Report on Agricultural and Forest Fires in West Bengal

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Chapter - 9

Burning Issues: A Comprehensive Study Report on Agricultural and Forest Fires in West Bengal

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Abstract

The agricultural and forest fires in West Bengal have emerged as pressing concerns, demanding a comprehensive study to understand their causes, impacts, and potential mitigation strategies. These fires pose a significant threat to both rural and ecological landscapes, adversely affecting agricultural productivity and biodiversity. The study delves into the root causes, which may include human activities, climatic factors, and land-use patterns. It also explores the socio-economic consequences on local communities and wildlife habitats. Identifying sustainable solutions, such as improved land management practices, early detection systems, and community awareness programs, is crucial to mitigating the adverse effects of these fires and ensuring the long-term environmental health of West Bengal.

Keywords: Agricultural fire, forest fire, sustainable solutions, West Bengal.

1. Introduction

The act of open fire burning poses a severe threat to the environment, manifesting various risks in diverse ways. The release of greenhouse gases, such as carbon dioxide and methane, intensifies global warming, resulting in detrimental effects on ecosystems across the globe. This practice not only disrupts climate patterns but also plays a role in deforestation, causing the loss of habitats for numerous species and disturbing ecological balances. The pollutants and particulate matter emitted during open fire burning have adverse effects on air quality, contaminating soil and water resources and posing risks to their overall health. In summary, open fire burning perpetuates a cycle of environmental degradation, compromising biodiversity, worsening climate crises, and endangering the overall health and resilience of our planet.

The burning of agricultural residue has traditionally been employed to clear fields post-harvest, but both this practice and forest fires have been recognized as notable contributors to air pollution. The combustion of crop

residue is particularly detrimental, releasing particulate matter, carbon monoxide, volatile organic compounds (VOCs), and nitrogen oxides into the air. These emissions compromise air quality, resulting in the formation of smog and diminished visibility. Forest fires, whether caused naturally or by human activities, also play a significant role in pollution (Mehmood *et al*, 2022). The smoke released from these fires contains particulate matter, carbon dioxide, methane, and other harmful substances that substantially contribute to regional and even global air pollution levels (Govardhan, G. *et al* 2023). These emissions not only impact local air quality but can also traverse great distances, affecting air quality in distant regions. This exacerbates health issues like respiratory problems and cardiovascular diseases while contributing to the greenhouse gases responsible for climate change.

In recent years, the incidence of forest and agricultural fires in West Bengal has significantly risen, partly attributable to the impact of climate change. The region has experienced a troubling surge in fire occurrences, affecting both forested areas and agricultural lands. Climate change has induced drier conditions, characterized by rising temperatures and unpredictable rainfall, fostering the initiation and rapid spread of fires (Dey, S., & Chowdhury, S., 2022). Prolonged dry spells, coupled with elevated temperatures, have heightened the susceptibility of forests and agricultural fields to ignition, creating an environment more conducive to the proliferation of fires. Furthermore, the altered climate has disrupted natural fire cycles, resulting in more frequent and intense blazes that pose a threat to ecosystems and livelihoods alike. The management and mitigation of these fires face challenges posed by the evolving climatic conditions, necessitating adaptive strategies that prioritize climate resilience.

2. Methodology

Specialized sensors with the ability to identify heat signatures and changes in land surface temperature are employed to detect open fires through satellite technology. Satellites equipped with thermal infrared sensors play a crucial role in pinpointing these fires by capturing the thermal radiation emitted from the Earth's surface. Through the analysis of temperature fluctuations, algorithms can distinguish between natural hotspots such as volcanoes and industrial sources, and those generated by open fires. NASA's Moderate Resolution Imaging Spectroradiometer (MODIS) and the European Space Agency's Sentinel satellites regularly capture images and data, enabling the continuous monitoring of fire occurrence, spread, and intensity. This information, coupled with the generation of fire maps, proves indispensable in firefighting efforts and efficient land management.

2.1 Data Collection

Information on open fires in West Bengal spanning from 2013 to 2022 was obtained from the Visible Infrared Imaging Radiometer Suite (VIIRS) satellite sensor, accessed through the NASA FIRMS website. VIIRS provides detailed data on thermal anomalies linked to wildfires, allowing for the identification and examination of open fires in the specified region.

2.2 Data Pre-processing

The collected VIIRS data underwent pre-processing to ensure precision and dependability. This included filtering out low-confidence, non-fire-related signals to enhance the quality of the dataset.

2.3 Temporal Analysis

Temporal patterns and trends related to open fires were scrutinized by organizing the fire data into temporal bins (such as monthly, seasonal, and yearly intervals). This approach facilitated the exploration of seasonal variations and long-term trends.

2.4 Spatial Analysis

Spatial distribution patterns of open fires were explored using Geographic Information System (GIS) techniques. The geospatial analysis involved overlaying fire occurrence data onto geographical features to reveal spatial correlations, identify hotspots, and assess fire-prone zones within West Bengal.

2.5 Analysis tool

The analysis was conducted using open-source tools like QGIS, and the R programming language.

3. Results

3.1 Rising Incidence of Fires in West Bengal

Over the past decade, West Bengal has witnessed a growing trend in the occurrence of fires. The linear trend analysis indicates an annual increase of 500 new fire events. The period of COVID-19 saw a notable decline in fire incidents, likely linked to the associated restrictions. Nevertheless, the overall rise in the number of fire occurrences is a cause for serious concern (Figure 1A).

Peak incidents were noted during summer and winter. Summer fires result from both forest fires and agricultural stubble burning, while winter fires are predominantly associated with agricultural stubble burning. Minimal fire

counts were observed during the monsoon season. The seasonal variation highlights winter and summer as the primary periods for fire occurrences (Figure 1B). Established in 2017, West Bardhaman district claimed the highest position in terms of fire occurrences. Despite being the fourth smallest district in West Bengal, West Bardhaman surpassed others in this regard. Following closely are Paschim Medinipur, Purba Medinipur, Jhargram, Bankura, and Purulia. In contrast, Kolkata, Coochbehar, and 24-Parganas registered the lowest number of district-wise fire events (Figure 2A).

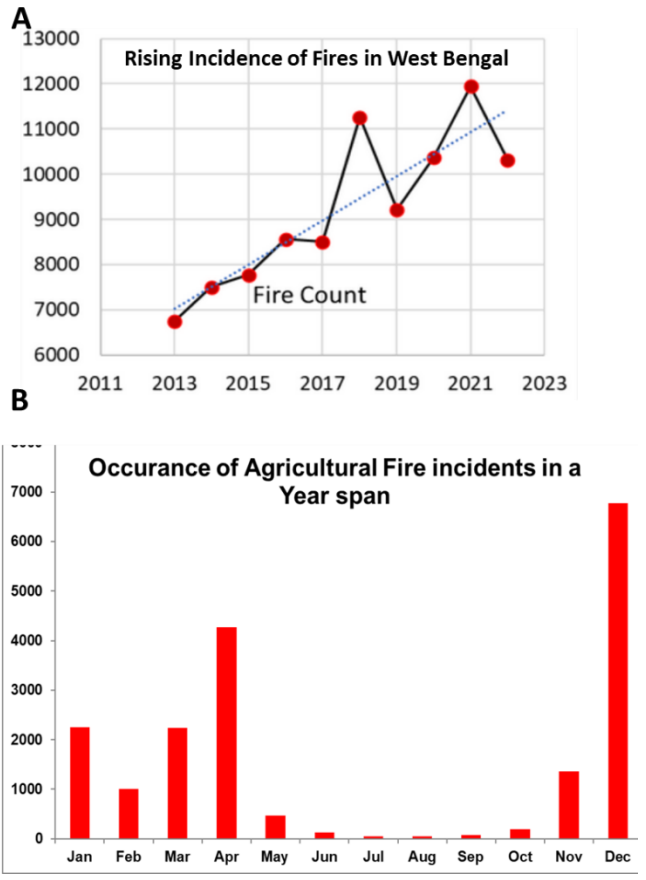


Fig 1: Incidence of Fires in West Bengal. A. Number of fire Incidence per year, B. Distribution of agricultural fire with respect to months in a year.

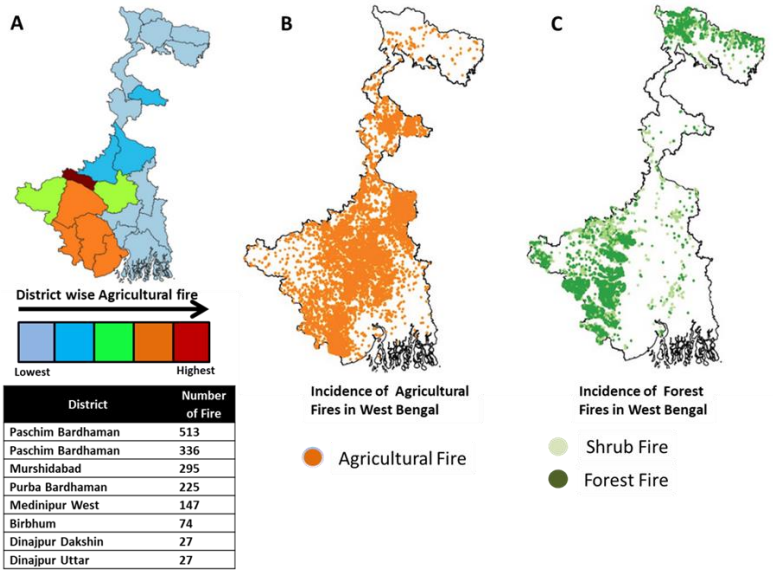
3.2 Distribution of forest and agricultural fire with in state

The fires were additionally categorized based on their land-use class, utilizing ESRI land-use and land-cover data with a resolution of 10 meters in

this study. Notably, forest fires are predominantly concentrated in the laterite zone (Purulia, Bankura, Paschim Medinipur, Jhargram), while agricultural fires are dispersed throughout South Bengal. Additionally, significant forest fires were observed in North Bengal (Figure 2B).

Moreover, trend analysis was conducted for both forest and agricultural fires, revealing an upward trajectory for both types. The observed rates of increase were 150/year for agricultural fires and 72/year for forest fires. However, post-2020, a significant decline in agricultural fires was noted for two consecutive years. This decline is attributed to awareness initiatives and the complete prohibition of agricultural residue burning implemented by the West Bengal government (Figure 2C).

During summer, particularly in March, the peak of forest fires becomes evident. Conversely, occurrences of agricultural fires are distributed throughout both summer and winter, notably in the months of April and December.



District	Number of Fire
Paschim Bardhaman	513
Paschim Bardhaman	336
Murshidabad	295
Purba Bardhaman	225
Medinipur West	147
Birbhum	74
Dinajpur Dakshin	27
Dinajpur Uttar	27

Fig 2: Distribution of Fires in West Bengal. A. District wise Distribution of Fires. B. Distribution of Agricultural Fires in West Bengal C. Distribution of Forest Fires in West Bengal.

4. Discussion

Reducing agricultural fires requires a combination of education, policy support, technological innovation, and collaborative efforts (Reddy. C, 2012). By fostering a culture of sustainability and providing the necessary tools and

incentives, we can work towards minimizing the environmental and health impacts associated with agricultural burning. Promoting the use of modern technologies can also contribute to reducing the need for burning (A, Mark *et al*, 2010). Village level fire alert set up, promoting organic based sustainable agricultural system, increase awareness among farmers may bring a change in the scenario. The implementation of a robust fire management system is crucial for safeguarding forests and mitigating the devastating impacts of wildfires (). A comprehensive approach involves a combination of technology, preparedness, and community engagement. Advanced technologies such as satellite imagery, drones, and remote sensing play a pivotal role in early detection of wildfires. These tools provide real-time information on fire locations and progression, enabling rapid response efforts. Automated systems that analyze data can help in identifying potential fire hotspots and triggering timely alerts. Preventive measures are equally important in effective fire management. Furthermore, a coordinated communication system is vital for efficient fire management. This includes the use of emergency alert systems to notify residents and stakeholders, as well as facilitating seamless communication among firefighting teams. Regular drills and simulations help ensure that responders are well-prepared to handle emergencies. The management of waste fires requires a proactive and comprehensive approach to minimize environmental pollution and health hazards associated with uncontrolled burning. A key element in improving fire management in waste is the establishment of proper waste disposal and recycling systems. Ensuring that communities have access to efficient waste collection and recycling facilities reduces the likelihood of illegal dumping and subsequent fires.

Advanced technologies, such as sensors and monitoring systems, can be employed to detect early signs of waste fires. Real-time data collection and analysis enable authorities to respond swiftly, preventing the escalation of fires and minimizing their impact. Additionally, incorporating fire-resistant materials in waste storage and disposal infrastructure can help contain and limit the spread of fires when they do occur. Community education plays a crucial role in waste fire prevention. Informing residents about the dangers of burning waste and promoting responsible waste disposal practices can contribute to reducing the occurrence of such fires. Awareness campaigns can also emphasize the importance of separating hazardous materials from general waste, minimizing the risk of toxic emissions during fires.

Reference

1. Dey, S., & Chowdhury, S. (2022). Air quality management in India using satellite data. In *Asian Atmospheric Pollution* (pp. 239-254). Elsevier.

2. Mehmood, K., Bao, Y., Saifullah, Bibi, S., Dahlawi, S., Yaseen, M., ... & Faraj, T. K. (2022). Contributions of open biomass burning and crop straw burning to air quality: current research paradigm and future outlooks. *Frontiers in Environmental Science*, *10*, 852492.

3. Appiah, Mark & Damnyag, Lawrence & Blay, Dominic & Pappinen, Ari. (2010). Forest and agroecosystem fire management in Ghana. Mitigation and Adaptation Strategies for Global Change. 15. 551-570.

4. Reddy, C. S., Krishna, P. H., Anitha, K., & Joseph, S. (2012). Mapping and Inventory of Forest Fires in Andhra Pradesh, India: Current Status and Conservation Needs. *ISRN Forestry*, *2012*, 1–10.

5. Sewak, R., Vashisth, M., & Gupta, L. (2021). Forest Fires in India: A Review. 23(07), 247–259.

6. Sewak, Reuben & Vashisth, Mehak & Gupta, Lovleen. (2021). Forest Fires in India: A Review. Journal of University of Shanghai for Science and Technology. 23. 247-259.

Chapter - 10
Aloe Vera: An In-Depth Exploration of Its Therapeutic and Medicinal Uses

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Chapter - 10

Aloe Vera: An In-Depth Exploration of Its Therapeutic and Medicinal Uses

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Abstract

Aloe vera, renowned for its medicinal properties, has been utilized across various cultures for centuries. This study offers a thorough overview of its therapeutic and medicinal uses. Aloe vera possesses a wide range of applications, including wound healing, skincare, gastrointestinal health, and immune modulation. Its gel contains bioactive compounds like polysaccharides, glycoproteins, and antioxidants, contributing to its healing properties. Topically, Aloe vera gel aids in wound healing, inflammation reduction, and managing skin conditions such as burns, psoriasis, and acne. Internally, Aloe vera juice supports gastrointestinal health and shows promise in conditions like irritable bowel syndrome (IBS) and ulcerative colitis. Additionally, it exhibits immunomodulatory effects, potentially aiding immune function and related disorders. Despite its traditional use and promising research, further clinical studies are needed to validate Aloe vera's efficacy and safety. Nonetheless, its versatility makes it a valuable asset in integrative medicine and holistic health practices. This review underscores the ongoing importance of exploring Aloe vera's therapeutic potential for improving human health.

Keywords: Aloe vera, bioactive compounds, medical application, therapeutic

Introduction

Aloe vera, originating from the Arabian Peninsula, has attracted considerable attention due to its extensive therapeutic and medicinal attributes. Across diverse cultures and over centuries, Aloe vera has been revered for its healing properties, leading to its widespread use in traditional medicine and sparking growing interest in scientific circles. Its gel, extracted from the leaves, contains a rich array of bioactive compounds, including vitamins, minerals, enzymes, polysaccharides, and phenolic compounds, which collectively contribute to its notable health benefits. Historically, Aloe

vera has been valued for its healing capabilities, with ancient civilizations such as the Egyptians, Greeks, and Romans leveraging its rejuvenating properties. Throughout the ages, Aloe vera has been applied to address various conditions, spanning from skin disorders and digestive issues to wound healing and infections. Presently, modern research continues to uncover the therapeutic potential of Aloe vera, elucidating its mechanisms of action and clinical applications. Recent scientific investigations have delved into the pharmacological properties of Aloe vera, unveiling its anti-inflammatory, antioxidant, immunomodulatory, antimicrobial, and wound-healing effects, among others. Furthermore, studies have highlighted its potential in managing numerous health conditions, including skin ailments like psoriasis and dermatitis, gastrointestinal disorders such as ulcerative colitis and irritable bowel syndrome, and metabolic conditions like diabetes and obesity. With the escalating interest in natural remedies and alternative medicine, Aloe vera emerges as a versatile botanical agent with broad therapeutic applications. Its accessibility, safety, and efficacy make it an attractive option for both preventive healthcare and complementary therapy. However, challenges persist, including the standardization of Aloe vera products, determination of optimal dosage and formulation, and rigorous clinical validation (Razia *et al*., 2021).

Aloe Vera: The multidimensiosal plant

Aloe vera, scientifically referred to as *Aloe barbadensis Miller*, is a succulent plant species from the Asphodelaceae family, originally native to the Arabian Peninsula but extensively cultivated worldwide for its medicinal and therapeutic attributes. The gel extracted from the succulent leaves of Aloe vera contains a plethora of biologically active constituents, including polysaccharides, vitamins (such as A, C, and E), minerals (like calcium, magnesium, and zinc), enzymes (such as amylase and lipase), phenolic compounds (including flavonoids and anthraquinones), and amino acids.

Among its components, polysaccharides, notably acemannan, are prominent for their immunomodulatory and anti-inflammatory characteristics. They stimulate macrophages, bolstering immune responses, and inhibit pro-inflammatory mediators, thereby mitigating inflammation. Aloe vera gel's antioxidant properties, attributed to vitamins C and E, flavonoids, and polyphenols, combat oxidative stress and cellular damage, promoting skin health and counteracting aging-related conditions. Enzymes within Aloe vera, including amylase, lipase, and catalase, contribute to digestion, lipid metabolism, and antioxidant defense mechanisms, respectively, enhancing digestive function and nutrient absorption. Anthraquinones present in Aloe

vera, such as aloin and emodin, exert laxative effects, traditionally utilized for gastrointestinal ailments, although prolonged use may lead to adverse effects like electrolyte imbalance.

Moreover, Aloe vera gel contains amino acids, essential for various physiological functions, with some, like glutamine and arginine, implicated in wound healing and tissue repair. The diverse array of bioactive compounds in Aloe vera gel underscores its multifaceted therapeutic properties, encompassing anti-inflammatory, immunomodulatory, antioxidant, antimicrobial, wound-healing, and digestive effects. These scientific insights highlight the potential health benefits of Aloe vera, prompting extensive research into its medicinal applications (Sonawane *et al*., 2021).

Medicinal properties

Aloe vera, a perennial succulent belonging to the Asphodelaceae family, has been revered for centuries for its medicinal properties. Originating from the Arabian Peninsula, North Africa, and certain parts of Asia, Aloe vera has been a staple in traditional medicine systems. Modern scientific inquiry has delved into the bioactive components of Aloe vera, shedding light on its pharmacological actions and potential therapeutic applications across various medical domains. The medicinal properties of Aloe vera encompass a broad spectrum of therapeutic effects, ranging from wound healing and anti-inflammatory action to skincare and gastrointestinal benefits. The synergistic interplay of its bioactive constituents underpins its efficacy in diverse medical applications. Further research endeavors are warranted to elucidate the underlying mechanisms of action and optimize the utilization of Aloe vera in pharmaceuticals, cosmetics, and complementary medicine (Sánchez *et al.*, 2020).

Wound healing

Among the well-established medicinal properties of Aloe vera is its prowess in wound healing. The gel extracted from the inner leaf of Aloe vera contains polysaccharides, predominantly glucomannans, which foster fibroblast proliferation and collagen synthesis, thereby expediting tissue regeneration. Additionally, bioactive molecules like gibberellins expedite wound closure by stimulating cell proliferation and migration. Clinical investigations have corroborated the efficacy of Aloe vera gel in hastening the healing process of burns, lacerations, and other dermal injuries.

Anti-inflammatory effects

Aloe vera manifests potent anti-inflammatory attributes attributed to its diverse array of bioactive constituents. Salicylic acid, a natural anti-

inflammatory compound found in Aloe vera, hampers the production of pro-inflammatory mediators such as prostaglandins and leukotrienes. Furthermore, the polysaccharides present in Aloe vera gel exert immunomodulatory effects, dampening the inflammatory cascade. These mechanisms collectively alleviate inflammatory conditions such as arthritis, dermatitis, and sunburn.

Skin moisturization

The moisturizing properties of Aloe vera render it a sought-after ingredient in skincare formulations. Its gel formulation creates a protective barrier on the skin, thwarting transepidermal water loss and preserving hydration. Additionally, Aloe vera gel encompasses humectant compounds that attract moisture to the skin, rendering it efficacious as an emollient for dry and sensitive skin types.

Sunburn relief

Aloe vera gel is extensively employed for its palliative effects on sunburned skin. Its anti-inflammatory and cooling properties furnish immediate relief from pain and erythema associated with sunburn. Moreover, Aloe vera gel expedites the amelioration of UV-induced skin damage by fostering epithelialization and curbing inflammation.

Acne treatment

Aloe vera holds promise as a natural remedy for acne vulgaris owing to its antibacterial, anti-inflammatory, and wound-healing properties. Its antimicrobial activity against acne-causing bacteria like Propionibacterium acnes, coupled with its capability to diminish inflammation and facilitate tissue regeneration, positions Aloe vera as a valuable adjunctive therapy for acne management.

Antibacterial and Antifungal activity

Aloe vera harbors bioactive compounds endowed with broad-spectrum antibacterial and antifungal properties, rendering it invaluable in combating microbial infections. Anthraquinones present in Aloe vera exert potent antibacterial effects against both Gram-positive and Gram-negative bacteria. Furthermore, phenolic compounds inherent in Aloe vera possess antifungal properties, inhibiting the proliferation of pathogenic fungi such as Candida albicans.

Digestive aid

Aloe vera juice, derived from the inner leaf latex, has traditionally been employed as a digestive tonic. Its laxative effects stem from anthraquinone

glycosides, such as aloin and barbaloin, which stimulate peristalsis and bowel movement. Additionally, the polysaccharides present in Aloe vera gel exert prebiotic effects, fostering the proliferation of beneficial gut flora and enhancing gastrointestinal health.

Oral health promotion

The antimicrobial properties of Aloe vera position it as a promising adjunctive therapy for maintaining oral hygiene. Aloe vera mouthwash impedes the growth of oral pathogens, mitigating plaque formation and gingival inflammation. Furthermore, its wound-healing attributes facilitate the resolution of oral ulcers and mucosal lesions, bolstering overall oral health.

Anti-aging effects

Aloe vera boasts antioxidant properties attributable to the presence of vitamins C and E, alongside flavonoids and phenolic compounds. These antioxidants scavenge free radicals and mitigate oxidative stress, thereby forestalling premature skin aging. Consistent application of Aloe vera gel augments collagen synthesis, enhances skin elasticity, and diminishes the appearance of wrinkles and fine lines, bestowing a youthful visage.

Bioactive compounds in aloe vera

Aloe vera harbors a rich array of bioactive compounds that underpin its diverse medicinal properties. These compounds include (Solaberrieta *et al*., 2020):

Polysaccharides

Acemannan: Acemannan, a β-(1,4)-linked acetylated mannan polysaccharide, is one of the primary polysaccharides present in Aloe vera gel. It exhibits immunomodulatory effects by stimulating macrophage activity, enhancing natural killer cell function, and promoting cytokine production. Acemannan also possesses wound-healing properties, facilitating fibroblast proliferation and collagen synthesis.

Glucomannans: Glucomannans are complex polysaccharides composed of glucose and mannose units. These polysaccharides contribute to the mucilaginous nature of Aloe vera gel and exhibit moisturizing effects when applied topically. Additionally, glucomannans have been shown to modulate the inflammatory response and enhance wound healing.

Gibberellins

Gibberellins are phytohormones that regulate various physiological processes in plants, including cell elongation and differentiation. In Aloe vera,

gibberellins such as gibberellin A1 (GA1) and gibberellin A3 (GA3) have been identified. These compounds play a crucial role in wound healing by promoting cell proliferation, migration, and tissue regeneration.

Salicylic Acid

Aloe vera contains salicylic acid, a phenolic compound with anti-inflammatory, analgesic, and antimicrobial properties. Salicylic acid exerts its anti-inflammatory effects by inhibiting the activity of cyclooxygenase enzymes and reducing the production of pro-inflammatory prostaglandins. Furthermore, it exhibits antimicrobial activity against various bacteria and fungi, making it effective in the treatment of acne and other skin infections.

Anthraquinones

Anthraquinones are phenolic compounds found predominantly in the latex of Aloe vera leaves. These compounds, including aloin, barbaloin (aloe-emodin glycoside), and emodin, possess laxative properties and contribute to the purgative effects of Aloe vera latex. Anthraquinones also exhibit antibacterial, antiviral, and antifungal activities, making them valuable in the treatment of gastrointestinal infections and skin conditions.

Phenolic Compounds

Aloe vera contains various phenolic compounds, including flavonoids (e.g., quercetin, kaempferol) and phenolic acids (e.g., caffeic acid, ferulic acid). These compounds possess antioxidant properties, scavenging free radicals and mitigating oxidative stress. Additionally, phenolic compounds exert anti-inflammatory effects by inhibiting pro-inflammatory cytokines and enzymes, such as cyclooxygenase and lipoxygenase.

Enzymes

Aloe vera gel contains several enzymes, including proteases (e.g., catalase, peroxidase), lipases, cellulases, and carbohydrases. These enzymes contribute to the breakdown of proteins, lipids, and carbohydrates, aiding in digestion and nutrient absorption. Proteolytic enzymes, such as bromelain and papain, facilitate the removal of dead skin cells and promote wound healing by debriding necrotic tissue.

Vitamins and Minerals

Aloe vera is a rich source of vitamins, including vitamin C (ascorbic acid), vitamin E (tocopherol), vitamin B12 (cyanocobalamin), and vitamin A (retinol). These vitamins exhibit antioxidant properties and play essential roles in skin health, immune function, and cellular repair. Additionally, Aloe vera

contains minerals such as zinc, magnesium, calcium, and selenium, which are necessary for enzymatic reactions and tissue integrity.

Amino Acids

Aloe vera contains all 8 essential amino acids, along with several non-essential amino acids, including arginine, glutamine, and glycine. These amino acids are the building blocks of proteins and peptides involved in tissue repair, collagen synthesis, and immune modulation. Amino acids also contribute to the moisturizing and soothing effects of Aloe vera gel when applied topically.

Antioxidant Effect

Aloe vera is celebrated for its robust antioxidant properties, stemming from its rich array of bioactive compounds, including vitamins, phenolic compounds, flavonoids, and polysaccharides. These antioxidants collectively play a pivotal role in neutralizing reactive oxygen species (ROS) and mitigating oxidative stress, thereby conferring numerous health benefits.

Vitamins: Aloe vera serves as a significant source of antioxidant vitamins, notably featuring vitamin C (ascorbic acid) and vitamin E (tocopherol). Vitamin C, functioning as a water-soluble antioxidant, scavenges free radicals and aids in regenerating vitamin E, enhancing its antioxidant capacity. Conversely, vitamin E, a fat-soluble antioxidant, shields cellular membranes against lipid peroxidation, a critical aspect of oxidative harm.

Phenolic compounds: Aloe vera boasts a spectrum of phenolic compounds, encompassing phenolic acids and flavonoids, renowned for their potent antioxidant prowess. These compounds act as effective scavengers of free radicals, impeding oxidative processes targeting lipids, proteins, and DNA. By thwarting oxidative damage, phenolic compounds significantly contribute to cellular resilience and longevity.

Flavonoids: Abundant within Aloe vera, flavonoids represent polyphenolic antioxidants with multifaceted therapeutic attributes, including anti-inflammatory and antimicrobial properties. By scavenging ROS and chelating metal ions, flavonoids ameliorate oxidative stress and inflammation. Furthermore, they modulate cellular signaling pathways implicated in oxidative stress response and immune modulation.

Polysaccharides: Aloe vera polysaccharides, notably acemannan and glucomannans, exert antioxidant effects by enhancing the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and

catalase. Moreover, these polysaccharides stimulate the synthesis of glutathione, a crucial cellular antioxidant, thereby reinforcing cellular defenses against oxidative insult. The antioxidant efficacy of Aloe vera extends across diverse physiological domains, offering protection against oxidative stress-related ailments and promoting holistic well-being. Emerging evidence suggests that regular consumption or topical application of Aloe vera may mitigate oxidative damage induced by environmental pollutants, ultraviolet radiation, and metabolic imbalances. Furthermore, Aloe vera antioxidants hold promise in mitigating the risk of chronic maladies, encompassing cardiovascular disorders, malignancies, and neurodegenerative conditions.

Mechanism of antioxidant action

The antioxidant effect of Aloe vera relies on a multifaceted mechanism orchestrated by its diverse array of bioactive compounds. These compounds, including vitamins C and E, phenolic compounds, flavonoids, and polysaccharides, operate in concert to combat oxidative stress through various pathways. Firstly, they act as scavengers of free radicals, donating electrons to unstable molecules and stabilizing them, thus preventing oxidative damage to cellular components. Additionally, Aloe vera's antioxidants facilitate the regeneration of endogenous antioxidants such as vitamin E, enhancing their capacity to neutralize free radicals effectively. Some antioxidants in Aloe vera, notably flavonoids, exert metal-chelating properties, sequestering transition metal ions and impeding their participation in the generation of highly reactive hydroxyl radicals. Moreover, Aloe vera polysaccharides stimulate the activity of endogenous antioxidant enzymes like superoxide dismutase and catalase, which convert harmful reactive oxygen species into less reactive forms. Furthermore, flavonoids and other phytochemicals in Aloe vera modulate intracellular signaling pathways involved in oxidative stress response and inflammation, thereby maintaining cellular homeostasis and mitigating oxidative damage. Additionally, antioxidants such as vitamin E protect cellular membranes from lipid peroxidation, preserving membrane integrity and function. Aloe vera compounds also bolster cellular antioxidant defense mechanisms by promoting the synthesis of endogenous antioxidants like glutathione. Together, these mechanisms underline the potent antioxidant effects of Aloe vera, offering protection against oxidative damage and contributing to overall health and well-being. Further elucidation of these mechanisms will advance our understanding and optimize the application of Aloe vera antioxidants in preventive and therapeutic interventions against oxidative stress-related diseases (Hęś *et al*., 2019; Kaparakou *et al*., 2021).

Future prospect

Research into the medicinal properties of Aloe vera opens up promising avenues for future exploration and application across various domains. In the realm of biomedical engineering, there's potential for the development of innovative drug delivery systems utilizing Aloe vera compounds. This could involve the creation of nanoformulations aimed at improving the bioavailability and targeted delivery of these bioactive components. Additionally, the integration of Aloe vera extracts into functional foods and nutraceuticals holds promise for enhancing overall health and well-being, offering convenient and effective means of harnessing its medicinal benefits. Further investigation into Aloe vera's skincare properties may lead to the development of advanced cosmeceuticals tailored to address specific dermatological conditions. Moreover, optimizing Aloe vera-based pharmaceutical formulations, coupled with rigorous clinical trials to validate their efficacy and safety, could expand treatment options for chronic inflammatory diseases and wound care. Biotechnological advancements, such as tissue culture and genetic engineering, present opportunities for improving the production and potency of Aloe vera compounds, ensuring sustainable sourcing for pharmaceutical and cosmetic applications. Establishing robust regulatory standards and promoting consumer education initiatives are crucial steps to ensure product quality, safety, and informed decision-making. By leveraging scientific progress and fostering interdisciplinary collaborations, Aloe vera stands poised to become a cornerstone of evidence-based medicine and wellness promotion in the future.

Conclusion

In conclusion, Aloe vera's antioxidant properties stem from a complex interaction among its bioactive components, comprising vitamins, phenolic compounds, flavonoids, and polysaccharides. These constituents collectively combat oxidative stress through various mechanisms, including scavenging free radicals, rejuvenating endogenous antioxidants, metal chelation, and modulation of intracellular signaling pathways. By effectively mitigating oxidative stress and preserving cellular integrity, Aloe vera contributes significantly to overall health and well-being. Future research aimed at understanding the specific mechanisms of action and therapeutic applications of Aloe vera antioxidants is essential to fully exploit their potential in addressing oxidative stress-related diseases. By gaining deeper insights into these mechanisms, we can optimize the incorporation of Aloe vera antioxidants into preventive and therapeutic strategies, thus advancing comprehensive approaches to health and wellness.

References

1. Hęś M, Dziedzic K, Górecka D, Jędrusek-Golińska A, & Gujska E. Aloe vera (L.) Webb: Natural Sources of Antioxidants - A Review. *Plant foods for human nutrition (Dordrecht, Netherlands)*, 2019; *74*(3), 255–265.
2. Kaparakou EH, Kanakis CD, Gerogianni, M., Maniati, M., Vekrellis, K., Skotti, E., & Tarantilis, P. A. (2021). Quantitative determination of aloin, antioxidant activity, and toxicity of Aloe vera leaf gel products from Greece. *Journal of the science of food and agriculture*, *101*(2), 414–423.
3. Razia S, Park H, Shin E, Shim KS, Cho E, & Kim SY. (2021). Effects of *Aloe vera* Flower Extract and Its Active Constituent Isoorientin on Skin Moisturization via Regulating Involucrin Expression: In Vitro and Molecular Docking Studies. *Molecules (Basel, Switzerland)*, *26*(9), 2626.
4. Sánchez, M., González-Burgos, E., Iglesias, I., & Gómez-Serranillos, M. P. (2020). Pharmacological Update Properties of *Aloe Vera* and its Major Active Constituents. *Molecules (Basel, Switzerland)*, *25*(6), 1324.
5. Solaberrieta, I., Jiménez, A., Cacciotti, I., & Garrigós, M. C. (2020). Encapsulation of Bioactive Compounds from *Aloe Vera* Agrowastes in Electrospun Poly (Ethylene Oxide) Nanofibers. *Polymers*, *12*(6), 1323.
6. Sonawane, S. K., Gokhale, J. S., Mulla, M. Z., Kandu, V. R., & Patil, S. (2021). A comprehensive overview of functional and rheological properties of aloe vera and its application in foods. *Journal of food science and technology*, *58*(4), 1217–1226.

Chapter - 11
Antimicrobial activity of Medicinal Plants

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Chapter - 11

Antimicrobial Activity of Medicinal Plants

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Abstract

Since ancient times, medicinal spices and herbs have been used in herbal medicine due to their healing properties. These is precious natural antimicrobial chemical substances that serve to treat infectious diseases brought on by bacteria and other pathogens. To treat a variety of diseases plant extracts, essential oils, and various secondary chemicals have been proven to have antiviral, antioxidant, antifungal & antibacterial attributes that have little or no unfavourable side effects. The antibacterial qualities of the compounds isolated from the medicinal plants show promise against a range of microorganisms, potentially impacting the food's shelf life and quality. Plants such as these have a wide range of therapeutic uses such as expectorants, diuretics, astringents, digestives, carminatives, and anti-inflammatory effects. The medical advantages of medicinal plants are explained by a variety of compounds found in them, as well as the structural features of the main compounds featured in this review. Both medicinal plants and antibiotics vary in the configuration and also in uses. Research on the components found in medicinal plants around their capacity to protect toward potentially harmful microbes needs to be conducted in order to enhance human health and promote the use of these products, which are less toxic and more affordable than conventional medicines. In this review we present a summery on the antibacterial properties of medicinal & herbal plants that are used widely for overcome illnesses in humans

Keywords: Medicinal plants, antimicrobial characteristics, medicinal plant extract, pathogens

Introduction

To defend against and cure many illnesses caused by bacterial, fungal, and viral infections. The growth of microbial immunity to currently available drugs was appropriate for the specific antimicrobial properties. Diseases consist of poisoning from food, toxic shock syndrome, osteomyelitis, and

other infections are caused by gram-positive germs like species of Staphylococcus. Gram-negative organisms such as Escherichia sp. produce bacterial illness, septicemia, influenza, and kidney and urinary tract infections (Amenu *et al*., 2012). Nature naturally bestowed plants with the capacity to produce medicinal properties and compounds. Based on their medicinal characteristics, specific plants have been utilized as medicinal products since the beginning (Ali *et al*., 2020). As therefore, these medicinal plants serve as crucial sources for naturally generated antibacterial substances that are used to treat illnesses brought on by infections and bacteria. WHO claimed, greatest place to obtain an enormous variety of remedies is from plants with medicinal properties (Manandhar *et al*., 2019). According to traditional and present medical concepts, plants and their biological factors were used to produce profitable drugs (Subhose *et al*., 2005). The essential oils, herbal extracts, and many other kinds of secondary chemicals have antibacterial, antifungal, antiviral, antioxidants, and little or no negative effects. These characteristics are essential for treatment of a variety of diseases (Hassan *et al*., 2019). Secondary compounds including flavonoids, alkaloid compounds, terpenoids, steroids, carotenoids, and other phenolic molecules are referred to as plant-based chemicals. It is commonly discovered in different components of plants. These plant-based compounds are used in the production of drugs to treat a variety of significant illnesses, such as gastrointestinal disorders, infections of the skin, respiratory illnesses, and UTIs. Their beneficial impact is significant (Mahato *et al*., 2018). Respiratory disorders are treated using herbal remedies such as Artemisia vulgaris, Boerhavia procumbens, Carum copticum, Euphorbia hirta, Hyoscyamus niger, and Zingiber officinale. (Khan *et al*., 2020). Many attempts have been performed over the past few years to recognize sophisticated antimicrobial substances from different natural resources (Ganapathy *et al*., 2016). In recent years, due to persistent factors that resulting in pathogen developing medicinal resistance, evaluating the antibacterial activity of various medicinal plants is particularly essential. Antimicrobial resistance needs to be restricted by utilizing a modest Therefore, analysing the components that assisted in the development of medicines with higher antimicrobial properties is the most essential phase (Ali *et al*., 2020). Plants are used to derive and utilize antimicrobial compounds for the preservation of food. From long ago, Chinese, Indians, and Egyptians have been preserving foods through spices and essential oils. Many spices, including pepper, nutmeg, cloves, cinnamon, ginger, garlic, mint, and turmeric, are native to Asia. (Dhiman *et al*., 2016). America produces nutmeg plants, sesame seeds, ginger, and pepper. The European continent is home to massive crops including basil, cilantro, thymus, dill tips, celery leaves, and

watercress (Gottardi *et al*., 2016). Herbs can also be used in cosmetics, fragrances, colouring, flavouring, and conventional food preparation techniques in the food sector because of their preservation qualities (Saxena *et al*., 2013). Spices may be used to identify a variety of chemicals with antibacterial action against particular types of bacteria that can affect its storage time and nutrient content. They provide plenty of positive perks, like increasing salivary flow, improving digestion, and helping to treat colds and also lowering the sensation of nausea and vomiting (Gottardi *et al*., 2016). The phytochemicals discovered in plant-based substances have been utilized for treating a variety of viral disorders. Plant displays minimal or no toxicity. We linked them with antibiotics to lessen antibiotic resistance because they may occasionally be as effective as antibiotics (Anand U *et al*., 2019). In this review we present a summery on the antibacterial properties of medicinal & herbal plants that are used widely for overcome illnesses in humans

Extract of medicinal plant

Numerous publications demonstrating the antibacterial qualities of medicinal plants have been published in recent years. Medicinal plants originate by extracting substances with biological activity from a range of plant sources, including roots, limbs, fruits, seeds, and flowers (Chamorro *et al*., 2022), Numerous techniques, such as maceration, percolation, infusion, decoction were used to extract the plants, in addition to microwave technology is used (Kamil *et al*., 2019). Moreover, the secondary metabolites undergo necessary sterilization and separation utilizing HPLC, TLC, GC, PC (Ebere *et al*., 2019). Then plant materials and their extract were mixed in a variety of solvents, such as methanol, ethanol, and ethyl acetate, water to evaluate their antibacterial efficacy against microbes (Nazzaro *et al*., 2013). The extraction method is also dependent on factors such as pH, temperature, and the ratio of sample to solvent. Then the antimicrobial capability of the substrates will be assessed.

Mechanism of plant extract

- **Alkaloids:** Alkaloids are heterocyclic nitrogen molecules with extremely varied chemical structures that have antimicrobial actions, pain reliever and cramps relieve effects. It demonstrates that the indoquinoline alkaloids possess activity against yeast and gram-negative bacteria. While quinine, an alkaloid that is well-known for being able to neutralize the malarial parasite via antiprotozoal action. The vast majority of alkaloids perform by inhibiting EP. The isoquinoline alkaloid called berberine is produced in cells which get

excited by the membrane's potential. It functions has an acceptable DNA intercalator that's functional in a range of bacteria and as a target for RNA polymerase, topoisomerase IV, and gyrase (Yi *et al*., 2007). As a result, berberine increases the permeability of bacterial membranes, which breaks the structure of the membrane (Peng *et al*., 2015).

- **Polyphenols:** Among the most varied categories of secondary metabolites that are bioactive that exist in medicinal plants are phenolic substances. Flavonoids, quinones, flavones, flavanols, and tannins are instances of phenolic substances (Friedman *et al*., 2006). The chemicals demonstrated distinct mechanisms of action against various types of microbes. Process that currently exist, consist of EP inhibitory activity, the ability to alter the permeable nature of the membranes of cells, The degradation of cellular viability due to a multitude of reactions taking place inside cell membranes, or the modification of various intracellular processes brought on by phenolic molecules attaching with enzymes. (Górniak *et al*., 2019).

- **Sulfur-Containing Compounds:** Research has demonstrated that compounds with sulfur in them derived from plants that had elevated polysulfuride levels had antibacterial, antiprotozoal, antiviral, and antifungal characteristics. (Rehman *et al*., 2013). Isothiocyanates, ajoene, and allicin are the three most significant components, It has been discovered that these substances work well against Gram-positive as well as Gram-negative bacteria (Barbieri *et al*., 2017). Sulfur-containing substances may also partially block the production of proteins and DNA as part of their antimicrobial properties (Lanzotti *et al*., 2014). Moreover, certain substances have the ability to alter the integrity of the cell wall and lead cellular metabolites to dissipate (Sofrata *et al*., 2011).

- **Coumarins:** The phenolic compounds known as coumarins have antibacterial qualities in both their organic and artificial forms (Vaou *et al*., 2021). Particularly, coumarin preparations from a number of medicinal plants are capable of successfully combating strains of *Staphylococcus aureus Enterobacter aerogenes, Bacillus subtilis,* & *Enterobacter cloacae. Klebsiella pneumoniae* & *Salmonella enterica Typhi* (Tan *et al*., 2017). Coumarins have the capacity to inhibit bacterial pathogens' QS network, which is the bacterial cells' ability to generate tiny signaling molecules. This can hinder the creation of virulent factors (Zang *et al*., 2018).

- **Terpenes:** Terpenoids are the derivatives of terpenes, which are sometimes referred to as these compounds, but with the addition of a second element, usually air. The key component in essential oil fractions that convey the distinct odor of plants is terpenes (Paduch *et al*., 2007). Essential oils work more effectively in combination with some active substances than they do independently which has contributed to increased antibacterial activity. *Salmonella sp*, *Vibrio parahaemolyticus*, *Helicobacter pylori*, *Escherichia coli,* and *Staphylococcus aureus* have all been shown to be susceptible to their antibacterial qualities. Additionally, they demonstrated varying levels of antibacterial efficacy towards sure dangerous fungus. (Paduch *et al*., 2007).

Antibiotic Properties of the Therapeutic Plant

Xtracted plant-based medications with a variety of biological actions, including antibacterial, anti-inflammatory, and antioxidant qualities (Mehta *et al*., 2001). Antimicrobial chemicals produced by plants with medicinal properties have the potential to stop the growth of bacteria, viruses, fungi, and protozoa through many processes in contrast to antibacterial medications that are presently on the market. Given that, they may be helpful in the therapeutic management of microbial variations that are resistant (Shankar *et al*., 2010). Antibiotic resistance in microbes can be countered when medicinal plants and drugs are combined. Utilizing complicated chemical molecules have significant value in therapeutic potential since they are less likely to cause side effects and resistance than manufactured drugs (Ruddaraju *et al*., 2020). Also, several substances present in in vitro medicinal plants have been revealed to have antibacterial properties (Almabruk *et al*., 2018).

Table 1: Some antimicrobial properties of the following organic compounds of plant extracts

S. No.	Plant source	Sub-category	Micro organisms	Mechanisms	References
1.	*Prangos hulusii*	Coumarin	*Bacillus subtilis*	Stop the replication process of DNA	(Tan *et al*., 2017)
2.	*Rubus ulmifolius*	Compounds containing sulphur	*Escherichia coli*	DNA synthesis inhibitor and destroy protein and bacterial membrane	(Wu *et al*., 2012)
3.	*Piper nigrum*	Alkaloid	*E.coli, Candida albicans Listeria*	Inhibit the translation process	(Boberek *et al*., 2010)

Future prospective and opportunities regarding antimicrobial activity

Many beneficial substances exist in medicinal plants, and just small amount of their potential has been explored. And there is also the challenge with antimicrobial many beneficial substances exist in medicinal plants, and just a small amount of herbs is that their effect might be limited by a variety of circumstances, such as maximum plasma concentrations and penetration into the tissue. To find out further about the antibacterial abilities of additional plants and determine their unique method of action, more study is being undertaken. Due to its medical benefits and minimal to negligible adverse effects, individuals could begin to focus more on plant-based medicines in the years. In order to keep individuals healthy and infection-free, more herbal plants and their extracts should be incorporated into their diets.

Conclusion

A new possibility for tackling the severe challenges posed by increase of antibiotic resistance is medicinal plant activity against microbes. Because these components have demonstrated their therapeutic potential in the treatment of antibiotic resistance, it is imperative that new bioactive substances from medicinal herbs be identified and isolated. These compounds have not yet undergone a complete analysis. It's not easy to effectively exploit these new bioactive substances. It seems that as biotechnology advances, we are going to enabled to examine the chemical makeup of medicinal plants in more depth and create ever-more intricate procedures for the extraction, separation, and identification of physiologically active compounds, which can be identified by a range of chemical structures and modes of action. It would be beneficial to establish uniform separation by in vitro testing procedures in order to streamline the process of searching while making the information easier to grasp. It is necessary to encourage studies on the mechanisms of action & combinations of medicines with other medicinal plants or substances.

Conflict of interest

The authors declare no conflict of interest.

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Reference

1. Ali, A. (2020). Herbs that heal: The philanthropic behavior of nature. *Ann Phytomed*, *9*(1), 7-17.

2. Almabruk, K. H., Dinh, L. K., & Philmus, B. (2018). Self-resistance of natural product producers: past, present, and future focusing on self-resistant protein variants. *ACS Chemical Biology*, *13*(6), 1426-1437.

3. Amenu, D. (2014). Antimicrobial activity of medicinal plant extracts and their synergistic effect on some selected pathogens. *American Journal of Ethnomedicine*, *1*(1), 18-29.

4. Anand, U., Jacobo-Herrera, N., Altemimi, A., & Lakhssassi, N. (2019). A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. *Metabolites*, *9*(11), 258.

5. Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S. F., & Nabavi, S. M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological research*, *196*, 44-68.

6. Boberek JM, Stach J, & Good, L. (2010). Genetic evidence for inhibition of bacterial division protein FtsZ by berberine. *PloS one*, *5*(10), e13745.

7. Chamorro F, Carpena, M., Fraga-Corral, M., Echave, J., Rajoka, M. S. R., Barba FJ, & Simal-Gandara, J. (2022). Valorization of kiwi agricultural waste and industry by-products by recovering bioactive compounds and applications as food additives: A circular economy model. *Food Chemistry*, *370*, 131315.

8. Dhiman R, Aggarwal N, Aneja KR, & Kaur M. 2016. *In vitro* antimicrobial activity of spices and medicinal herbs against selected microbes associated with juices. *International Journal of Microbiology*, *2016*.

9. Ebere EC, Wirnkor VA, & Ngozi VE. Uptake of microplastics by plant: a reason to worry or to be happy?. *World Scientific News*, 2019; (131), 256-267.

10. Friedman M, Henika PR, Levin CE, Mandrell RE, & Kozukue, N. (2006). Antimicrobial activities of tea catechins and theaflavins and tea extracts against Bacillus cereus. *Journal of food protection*, *69*(2), 354-361.

11. Górniak, I., Bartoszewski, R., & Króliczewski, J. (2019). Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry reviews*, *18*, 241-272.

12. Gottardi, D., Bukvicki, D., Prasad, S., & Tyagi, A. K. (2016). Beneficial effects of spices in food preservation and safety. *Frontiers in*

microbiology, *7*, 1394.

13. Hassan, A., & Ullah, H. (2019). Antibacterial and antifungal activities of the medicinal plant veronica biloba. *Journal of chemistry*, *2019*, 1-7.
14. Kamil Hussain, M., Saquib, M., & Faheem Khan, M. (2019). Techniques for extraction, isolation, and standardization of bio-active compounds from medicinal plants. *Natural Bio-active Compounds: Volume 2: Chemistry, Pharmacology and Health Care Practices*, 179-200.
15. Khan, T., Khan, M. A., Ullah, N., & Nadhman, A. (2021). Therapeutic potential of medicinal plants against COVID-19: The role of antiviral medicinal metabolites. *Biocatalysis and agricultural biotechnology*, *31*, 101890.
16. Lanzotti, V., Scala, F., & Bonanomi, G. (2014). Compounds from Allium species with cytotoxic and antimicrobial activity. *Phytochemistry Reviews*, *13*, 769-791.
17. Lewis, K., & Ausubel, F. M. (2006). Prospects for plant-derived antibacterials. *Nature biotechnology*, *24*(12), 1504-1507.
18. Mahato, T. K., & Sharma, K. (2018). Study of medicinal herbs and its antibacterial activity: A review. *Journal of Drug Delivery and Therapeutics*, *8*(5-s), 47-54.
19. Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. *Journal of tropical medicine*, *2019*.
20. Mehta SR, Yusuf S, Peters RJ, Bertrand ME, Lewis BS, Natarajan MK, & Fox KA. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. The Lancet, 2001; *358*(9281), 527-533.
21. Nazzaro F, Fratianni F, De Martino L, Coppola R, & De Feo V. Effect of essential oils on pathogenic bacteria. *Pharmaceuticals*, 2013; *6*(12), 1451-1474.
22. Ody P. (2017). *The complete medicinal herbal: a practical guide to the healing properties of herbs*. Simon and Schuster.
23. Paduch, R., Kandefer-Szerszeń, M., Trytek, M., & Fiedurek, J. (2007). Terpenes: substances useful in human healthcare. *Archivum immunologiae et therapiae experimentalis*, *55*, 315-327.

24. Peng, L., Kang, S., Yin, Z., Jia, R., Song, X., Li, L., ... & Jing, B. (2015). Antibacterial activity and mechanism of berberine against Streptococcus agalactiae. *International journal of clinical and experimental pathology*, *8*(5), 5217.

25. Rehman, F., & Mairaj, S. A. M. Y. A. (2013). Antimicrobial studies of allicin and ajoene. *Int J Pharm Bio Sci*, *4*(2), 1095-1105.

26. Ruddaraju, L. K., Pammi, S. V. N., sankar Guntuku, G., Padavala, V. S., & Kolapalli, V. R. M. (2020). A review on anti-bacterials to combat resistance: From ancient era of plants and metals to present and future perspectives of green nano technological combinations. *Asian Journal of Pharmaceutical Sciences*, *15*(1), 42-59.

27. Saxena, M., Saxena, J., Nema, R., Singh, D., & Gupta, A. (2013). Phytochemistry of medicinal plants. *Journal of pharmacognosy and phytochemistry*, *1*(6), 168-182.

28. Shankar, S. R., Rangarajan, R., Sarada, D. V. L., & Kumar, C. S. (2010). Evaluation of antibacterial activity and phytochemical screening of Wrightia tinctoria L. *Pharmacognosy Journal*, *2*(14), 19-22.

29. Sofrata, A., Santangelo, E. M., Azeem, M., Borg-Karlson, A. K., Gustafsson, A., & Pütsep, K. (2011). Benzyl isothiocyanate, a major component from the roots of Salvadora persica is highly active against Gram-negative bacteria. *PloS one*, *6*(8), e23045.

30. Subbaiah, S. G. P., S. S. Dakappa, and R. Y. Lakshmikantha. "Antibacterial and molecular docking studies of bioactive component from leaves of Stachytarpheta cayennensis (Rich.) Vahl." (2017): 28-34.

31. Subhose, V., Srinivas, P., & Narayana, A. (2005). Basic principles of pharmaceutical science in Ayurvĕda. *Bulletin of the Indian Institute of History of Medicine (Hyderabad)*, *35*(2), 83-92.

32. Tan, N., Yazıcı-Tütüniş, S., Bilgin, M., Tan, E., & Miski, M. (2017). Antibacterial activities of pyrenylated coumarins from the roots of Prangos hulusii. *Molecules*, *22*(7), 1098.

33. Vaou, N., Stavropoulou, E., Voidarou, C., Tsigalou, C., & Bezirtzoglou, E. (2021). Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms*, *9*(10), 2041.

34. Wu, H. Z., Fei, H. J., Zhao, Y. L., Liu, X. J., Huang, Y. J., & Wu, S. W. (2012). Antibacterial mechanism of sulforaphane on Escherichia

coli. *Sichuan da xue xue bao. Yi xue ban= Journal of Sichuan University. Medical Science Edition*, *43*(3), 386-390.

35. Yi ZhiBiao, Y. Z., Yu Yan, Y. Y., Liang YiZeng, L. Y., & Zeng Bao, Z. B. (2007). Evaluation of the antimicrobial mode of berberine by LC/ESI-MS combined with principal component analysis.

36. Zhang, Y., Sass, A., Van Acker, H., Wille, J., Verhasselt, B., Van Nieuwerburgh, F., ... & Coenye, T. (2018). Coumarin reduces virulence and biofilm formation in Pseudomonas aeruginosa by affecting quorum sensing, type III secretion and C-di-GMP levels. *Frontiers in Microbiology*, *9*, 1952.

Chapter - 12
Chromium Remediation from Leather Industry Wastewater: The Microbial Approach

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Chapter - 12

Chromium Remediation from Leather Industry Wastewater: The Microbial Approach

Rupesh Dutta Banik, Addityaa Sinha, Akash Saha and Pritha Pal

Abstract

Due to ineffective management of industrial effluent treatment and administration, the issue of contaminated industrial wastewater has gotten worse in third-world nations. One of the leather industry's long-term issues is complying with environmental requirements regarding the liquid and solid waste produced during leather goods production. The ecological and human health will be at risk from the improper handling of this waste water, which contains chromium (Cr) among other hazardous heavy metals. Various oxide forms ranging from -2 to +6 are present in the environment containing this dangerous heavy metal. Nevertheless, trivalent and hexavalent chromium have the highest level of stability. Trivalent chromium has lower cellular absorption compared to hexavalent Cr. It has a harmful influence on the physiological functions of plants, including photosynthesis, development and growth, water relations, and metabolism etc. Ingestion, skin contact, and inhalation are the three most prevalent ways that people are exposed to chromium. Chromium is mostly harmful to health because it can cause lung and nasal ulcers, skin allergies, issues with reproduction and development, bronchial asthma and so on. Moreover, it has carcinogenic properties that may lead to various cancer. When used in excess, it can potentially be fatal. “Bioremediation” is a stimulating method that employs microorganisms to reduce or remove toxic chemicals from the environment. Bioremediation is a process that can degrade or convert hazardous metals into less complex and safer chemicals. Industrial waste water microbial treatment has recently emerged as an economical and eco-friendly substitute to existing procedures, gaining widespread acceptance. This study provides a comprehensive overview of the role of microbial remediation of Cr from leather industrial wastewater.

Keywords: Industrial wastewater, leather industry, Hazardous heavy metal, carcinogenic, bioremediation

Introduction

Many industrial wastes are thrown into water daily. The majority of these wastes are gradually broken down by living things into smaller, harmless molecules; but some are more difficult to break down and instead build up to levels where they might seriously endanger the health of those living things. (Shekhawat *et al*. 2015). A multivalent metallic element with a glossy, brittle, hard texture, and resistance to tarnishing and corrosion is chromium. It has an atomic weight of 52, making it a transition metal. Because chromium is unstable in oxygen, it forms an oxygen-tight oxide layer quickly to shield the metal underneath (Teklay 2016). The most common forms of chromium (Cr) in water are trivalent [Cr (III)] or hexavalent [Cr (VI)] species, which are known for their stability. Chromium (VI) is a recognized toxic substance and cancer-causing agent, while Chromium (III) is required in small quantities for the breakdown of sugar and fats in the human body. Insufficient levels of Chromium (III) can lead to a condition called "chromium deficiency". But it has been discovered that both kinds of chromium impede biological processes in humans, smaller aquatic creatures, and microbes (Vaiopoulou *et al*. 2012). Compounds containing chromium (VI) present the greatest health risks. Their water solubility affects how dangerous they are. Since less hexavalent chromium is likely to be released into the body, the least soluble compounds—such as lead or barium chromates—are generally less hazardous (Teklay 2016). It is recommended to eliminate Cr from wastewater or alter the form of Cr to less dangerous variants, as the release of chromium into water bodies can have negative effects on aquatic organisms. The two basic methods for accomplishing chromium detoxification are biological and conventional. The removal of chromium from waste waters can be achieved through various methods such as alkaline addition and chemical precipitation, reducing agents to convert Cr (VI) to Cr (III) before precipitation, and filtration, chelating, ion exchange, adsorption on natural or synthetic media, solvent extraction, membrane separation, electrolysis, or evaporation (Vaiopoulou *et al*. 2012). With melting and breaking points of 1907°C and 2671°C, it is strong and brittle, with metallic brightness and steely-dark shading that resists staining. Oxidation reactions cause chromium to passivate, creating a thin, surface coating that prevents oxygen from spreading to the base metal. Numerous industries produce a lot of metals, including tempered steel welding, chromate preparation, metal preparation, tannery treatment, and color creation of chrome and ferrochrome. According to many official and non-managerial groups, hexavalent chromium is still considered an operator that causes malignant growths in humans (GracePavithra *et al*. 2019). Traditional approaches, including chemical, physical, and thermal actions, have numerous

significant disadvantages. These include the generation of dangerous byproducts, the need to transport contaminated soil or water for treatment, together with the high cost associated with the treatment procedure, and the inadequate restoration of wildlife and natural ecosystems. Via using normal biological processes, either via aerobic or anaerobic processes, organisms such as microorganisms (bacteria, fungus, and Actinomycetes, etc.) or their byproducts can be used to decrease and neutralize pollutants from industrial effluent (Arora and N.K. 2018) (Saxena *et al*. 2017) (Banik *et al*. 2023).

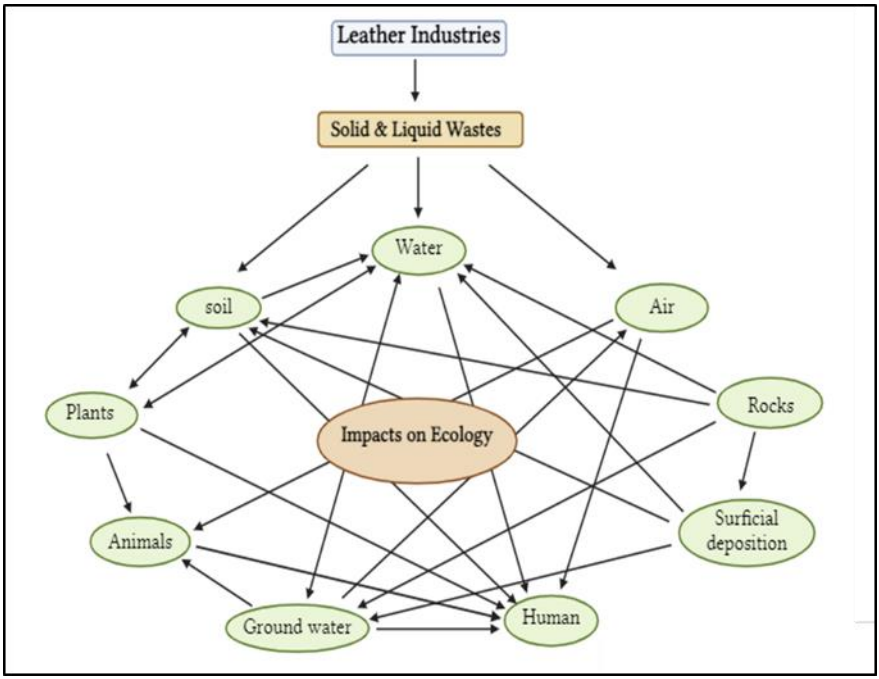

Fig 1: Ecological components that are affected by leather industrial wastes (Banik *et al*. 2023) (Tadesse *et al*. 2017)

Materials and Methods: The pertinent data for this review paper was located by searching PubMed, PubMed Central, Google, and published research papers and review articles from around the globe on the environmental pollutants produced by the leather industries and the microbial bioremediation strategy to remove the pollutants for a cleaner world. We eliminated speculative statements regarding exposure and solely used published evidence. One of the inclusion conditions is the exploitation of information from recognized publications on the issue. The study eliminated all languages other than English.

Results and Discussion

Chromium releasing industries: Chromium is widely utilized in many different sectors. An estimated 18,000-30,000 × 103 t/year is produced of Cr. Ninety percent of this total production is used in the metallurgical sectors, which include the manufacturing of Cr metal and chrome alloy. The remaining 5–5% are employed in foundries, refractory industries, and chemical industries. Cr is utilized in the chemical industries for wood preservation, textile dyeing, metal corrosion inhibition, and leather tanning. A wide range of industries, including the chemical, plastic and resin, pulp and paper, leather, petroleum, distillery, sugar, soap and detergent industries, discharge a significant amount of chromium through their wastewater. Tanneries are the specific source of the Cr pollution among all of them. Because tanneries' waste water control and treatment facilities are insufficient to adequately clean the wastes, the discharge of raw waste into the environment increases the pollution of the environment with Cr (Rahman *et al*. 2017) (Dutta *et al*. 2021).

Effects of Cr discharge onto the environment and human health from industrial wastewater: Cr is a substance that is found in nature in varying concentrations depending on the surroundings. Although studies have shown that Cr (III) is crucial for the metabolism of proteins and fats, one study found that Cr (III) can be harmful to people when swallowed. Stomach cancer is also caused by chromium-containing substances such as mineral fiber, diesel emissions, aluminum powder, and arsenic. The majority of cases of stomach cancer occur in those under 60 (Shekhawat *et al*. 2015).

Table 1: Effects of chromium dust in human being through inhalation (Teklay 2016).

Effects of chromium dust inhalation that cause pulmonary irritation include	Asthma
	Chronic bronchitis
	Chronic irritation
	Chronic pharyngitis
	Chronic rhinitis
	Congestion and hyperemia
	Upper respiratory tract polyps
	Nasal mucosa ulceration, potentially leading to septal perforation.
	Tracheobronchitis

The nose, throat, and lungs are all sensitive to chromium's irritation of the respiratory system. Runny nose, sneezing, coughing, itching, and a burning feeling are some possible symptoms. Nasal sores might form and nosebleeds

can occur with repeated or extended exposure. The nasal septum, which is the wall that divides the nasal passageways, becomes entire (a perforation) if the damage is major (Teklay 2016). Teenagers and children are more susceptible to accidents and hazardous metal exposures. The injury rates among 15–17-year-olds were almost twice as high as those among persons 25 years of age and above. Chemical exposure can seriously disturb the body's processes of a child. Children who are still developing are particularly susceptible, and their susceptibilities may vary depending on the stage of life they are in, particularly during "critical windows of development.". Immune system dysfunction, premature or delayed puberty, and neurobehavioral abnormalities have all been related to developmental exposures. These consequences can have long-lasting effects and be irreversible (Scott *et al*. 2021).

Eco-friendly method for Chromium extenuation: Recent international attempts to clean up many of these settings have increased in part because of the frequency with which environmental degradation occurs, the anticipated quantity of polluted regions, and the continuous identification of these sites. This is either done to prepare the area for reconstruction or restoration for use, or to reduce the likelihood of harmful health consequences or environmental problems brought on by pollution (Luka *et al*. 2018) (Arora 2018). One economical and ecologically sustainable way to potentially address environmental degradation is through bioremediation. Bioremediation is the process of removing or reducing pollutants from the air, soil, and water by using biological means. To reduce or eliminate the harmful component, the approach makes use of an organism that was either imported from another system or obtained from the pertinent environment (Banik *et al*. 2023) (Sher *et al*. 2019) (Luka *et al*. 2018) (Arora2018) (Ashraf *et al*. 2018) (Saravanan 2022). Most of this greener process is dependent on microbes that spell contaminants and convert them into harmless compounds through the action of enzymes. Applying bioremediation often means changing the environment to hasten microbial development and disintegration since bioremediation occurs only in environments that facilitate microbial development and activity. (Karigar *et al*. 2011). The ability to generate isolated microbes in practically any environmental setting is absolutely necessary. Microorganisms may adapt to and flourish in a variety of environmental circumstances, including high heat, extreme cold, the desert, water, anaerobic conditions, high oxygen levels, the presence of toxic substances, and any waste discharge. An energy supply and a carbon supply are the two essential elements. (Luka *et al*. 2018). This approach relies on environmental factors and inputs such as nutrition and oxygen to promote microbial activity for Cr remediation. Through immobilization procedures, a range of microbial strains carry out

chromium bioremediation. In order to protect themselves from the negative effects of chromium, the bacteria have developed several defense mechanisms. A removal-facilitating technique called bioleaching. Selective adsorption, acid extraction, calcination, and membrane dialysis are examples of conventional bioleaching methods. Using this tactic can make other efforts more successful. For the purposes of metal bioleaching, it is a single precipitation technique using microorganisms and dangerous metal ions. The mainstay of the technology, microbially induced calcite precipitation (MICP), can eradicate all traces of arsenic from the environment. (Pratush *et al*. 2018) (Sher *et al*. 2019).

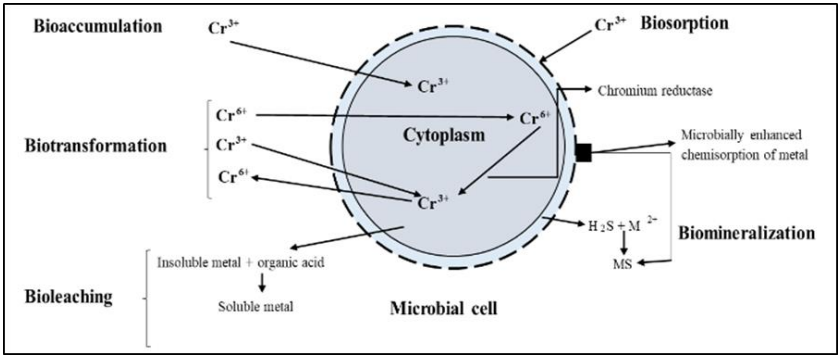

Fig 2: Showing various processes used to remove hazardous metal ions from contaminated areas (Banik *et al*. 2023) (Sher *et al*. 2019).

Remediation of chromium using bacterial species: Microbial remediation, or lowering the concentration of Cr in the environment, is accomplished by using native microorganisms. The major chromium transformers are *Pseudomonas sp., Methylococcus capulants, Streptomyces nouresei, P. putida, Aeromonas caviae, Bacillus circulans, Bacillus megaterium, Pantoea sp., Zoogloea ramigera, Staphylococcus xylosus and Bacillus coaglans* (Kumar *et al*. 2020) (Pratush *et al*. 2018). The absorption of Cr by free *Bacillus* coagulants was contrasted with biomass that had been immobilized in various matrices. The greatest biosorption was discovered at pH 2.5. Therefore, maintaining matrix stability during biosorption is crucial without compromising Cr (VI) sorption effectiveness. The immobilization of biomass in polyacrylamide and agarose was found to have minimal impact on Cr adsorption, resulting in a very stable matrix. *Enterobacter cloacae* demonstrated resistance to Cr (VI). *Shewanella* MR-1 growth was inhibited at a concentration of 0.035 mmol Cr (VI) at aerobic conditions and at a concentration of 0.015 mmol Cr (VI) at anaerobic conditions. When 0.035 mmol Cr (VI) was added, midlog phase *Shewanella sp.* cultures grew slower,

however, when Cr (VI) was added under anaerobic conditions, growth ceased right away. (Viamajala *et al*., 2004), (Viamajala *et al*. 2004).

Table 2: Showing the bacterial species that have Cr removing capacity (Pushkar *et al.* 2021).

Name of bacteria	Percentage of Cr removal	Optimum temperature for removal
Cellulosimicrobium sp.	99.33% at 50 mg/L And 62.28% at 300 mg/L	37ºC
Pseudomonas stutzeri	27.47 mg/g	30ºC
Bacillus cereus	100% at 200 mg/L	37ºC
Citrobacter freundii	(52%) 44.61 mg/L to 12.17 mg/L	(effluent)
Brevibacterium iodinum S2	2600 mg/g	37ºC
Cellulosimicrobium funkei	86.67% reduction at 200 mg/L	35ºC
Stenotrophomonas maltophilia	260 mg/L from 490 mg/L	NM
Enterobacter sp. SL	91.10% of 100 mg/L	45ºC
Shewanella sp.	89% of 500 mg/L	37ºC
Morganella morganii	92% of 4600 mg/L	37ºC
Halomonas sp	81% of 100 mg/L	30ºC

The efflux system, believed to be regulated by the ChrA gene, is a key mechanism responsible for Cr susceptibility in bacteria. Deletion of the ChrA gene diminishes the persistence capacity of bacteria in the existence of Cr due to the deactivation of the efflux mechanism. The resistance of many bacteria to both antibiotics and Cr can be attributed to the idea that tetracycline and Cr are ejected from cells through active efflux pumps. After $Cr6^{+}$ has been transported into the cell via the sulfate ion channel, the ChrA transmembrane protein facilitates its release. The ChrA protein utilizes H^{+} to create a transmembrane electrochemical proton gradient, resulting in the outflow of Cr from the cell. The ChrA transmembrane protein aids in the efflux of $Cr6^{+}$ from the cell following its entry via the sulfate ion channel. The ChrA protein facilitates the efflux of chromium from the cell by using a transmembrane electrochemical proton gradient generated by H^{+}. Cloned ChrAT and ChrA bacteria exhibit greater resistance to Cr when compared to bacteria that were specifically engineered to be resistant to ChrT. The importance of the ChrA-dependent chromium efflux mechanism in bacteria is demonstrated, as well as the role of the ChrA protein in transporting $Cr3^{+}$ out of the cell after reduction (Gu *et al*. 2020) (Pushkar *et al*. 2021) (Baaziz *et al*. 2017).

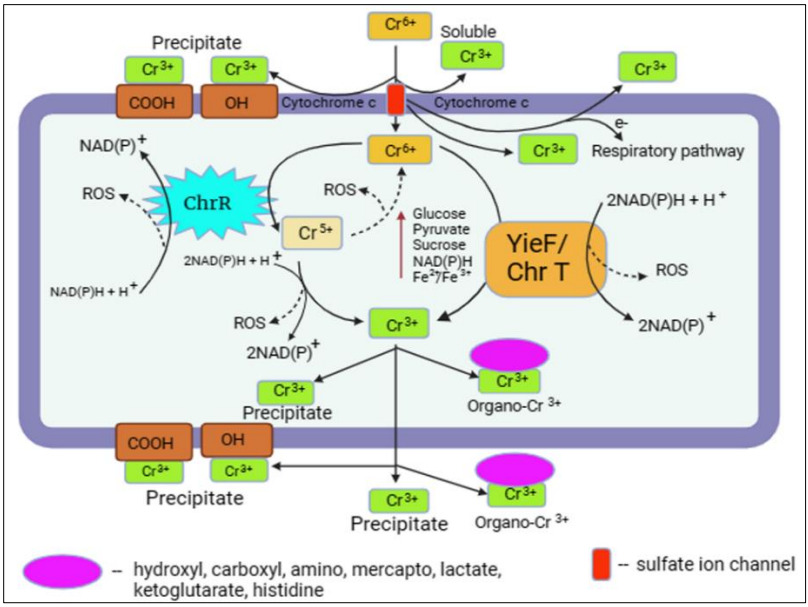

Fig 3: Intra and extracellular Cr 6^{+} reduction in bacteria by and accumulation of reduced product (Pushkar *et al.* 2021)

Remediation of chromium using fungal species: The capacity of fungi to biosorb and bioaccumulate Cr(VI) is widely recognized (Pillichshammer *et al.* 1995) (Dursun *et al.* 2003b) (Park *et al.* 2005). *Aspergillus* is one of the fungi that have been researched for use in Cr (VI) bioremediation. (Dursun *et al.* 2003a) (Park *et al.* 2005) (Prasenjit and Sumathi 2005) (Jayanthi *et al.* 2014). *Fusarium*, (Zafar *et al.* 2007) (Ghosh *et al.* 2011), *Mucor*, (Yan and Viraraghavan 2003) (Tewari *et al.* 2005), *Rhizopus*, (Abraham *et al.* 2001) (Ahmad *et al.* 2005) (Zafar *et al.* 2007), *Penicillium*, (Ahmad *et al.* 2006) (Jayanthi *et al.* 2014) (Samuel *et al.* 2015), *Trichoderma*, (Morales and Cristiani 2006) (Morales and Cristiani 2008), and *Paecilomyces*. (Ca´rde nas and Acosta 2010) (Sharma and Adholeya 2011), These microbes have also been documented for their capacity to convert Cr (VI) to Cr (III).

Table 3: Cr tolerance by different fungal species (García-Hernández *et al.* 2017)

Fungal sp.	Tolerance g/mL	Culture condition
Cladosporium perangustum	>300	Solid and liquid
Aspergillus niger	350 - 400	Solid
Penicillium chrysogenum	800	Solid and liquid
Aspergillus sp.	600	Liquid
Aspergillus flavus	800	Solid

Fusarium solani	1000	Liquid
Penicillium sp.	1040	Solid
Aspergillus versicolor	1000	Solid
Alternaria sp.	900	Solid

The tolerance of Cr (VI) in species belonging to the Fusarium genus has been reported to range from 1000, 1300, to 5000 μg/mL. (Iram *et al*. 2012) (Ezzouhri *et al*. 2009) (Zafar *et al*. 2007). For example, *F. solani* is known to have a tolerance of 1000 μg/mL (Ghosh *et al*. 2011). However, it has been noted that Penicillium species may tolerate between 1040 and 7000 μg/mL of total chromium (Zafar *et al*. 2007). Fungi in the *Rhizopus* genus, have been shown to be able to tolerate 400 μg/mL of total chromium and even up to 7000 μg/mL at a lower proportion. (Ahmad *et al*. 2006) (Zafar *et al*. 2007).

Conclusion

A comprehensive analysis indicates that the substantial quantity of chromium discharged by wastewater from various enterprises has a noteworthy impact on the ecosystem overall. The destructive consequences of the leather industry have taken consideration to the necessity for environmentally friendly technology. The ecologically friendly physio-chemical therapy approaches also involve the practice of a significant amount of chemicals. Bioremediation methods can be a beneficial alternative for safely breaking down and detoxifying solid wastes and wastewater that include heavy metals, which are produced by industry. These technologies are environmentally sustainable, financially viable, and provide a capable method to improve environmental value. The study showcases a variety of microorganisms that have proven to be successful in eradicating or reducing detrimental issues found in waste products formed by different industries. Despite the presence of numerous microorganisms and the availability of effective eco-friendly and cost-efficient techniques, industrial wastes and wastewater persistently contribute to environmental contamination and toxicity complications. This study assists as a social catalyst for the betterment of the general public. In order to address this urgent matter, it is crucial to adopt sustainable and eco-friendly practices for managing industrial wastewater and solid waste. This includes reducing the usage of hazardous chemicals and raising awareness among different companies and governments.

Potential for future development and expansion

It is imperative to pursuit for more active microorganisms for the purification and decomposition of chromium prior to its ultimate discharge

into the environment. Chromium affects soil, aquatic ecosystems, and human health. Understanding their biochemistry and genetic makeup is essential to developing effective bioremediation techniques. As a result, there will be a greater chance for long-term survival for both surface and aquatic natural ecosystems. All industries that release chromium into wastewater must use less expensive and less harmful to the environment methods of waste handling.

Conflict of interest: There isn't any conflict of interest with this study.

Author contributions: The data is acquired and analyzed by Rupesh Dutta Banik, Addityaa Sinha and Akash Saha. Dr. Pritha Pal is responsible for the article's ideation, design, and revisions.

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References

1. Abraham, T. E. (2001). Biosorption of Cr (VI) from aqueous solution by *Rhizopus nigricans*. *Bioresource technology*, 79(1), 73-81.
2. Ahmad, I., Ansari, M. I., & Aqil, F. (2006). Biosorption of Ni, Cr and Cd by metal tolerant *Aspergillus niger* and *Penicillium sp*. using single and multi-metal solution.
3. Ahmad I, Zafar S, & Ahmad, F. (2005). Heavy metal biosorption potential of *Aspergillus* and *Rhizopus sp.* isolated from wastewater treated soil.
4. Arora, N. K. (2018). Bioremediation: a green approach for restoration of polluted ecosystems. *Environmental Sustainability*, *1*(4), 305-307.
5. Ashraf S, Naveed M, Afzal M, Ashraf S, Rehman K, Hussain A, & Zahir, Z. A. (2018). Bioremediation of tannery effluent by Cr-and salt-tolerant bacterial strains. *Environmental monitoring and assessment*, *190*, 1-11.
6. Baaziz, H., Gambari, C., Boyeldieu, A., Ali Chaouche, A., Alatou, R., Méjean, V., ... & Fons, M. (2017). ChrASO, the chromate efflux pump of *Shewanella oneidensis*, improves chromate survival and reduction. *PLoS One*, *12*(11), e0188516.
7. Banik, R. D., Pal, P., & Baksi, S. (2023). Green strategy for Reclaiming Ecosystem: Microbial Remediation of Leather Industry Wastes. *Journal of Survey in Fisheries Sciences*, 10(1S), 6432-6441.
8. Cárdenas-González, J. F., & Acosta-Rodríguez, I. (2010). Hexavalent chromium removal by a *Paecilomyces sp.* fungal strain isolated from environment. *Bioinorganic Chemistry and Applications*.

9. Dursun, A. Y., Uslu, G., Cuci, Y., & Aksu, Z. (2003). Bioaccumulation of copper (II), lead (II) and chromium (VI) by growing *Aspergillus niger*. *Process biochemistry*, 38(12), 1647-1651.

10. Dursun, A. Y., Uslu, G., Tepe, O., Cuci, Y., & Ekiz, H. I. (2003). A comparative investigation on the bioaccumulation of heavy metal ions by growing *Rhizopus arrhizus* and *Aspergillus niger*. *Biochemical Engineering Journal*, *15*(2), 87-92.

11. Dutta D., Arya, S., & Kumar, S. (2021). Industrial wastewater treatment: Current trends, bottlenecks, and best practices. *Chemosphere*, 285, 131245.

12. Ezzouhri L, Castro E, Moya M, Espinola, F., & Lairini, K. (2009). Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. *African journal of microbiology research*, *3*(2), 35-48.

13. García-Hernández, M. A., Villarreal-Chiu, J. F., & Garza-González, M. T. (2017). Metallophilic fungi research: an alternative for its use in the bioremediation of hexavalent chromium. *International Journal of Environmental Science and Technology*, *14*, 2023-2038.

14. GracePavithra, K., Jaikumar, V., Kumar, P. S., & SundarRajan, P. (2019). A review on cleaner strategies for chromium industrial wastewater: present research and future perspective. *Journal of Cleaner Production*, *228*, 580-593.

15. Gu, S., Kelly, B., & Xiu, D. (2020). Empirical asset pricing via machine learning. *The Review of Financial Studies*, *33*(5), 2223-2273.

16. Iram, S., Parveen, K., Usman, J., Nasir, K., Akhtar, N., Arouj, S., & Ahmad, I. (2012). Heavy metal tolerance of filamentous fungal strains isolated from soil irrigated with industrial wastewater. *Biologija*, *58*(3).

17. Jayanthi, M., Kanchana, D., Saranraj, P., & Sujitha, D. (2014). Bioadsorption of chromium by *penicillium chrysogenum* and *Aspergillus niger* isolated from tannery effluent. *Int J Microbiol Res*, *5*(1), 40-7.

18. Karigar, C. S., & Rao, S. S. (2011). Role of microbial enzymes in the bioremediation of pollutants: a review. *Enzyme research*.

19. Kumar, A., Kumar, A., MMS, C. P., Chaturvedi, A. K., Shabnam, A. A., Subrahmanyam, G., ... & Yadav, K. K. (2020). Lead toxicity: health hazards, influence on food chain, and sustainable remediation approaches. *International journal of environmental research and public health*, 17(7), 2179.

20. Luka Y, Highina BK, & Zubairu, A. Bioremediation: A solution to environmental pollution-a review. *Am J Eng Res*, 2018; 7(2), 101-109.

21. M Ghosh, D. (2011). Biosorption of Cr [VI] by resting cells of *Fusarium solani*.

22. Morales-Barrera L, & Cristiani-Urbina, E. (2006). Removal of hexavalent chromium by *Trichoderma viride* in an airlift bioreactor. *Enzyme and Microbial Technology*, *40*(1), 107-113.

23. Morales-Barrera, L., & Cristiani-Urbina, E. (2008). Hexavalent chromium removal by a *Trichoderma inhamatum* fungal strain isolated from tannery effluent. *Water, Air, and Soil Pollution*, 187, 327-336.

24. Park D, Yun YS, Jo JH, & Park, J. M. (2005). Mechanism of hexavalent chromium removal by dead fungal biomass of *Aspergillus niger*. *Water Research*, 39(4), 533-540.

25. Pillichshammer M, Pümpel T, Pöder, R., Eller, K., Klima, J., & Schinner, F. (1995). Biosorption of chromium to fungi. *Biometals*, *8*, 117-121.

26. Prasenjit, B., & Sumathi, S. (2005). Uptake of chromium by *Aspergillus foetidus*. *Journal of Material Cycles and Waste Management*, 7, 88-92.

27. Pratush, A., Kumar, A., & Hu, Z. (2018). Adverse effect of heavy metals (As, Pb, Hg, and Cr) on health and their bioremediation strategies: a review. *International Microbiology*, 21, 97-106.

28. Pushkar, B., Sevak, P., Parab, S., & Nilkanth, N. (2021). Chromium pollution and its bioremediation mechanisms in bacteria: A review. *Journal of Environmental Management*, 287, 112279.

29. Rahman, Z., & Singh, V. P. (2019). The relative impact of toxic heavy metals (THMs)(arsenic (As), cadmium (Cd), chromium (Cr)(VI), mercury (Hg), and lead (Pb)) on the total environment: an overview. *Environmental monitoring and assessment*, 191, 1-21.

30. Samuel, M. S., & Chidambaram, R. (2015). Hexavalent chromium biosorption studies using *Penicillium griseofulvum* MSR1 a novel isolate from tannery effluent site: Box–Behnken optimization, equilibrium, kinetics and thermodynamic studies. *Journal of the Taiwan Institute of Chemical Engineers*, *49*, 156-164.

31. Saravanan, A., Kumar, P. S., Duc, P. A., & Rangasamy, G. (2023). Strategies for microbial bioremediation of environmental pollutants from industrial wastewater: A sustainable approach. *Chemosphere*, 313, 137323.

32. Saxena, G., Chandra, R., & Bharagava, R. N. (2017). Environmental pollution, toxicity profile and treatment approaches for tannery wastewater and its chemical pollutants. *Reviews of Environmental Contamination and Toxicology*, 240, 31-69.

33. Scott, N. B., & Pocock, N. S. (2021). The health impacts of hazardous chemical exposures among child labourers in low-and middle-income countries. *International Journal of Environmental Research and Public Health*, 18(10), 5496.

34. Sharma S, & Adholeya A. Detoxification and accumulation of chromium from tannery effluent and spent chrome effluent by *Paecilomyces lilacinus* fungi. *International Biodeterioration & Biodegradation*, 2011; 65(2), 309-317.

35. Shekhawat, K., Chatterjee, S., & Joshi, B. (2015). Chromium toxicity and its health hazards. International *Journal of Advanced Research*, 3(7), 167-172.

36. Sher, S., & Rehman, A. (2019). Use of heavy metals resistant bacteria—a strategy for arsenic bioremediation. *Applied microbiology and biotechnology*, 103, 6007-6021.

37. Singh, A. L. (2008). Removal of chromium from waste water with the help of microbes: a review. e-*Journal of Science & Technology*, 3(3).

38. Tadesse, G. L., Guya, T. K., & Walabu, M. (2017). Impacts of tannery effluent on environments and human health: a review article. *Advances in Life Science and Technology*, *54*(10).

39. Teklay, A. (2016). Physiological effect of chromium exposure: a review. *Int J Food Sci Nutr Diet S*, 7, 1-11.

40. Tewari, N., Vasudevan, P., & Guha, B. K. (2005). Study on biosorption of Cr (VI) by *Mucor hiemalis*. *Biochemical Engineering Journal*, *23*(2), 185-192.

41. Vaiopoulou, E., & Gikas, P. (2012). Effects of chromium on activated sludge and on the performance of wastewater treatment plants: a review. *water research*, 46(3), 549-570.

42. Viamajala, S., Peyton, B. M., Sani, R. K., Apel, W. A., & Petersen, J. N. (2004). Toxic effects of chromium (VI) on anaerobic and aerobic growth of *Shewanella oneidensis* MR-1. *Biotechnology progress*, 20(1), 87-95.

43. Yan, G., & Viraraghavan, T. (2003). Heavy-metal removal from aqueous solution by fungus *Mucor rouxii*. *Water research*, 37(18), 4486-4496.

44. Zafar, S., Aqil, F., & Ahmad, I. (2007). Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. *Bioresource technology*, 98(13), 2557-2561.