MICROPROPAGATION OF MEDICINAL PLANTS

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Conservation of Medicinal Plant Bramhi- *Bacopa monnieri*(L.) Wettstein Through *in vitro* Cultures

Author(s): Kiranmai Chadipiralla*, Boddupalli Krishna Jaswanth and Pichili Vijaya Bhaskar Reddy

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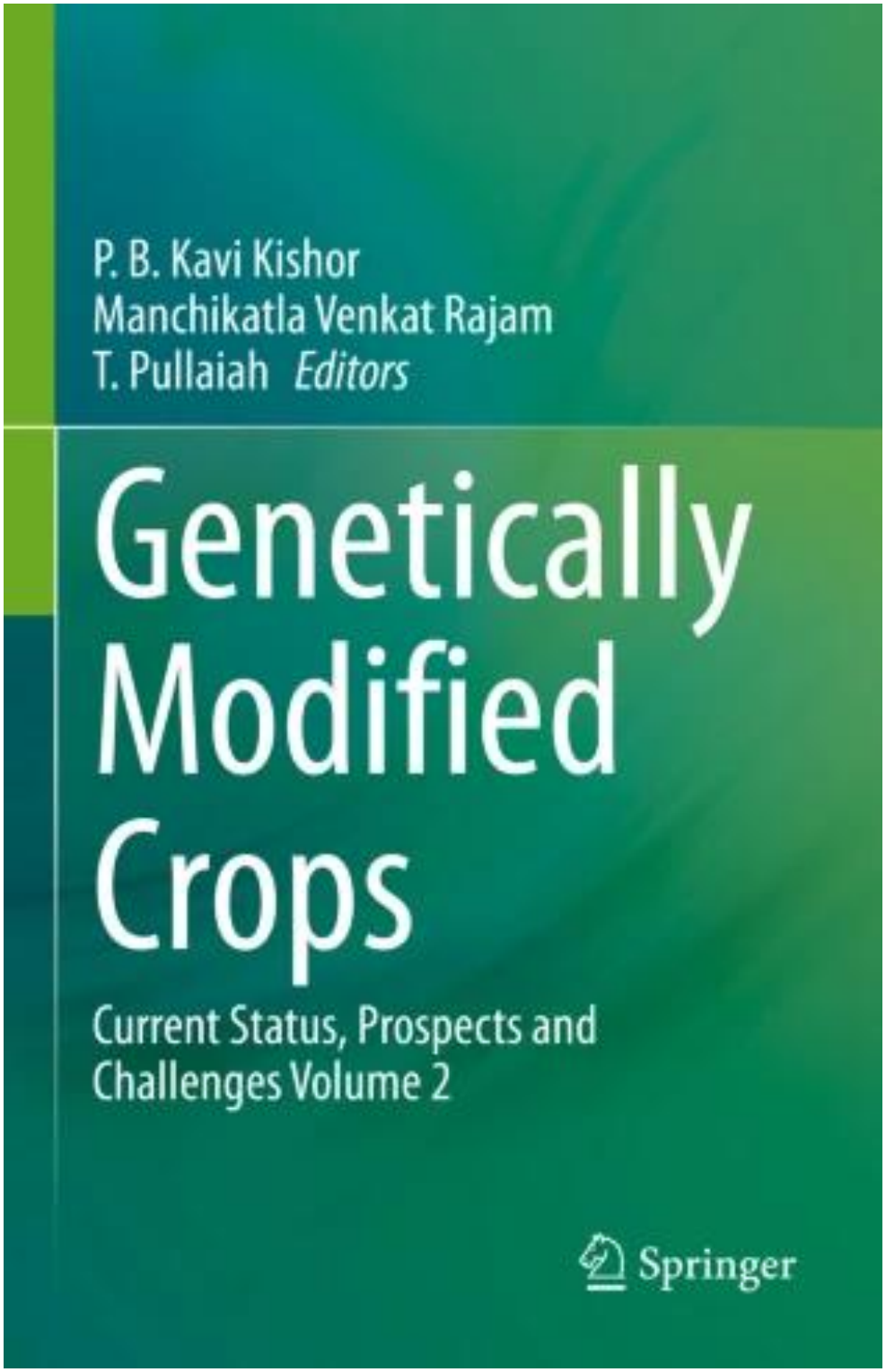
Abstract

Bacopa monnieri (L.) Wettstein is a medicinal herb from the family Plantaginaceae widely known as 'water hyssop' or 'brahmi'. The therapeutic potential of plants is due to the presence of many bioactive secondary metabolites, majorly brahmine, herpestine, alkaloids, and saponins (bacosides), which are responsible for pharmacological effects including neuroprotective, hepatoprotective, gastroprotective, antioxidant, anti-inflammatory, and antimicrobial properties. Vegetative cultivation of Bacopa on a large scale has its limitations due to the lack of viability of seeds during propagation and the unpredictable nature of the production of phytochemicals for commercial purposes, which can be overcome by tissue culture mechanism. Over the past few decades, many studies on the tissue culture of Bacopa in establishing a standardized protocol were reported. This chapter deals with de novo organogenesis of the root and shoot along with the callus induction and somatic embryogenesis from different explants of B. monnieri on MS basal nutrient medium supplemented with Plant Growth Regulators.

Keywords: Bacopa monnieri, Micropropagation, Plant growth regulator.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Genetically Modified Brinjal (*Solanum melongena* L.) and Beyond

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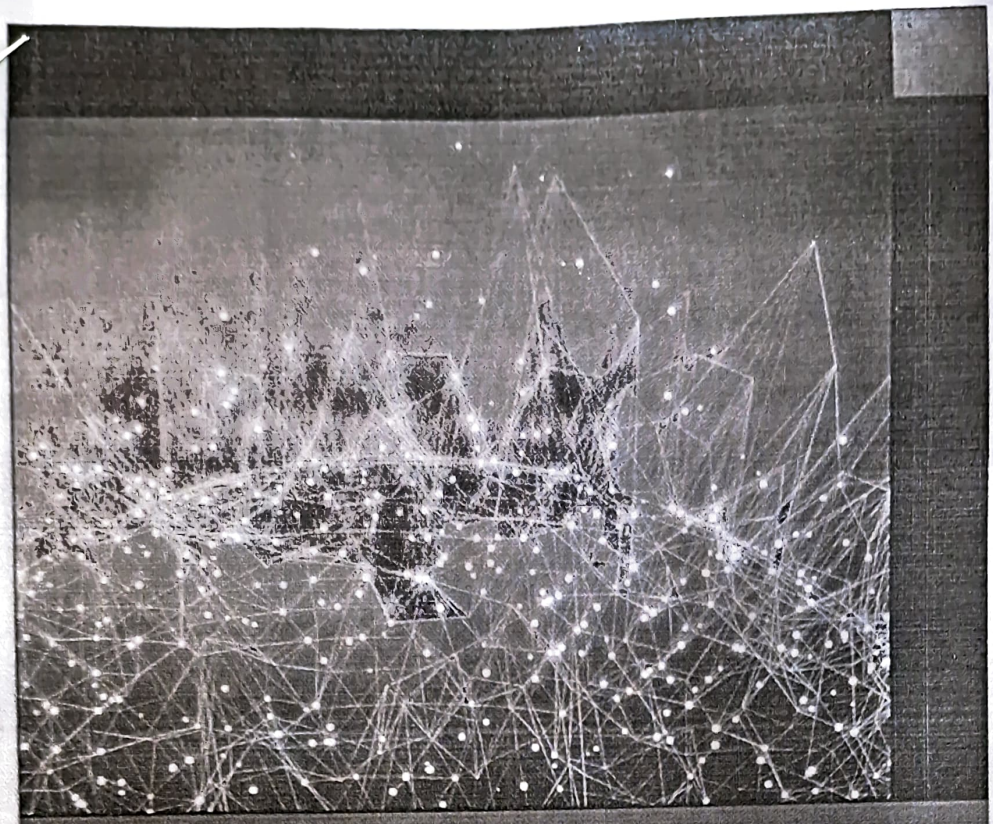
Abstract

- *Solanum melongena* L., commonly called as brinjal/eggplant, occupies an important position in vegetable rearing across the globe and has been regarded as the poor man's crop. The estimated production goes over 52,309,119 metric tonnes annually. Traditional plant breeding techniques have played a vital role in developing new cultivars, thereby improving the overall crop production that catered to the needs of the global requirement. However, in the long run, the requirement has risen enormously due to the rapidly growing population. Simultaneously, the reduction in the yield due to various factors including soil quality, environmental vagaries, diseases and pest attacks posed new challenges in the production-consumption landscape. Of all the factors, the threat of the notorious insect pest, *Leucinodes orbonalis*, commonly known as brinjal shoot and fruit borer (BSFB) which belongs to the phylum Arthropoda and to the order Lepidoptera stood as the greatest challenge to counter as it withstood several broad range insecticides. This situation demanded for BSFB-resistant varieties of brinjal, eventually leading to the development of the genetically modified Bt brinjal. The development of such an insect-resistant variety has been a landmark in brinjal production. The present chapter focuses on transgenic brinjal with improved agronomic traits, particularly insect-resistant Bt varieties, the basic biology of Bt and the major methodologies, the mechanism of action involved in the development of the Bt brinjal.



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17 Recent advancements in the bioremediation of heavy metals from the polluted environment by novel microorganisms

G. Mary Sandeepa, B. Lakshmanna, and M. Madakka

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17.1 INTRODUCTION

The environmental pollution caused by the handling and exploration of heavy metals and crude oil is greatly threatening the health of human and ecosystem. These threats have deleterious effects on life forms which play an essential role

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20 Microbial remediation of agricultural soils contaminated with agrochemicals

M. Madakka and G. Mary Sandeepa

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20.1 INTRODUCTION

During 'Green revolution (GR) period' (1966–1985), the agriculture innovations had transformed the agriculture practice and productivity. A remarkable growth in world crop production has occurred in the past six decades. It is mainly because of increase in the utilization of fertilizers, pesticides, new plant varieties and development in technologies like irrigation. In agriculture this development feeds more or less six billion people in the world. World population is rising day by day and the change in diets also demand the 70% of food production rates (FAO, 2009). According to Oerke (2006) 35% of global food production yield is decreasing because of pests. The fast improvement of the agrochemical field was occurred after 1945 many pesticides like

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CONTENTS

BACTERIAL QUORUM SENSING SYSTEM AND THEIR ROLE OF SIGNALLING IN BACTERIA

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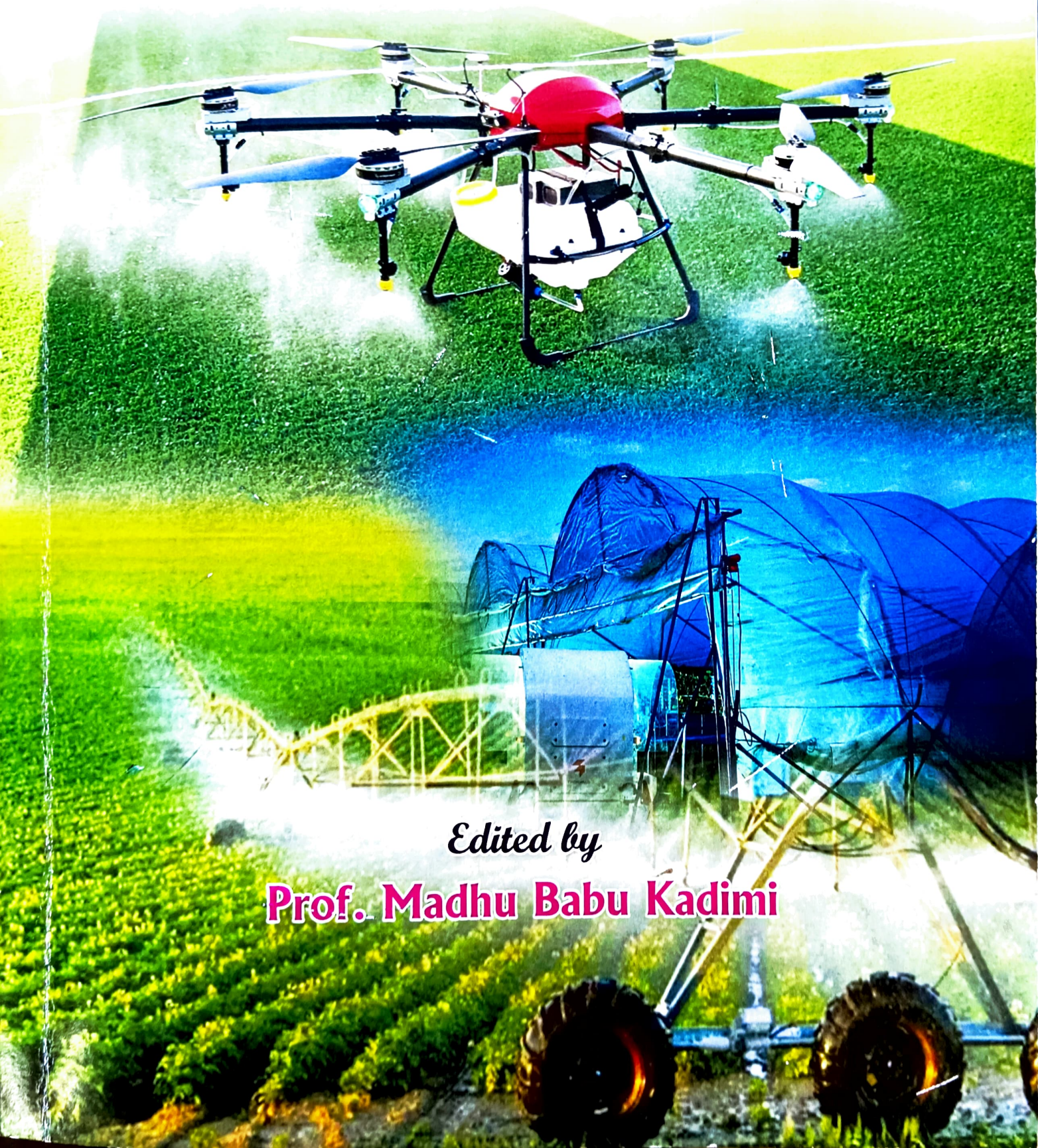
Abstract

Microscopic organisms were discovered by Robert Hooke and Anton Van Leeuwenhoek between 1665 and 1683. It took over 150 years after this discovery to develop microscopy, which became the foundation for understanding microbes (Gest et al.,2004). During the period 1950-2000, extraordinary changes in microbiology occurred, including the discovery of DNA structure by Watson and Crick in 1953. Thus, with the advancement of microscopy and other related subjects such as microbial metabolism, scientists were able to work on microorganisms and link them for industrial purposes (Wainwright and Lederberg, 1992). The importance of microbes in human life is vast. It influences me as a friend and as a foe at times. Many microbe products contribute to human nutrition and some diseases. Others help to improve the standard of living. As a result, microbes and microbiology play an important role in the advancement of human health and welfare. Among them are bacteria are more closely related to human life than the microbial community. We are supported by natural flora from birth, and it grows and helps to build immunity during growth (Nuriel et al., 2016). Similarly, many bacteria are beneficial to humans' daily lives by serving as probiotics and assisting in the fermentation of many food products.

Quorum Sensing

Bacteria use this mechanism to communicate with one another and thus coordinate their behaviour. Typically, the mechanisms involve the regulation of specific genes in response to population density. Coordination of gene expression is accomplished through the production and release of specific signal molecules. Autoinducers are signal molecules that are released and detected by specific receptors. Population density is low. The amount of autoinducers produced in the cell is diffused to the surrounding environment. As the bacterial population grows, so does the production of autoinducers. The resulting auto-inducers significantly activate the protein that acts as a transitional regulator. When the regulators are activated, they interact with the target DNA sequence by increasing or decreasing the transcription of QS-regulated genes. As a result, the bacterial population exhibits phenotypic expression. (Podbielski et al., 2004; Gonzalez et al., 2006; Henke et al., 2004).

An Essays on
Advanced Technology in Agriculture



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Contents

CROP INSURANCE IN INDIA: PROGRESS AND CHALLENGES

Dr. P. Srinivas
Prof.N.R.Venkataramana Reddy
A. Muni Sankara Swamy

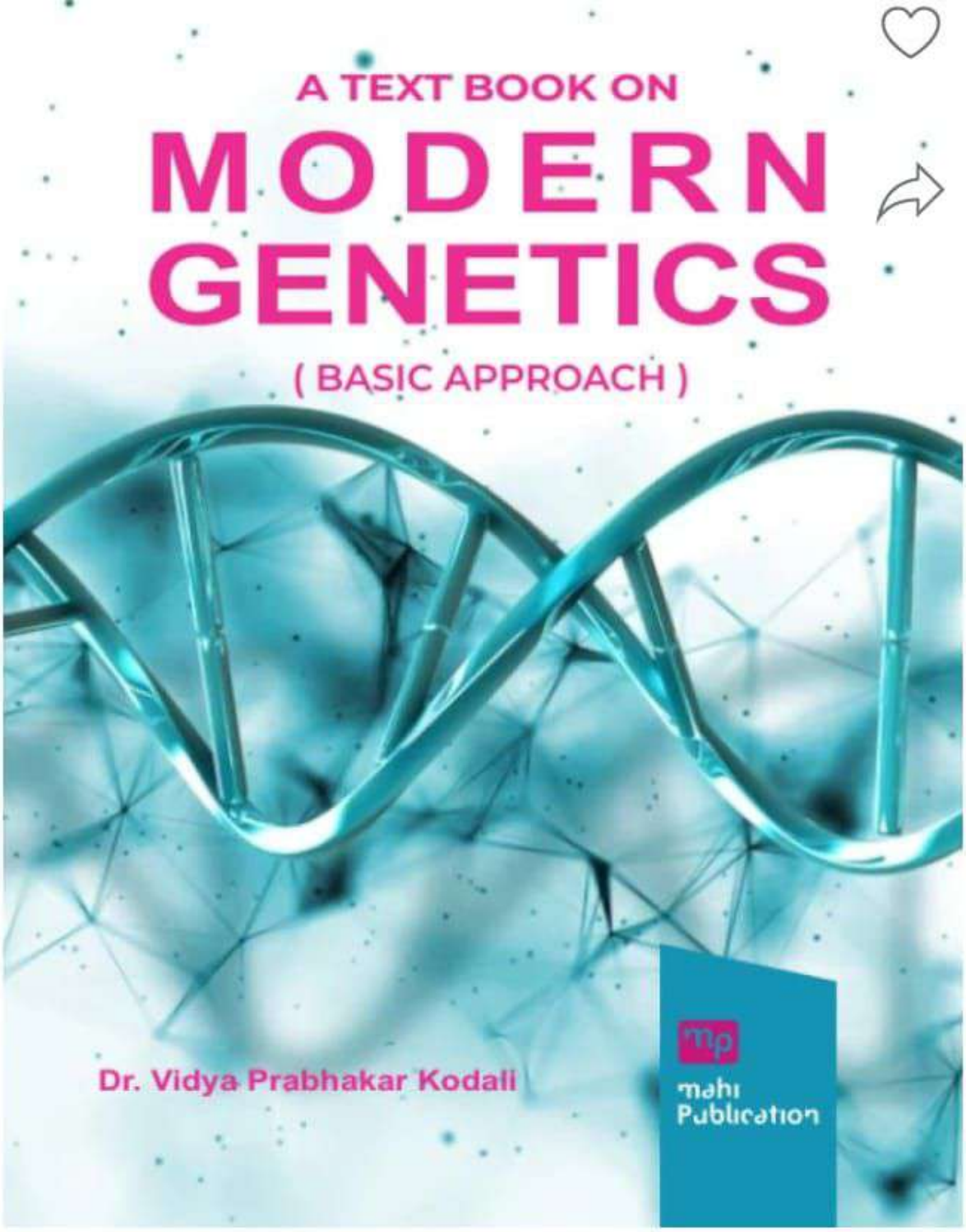
I. Introduction

According to the World Bank, rural areas are home to 46% population of the world and 70% of the poor population lives in rural areas only (Sovacool, 2018) with agricultural as their primary contributors to economic growth such as livelihood, income and employment (Ferdous *et al.*, 2021). India's agricultural sector, which contributes 16.5 percent of the country's Gross Value Added (GVA) for the year 2019-20 (at current prices), supports 54.6 percent of the total workforce in the country (Census 2011) and contributes significantly to food security. However, it faces numerous challenges, particularly its vulnerability to climate risks. India's agricultural landscape is heavily dependent on monsoon rainfall, making it susceptible to droughts, floods, erratic weather patterns, and other climatic uncertainties (Todmal, 2022; Birthal and Hazrana 2019). These risks pose significant threats to crop production, farmer incomes, and overall agricultural stability. In managing these inherent risks, crop insurance has emerged as a crucial instrument to safeguard the interests of farmers and ensure the sustainability of Indian agriculture. Crop insurance provides financial protection against losses caused by natural risks like natural calamities, pests, diseases, etc. (Bhise *et al.,* 2007). By providing a safety net, crop insurance helps farmers recover from losses, stabilize their incomes, and sustain agricultural productivity, thereby contributing to the overall resilience of the sector.

This research paper aims to study the evolution of the crop insurance in India, progress of crop insurance schemes with special reference to Pradhan Mantri Fasal Bima Yojana and Challenges in implementation of crop insurance schemes in India.

II. Evolution of crop insurance in India

In the earlies of 1915, Shri J.S. Chakravarthi of Mysore State proposed a rain insurance scheme, which was based on the area approach, aiming to insure farmers against drought (Raut and Bhandari 2021). He published papers in the Mysore Economic Journal and authored a book titled "Agricultural Insurance: Practical Scheme suited to Indian Conditions" in 1920. In the post-independence period, a special study was commissioned in 1947-48 to explore the modalities of crop insurance. The study considered two approaches: the individual approach and the homogeneous area approach. The study recommended the adoption of the homogeneous area approach due to the lack of reliable data on individual farmers and the moral hazards involved in the individual approach. In October 1965, the Government of India decided to introduce a Crop Insurance Bill and a Model Scheme of Crop Insurance, allowing states to implement crop



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Environment can be defined as a sum total of all the living and non-living elements and their effects that influence human life. While all living or biotic elements are animals, plants, forests, fisheries, and birds, non-living or abiotic

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BHAII-MS-1

Bridging biology and technology: what is known and what is to be known?

S.B. Sainath
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Abstract

Biotechnology is a biology-based technology wherein organisms, cells or cellular components are exploited to develop new technologies that are useful in medicine, research, aquaculture, agriculture, industry and the clinic. With the advent of advanced molecular and analytical technologies, many ground breaking technologies happened and eventually, the perspective of biology. The present review provides a telescopic view of ground breaking bio-based technologies and their impact on humans and agriculture. One of the cogent examples of this trend are CRISPR technology used for gene editing applications not only in biomedicine but also in agriculture. The present review also highlighted different themes of biotechnology such as animal biotechnology, plant biotechnology, food biotechnology, energy biotechnology, environmental biotechnology, systems biology, nanobiotechnology, tissue, cell and pathway engineering, and pharmaceutical biotechnology. Finally, the gaps and challenges are discussed.

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Introduction

It is well accepted that the second half of this century was completely dominated by software revolution. Software revolution, known for building the electrons on a material known as silicon revolutionized technologies, companies and industries which at one point of time was unimaginable. On the other hand, we are going to witness that the second half of this century by approximately, 2050 will also be dominated by living software revolution known for its ability to program biochemistry and molecular networks on a material known as biology. Biotechnology comprises the study and manipulation of living organisms with the goal of creating new products and processes that can improve our health and/or standards of living. The biotechnology as we know today is the perfect amalgam of 'Biology' and its applications by utilizing advances in different fields such as engineering, physics, chemistry, and mathematics/information technology. The field of biotechnology includes emerging technologies that can address problems related to healthcare to food production to waste management.

Today, the field of biotechnology is considered as a broad discipline and popularly known as tinkering biology in which biological processes, cells or cellular components, organ or organ systems, organisms, are exploited to develop new technologies. Recent advances and developments in areas such as nanotechnology, molecular biology and OMICS, synthetic-biology, and genetic engineering tools further pave the way to achieve different selective criteria and development of 'tailor-made' microbe-metabolites that can be effectively applied for different applications. It is worth mentioning the impact of substantial academic and industrial research and developments in last decade on several attributes of different branches of biotechnology, especially in nanotechnology for health and enzyme research, environmental applications, applications of extremophiles, proteomics, uses of toxins, and many more remarkable fields. The products developed through biotechnological tools by the scientists are useful in research, agriculture, industry and the clinic. There are two important areas, the medical and agriculture fields wherein the technological advancements are taking place in biology. Imagine plants that fix Nitrogen very efficiently or plants that resist against harmful pathogens or even development of plants in a perineal manner rather than annual through which crop yield increases twice a year, would certainly revolutionize agriculture. On the other hand, imagine devices that modulate your immune system and prevent against pathogens in an effective manner, would certainly revolutionize medicine. Thus, technological developments in these areas would certainly promote ever growing population fed and also aging population healthy. Some of the cogent examples are the production of transgenic animals, transgenic plants, gene therapy, tissue engineering, stem cell technology, monoclonal antibodies, somatic cell nuclear transfer technology, CRISPR, and omics technology. The Milestones of biotechnology in the area of health care and translational research were shown below (source: Liao et al., 2023)

1928: Discovery of transforming principle by Griffith experiments

1943: Discovery of transforming principle as DNA by McLoed, Mc

1952: Creation of continuous HeLa cell lines by George Otto Gey

1953: Discovery of helical model of DNA by Watson and Crick

1954: Kidney transplantation between identical twins by Joseph Murray

1957: An alteration in a single amino acid within hemoglobin cells induces sickle-cell anemia

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1958: Creation of DNA in a test tube by Arthur Kornberg
1960: Discovery of mRNA
1961: Concept of operon by Francois Jacob and Jacques Monad
1962: green fluorescent protein in the jellyfish by Osamu Shimomura
1963: Samuel Katz and John F Enders developed the first measles vaccine
1963: Insulin production by USA, Germany, and China
1964: The prediction of the existence of reverse transcriptase
1967: Vaccine for mumps/rubella was developed
1968: The discovery of regulation of the cell's synthesis of proteins
1970: Discovery of restriction endonucleases
1972: Discovery of DNA ligases
1973: Southern blotting/ hybridization by Sir Edwin Mellor Southern
1974: Japan scholars developed the first vaccine for chicken pox
1975: Monoclonal antibodies BY Wholer and Milstein
1976: The NIH published the first guidelines for rDNA research
1977: development of the first pneumonia vaccine by the University of Pennsylvania
1978: The first test-tube baby, Louise Brown, was born in the UK
1980: Automatic gene machine was developed in California
1981: Cloning of mice by Switzerland research team
1982: First recombinant protein approved by FDA
1983: Kary Mullis discovered the polymerase chain reaction (PCR) technique
1984: Development of recombinant hepatitis vaccine
1985: Genentech launched its biopharmaceutical product
1986: DNA sequencing analysis using automated DNA sequencer
1987: FDA approved diagnostic kit (serum tumor marker test) development for ovarian cancer
1988: Human Genome Project
1989: First biologically derived human therapeutic developed by Amgen approved by FDA
1990: Hepatitis C antibody test approved by FDA
1992: Recombinant proteins in the treatment of renal cell cancer approved by FDA
1993: Recombinant proteins in the treatment of multiple sclerosis approved by FDA
1994: Recombinant proteins in the treatment of growth hormone deficiency approved by FDA
1995: The first baboon-to-human bone marrow transplant was carried out on an AIDS patient
1996: Gene Chips by Stanford University and Affymetrix
1997: Discovery of first human artificial chromosome
1998: Human skin was created in the laboratory
2000: Synthesis of DNA in a test tube by Har Gobind Khorana
2001: Human genome sequence
2002: The rapid shotgun sequencing of major genomes
2003: The sequencing of the human genome
2004: Monoclonal antibody and DNA microarray analysis system approved by FDA
2006: Recombinant vaccine for human papillomavirus approved by FDA
2007: Human skin cells were manipulated to produce embryonic stem cells
2008: Artificial DNA prepared entirely of artificial parts
2010: Development of prostate cancer medicine approved by FDA
2011: First cord blood therapy approved by FDA
2012: Draft regulations for biosimilar drugs approved by FDA

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2013: CRISPR-Cas9 technology and successful gene editing in human cells
2015: First nasal spray version of naloxone hydrochloride approved by FDA
2016: Buprenorphine implant Probuphine approved by FDA
2018: CART Cell therapy (Novartis) for acute lymphoblastic leukemia approved by FDA
2019: Development of Agg-tag tagging system to identify misfolded and aggregating proteins
2020: AlphaFold2 technology based on AI algorithm to predict protein folding
2021: Development of COVID 19 diagnostic kits approved by FDA
2022: Development of COVID-19 vaccine approved by FDA

The present review provides a telescopic view of bio-based technologies and their impact on humans and agriculture. In the current review, outlines of different themes of biotechnology such as animal biotechnology, plant biotechnology, food biotechnology, energy biotechnology, environmental biotechnology, systems biology, nanobiotechnology, tissue, cell and pathway engineering, and pharmaceutical biotechnology was highlighted. For more information about the history, scope, basic concepts and development of biotechnology, the readers are informed to refer excellent reviews by Bhatia (2018), **Chekol and Gebreyohannes (2018) and Liao et al. (2023).**

Animal biotechnology

Animal Biotechnology deals with the technological or genetic engineering principles applied to the animals which in turn have implications for human health and welfare. Animal biotechnology is a broad field encompassing the polarities of fundamental and applied research, including molecular modeling, gene manipulation, development of diagnostics and vaccines, and manipulation of tissue. Many areas of animal biotechnology such as transgenic animal models, tissue engineering, nanobiotechnology, and proteomics are some of the examples. Further, in-depth examples of applications in human health and prospects for the future, including cytogenetics and molecular genetics, xenografts, and treatment of HIV and cancers.

Plant biotechnology

Plant biotechnology can be defined as the introduction of desirable traits into plants through genetic modification. It encompasses a multitude of scientific tools and techniques for screening and genetic manipulation of plants to develop beneficial or useful plant/plant products. An overview of literature on plant biotechnology innovations and the need to steer agriculture towards sustainability introduces a series of perspectives on how plant biotech can contribute to the major challenge of feeding our super population with enough nutritious food without further compromise of the environment. With all aspects of fundamental and applied research in the field of plant biotechnology, which includes molecular biology, genetics, biochemistry, cell and tissue culture, production of secondary metabolites, metabolic engineering, genomics, proteomics, and metabolomics.

Environmental biotechnology

Environmental biotechnology is the branch of biotechnology that addresses environmental problems, such as the removal of pollution, renewable energy generation or biomass production, by exploiting biological processes. The technological innovations introduced in this area could be effectively clean up many wastes than conventional methods and greatly reduce the dependence on methods for land-based disposal. For example, environmental biotechnology acts as a platform for researchers integrating apparently disjointed biological knowledge, and tailoring this knowledge to address specific engineering challenges related to the environmental pollution. This will be helpful to assess and

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reduce the risk to human health using molecular based forensic tools in the identification of specific environmental problems including microbiological pollution.

Food biotechnology

The issue of food security and safety is a global issue that affects the whole food system. Questions arise on how to change food habits and ways to reduce waste in the food chain, from harvest to the moment of consumption. Although it is important to tackle these issues, it does not change the fact that we will need to change our dominant agricultural model in order to feed a growing global population in a way compatible with the sustainable use of global resources. Innovative agriculture and food systems must be tailored to a diverse global population whilst preserving the variety of its cultures i.e., fitting the characteristics and needs of various individuals, cultures, and social groups. Science alone will not solve food problems and hence, three major forces - science, the economy and society - shape our modern world needs to be harmonize. Food biotechnology deals with the current and emerging developments and applications of modern genetics, enzymatic, metabolic and systems-based biochemical processes in food and food-related biological systems which ultimately helps to produce and improve foods, food ingredients, and functional foods at the processing stage and beyond agricultural production. This area also includes microbial and fermentation based metabolic processing to improve foods, food microbiomes for health, metabolic basis for food ingredients with health benefits, and biochemical processes for food waste management. Food biotechnology also encompasses fundamental and applied educational and research aspects related to food quality and value-added processing challenges.

Systems biology

Systems biology is a branch of biotechnology that acts as an intellectual resource for integrative biology. It is a comprehensive approach in biomedical research to decipher the complexity of biological systems to get a clear-cut picture, be it at the level of the organism, tissue, or cell. Systems biology is an integration of many scientific disciplines – biology, computer science, engineering, bioinformatics, physics and others to understand the complexity of biological systems. One of the major advantages of systems biology is to predict how these complex systems change over time and under varying conditions, and to develop solutions to the world's most pressing health and environmental issues. For example, systems biology wherein innovation in biology-based technology and computation is helpful to discover new biomarkers for disease, stratify patients on genetic profiles and development of targeted drugs. Another example of systems biology is to predict the drug targets in microbes that are resistance against antibiotics. This area is popularly known as reverse vaccinology.

Pharmaceutical biotechnology

It is well known that the designing stable and effective therapeutic proteins requires knowledge of protein structure and the interactions that stabilize the structure necessary for function. Pharmaceutical biotechnology is deals with the development of bioformulations like therapeutic molecules including antibodies, nucleic acid products and vaccines. Such bioformulations are developed through the principles of biotechnology and include several stages: understanding the principles underlying health and disease; the fundamental molecular mechanisms governing the function of related biomolecules; synthesis and purification of the molecules; determining the product shelf life, stability, toxicity and immunogenicity; drug delivery systems; patenting; and clinical trials. In the coming years, protein-based therapeutics is an emerging area of pharmaceutical biotechnology. Some examples of

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pharmaceutical compounds are cytokines, interferons (eg Rebif, interferon beta-1a), interleukins (eg Ontak, denileukin difitox), tumour necrosis factors (eg Beromun, tasonermin), growth factors (eg Neupogen, filgrastim), hormones (eg Humalog, insulin lispro), enzymes (eg Benefix, nonacog alfa), antibodies (eg Avastin, bevacizumab) and vaccines (eg Engerix B, hepatitis B virus coat).

Nanobiotechnology

Nanobiotechnology is one of the revolutionary areas of biotechnology that encompasses physicochemical and biological properties including size (diameter), surface (porosity), pH, solubility, complexation (binding/ligand capacity), bioavailability, toxicity, and cellular and molecular effects of nanostructures and their applications in various areas such as medicine and agriculture. It deals with the control, manipulation, synthesis, and biofunctionalization of structures/devices at the nanometer scale. It is an interdisciplinary science that utilize the use of biocompatible and biodegradable natural and synthetic polymers and their applications range from diagnosis (imaging), drug delivery, and targeting of extremely serious disorders, such as cancer, Parkinson and Alzheimer diseases, chronic inflammations, ocular dysfunctions, and microbial/viral infections. Currently, nanobiotechnology is progressing towards the development of modern nanosystems, including niosomes, polymeric nanoparticles, nanocomposite (colloidal) hydrogels, polymeric micelles, dendrimers, aptamers, capsosomes, nanoneedles, molecularly imprinted polymers, stimuli-responsive polymers, therapeutic polymers (polymer–drug conjugates), and polymeric artificial cells.

Tissue cell and pathway engineering

Tissue, cell and pathway engineering technology deals with regenerative medicine, wherein strategies aim at delivering solutions for those diseases and traumatic accidents that occur in our life and impair the capacity of our tissues to repair and regenerate themselves, restoring their original functionality. Recent developments include clinic spanning from skin grafts to stem cell-based therapies for cornea regeneration and instructive biomaterials for bone regeneration. These models are instrumental to understand more fundamentally the cell-material interface and also future avenues of tissue regeneration combining developmental biology, organoids, and 3D bioprinting. With reference to the integrating developmental re-engineering with 3D bioprinting, regulation of several signaling pathways through the fabrication of mini-organ constructs for transplantation or *in vitro* screening of drugs using an organ-on-a-chip platform.

CRISPR technology

Thanks to the two women scientists Jennifer Doudna an American Biochemist, University of California, Berkeley and Emmanuelle Charpentier a French Microbiologist, Max Planck Institute for Infection Biology who make history for their discovery that an intrinsic immune system of bacteria and archea fend off viruses and plasmids which invade them using a molecular programming mechanism known as Clustered Regulatory Interspread Palindromic Repeats (CRISPR). Accordingly in 2020, the two female CRISPR scientists won Nobel Prize in chemistry for their discovery. It is worth to state that the microorganisms often surprise the scientists with plethora of molecular gadgets that have potential biotechnological applications and most notable example of this trend is the application of restriction enzymes as a molecular scissor in genetic engineering strategies. Currently, targeted gene editing is gaining popularity because of its simplicity in design and usage in biomedical and industrial applications.

Other ground breaking technologies include, a) AlphaFold, a protein folding

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program developed by Google's DeepMind to understand the concepts like how proteins fold and better understanding of proteins may be helpful to human health; b) CRISPR-Based Gene Editing of DNA is offering potential cures for genetic disorders and also addressing inheritable diseases like cystic fibrosis to revolutionizing agriculture eg: grow drought-resistant crops; c) Epigenetics and digital therapeutics is another emerging areas of biotechnology wherein there is a scope for the development of precision medicine and tailoring treatments to individual genetic profiles. d) In order to get invaluable insights into drug effects and disease progression, a real time visualization of cellular processes in living organisms, Bioluminescent imaging is widely used; e) 3D bioprinting technology is a tissue crafting three dimensional technology that revolutionize the organ transplantation; f) Biosensors that track various biosignatures such as blood pressure, pulse, breathing, and body temperature will revolutionize precise monitoring system of our health; g) development of bioplastics is one of the profound technologies wherein the safety of humans, plants, wildlife and eventually environmental health against the toxicity of plastics and their metabolites is of prime concern.

Conclusion

Harmonization of biotechnological developments collated exactly what is known and what is to be known about the improvement of human health and also for sustainable agriculture and augment the researchers and clinicians to unlock the power of genome. Given the tools that are currently available and the translational potential for these studies, the power of technological developments in biosciences has become one of the most essential areas for those studying life sciences.

References

The present topic deals with the recent developments in the area of biotechnology. Due to limited space and also to provide a comprehensive data on the biotechnological developments, a broad outline on the selected topics were described in the current review. Hence, possible links to the articles are provided for further information on the aforementioned topics.

1. https://www.frontiersin.org/articles/10.3389/fimmu.2020.585354/full
2. https://www.technologyreview.com/2023/12/12/1084492/mapping-the-micro-and-macro-of-biology-with-spatial-omics-and-ai/
3. https://institut-curie.org/popin/spatial-omics
4. https://pubmed.ncbi.nlm.nih.gov/10592781/
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6. https://biotechnology.report/Resources/Whitepapers/28d904dc-b8e3-4b1a-9c76-bf32a6734c47_fulltext_ajbtbe.pdf
7. https://carnegieendowment.org/2020/11/20/blessing-and-curse-of-biotechnology-primer-on-biosafety-and-biosecurity-pub-83252
8. https://www.nsf.gov/bio/pubs/reports/stctechn/stcmain.htm
9. https://www.pnas.org/doi/10.1073/pnas.191380098
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13. https://greentumble.com/advantages-and-disadvantages-of-using-biotechnology-in-agriculture
14. https://link.springer.com/article/10.1007/s41207-020-00240-5
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Xenobiotics and human health with special reference to pesticides

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Abstract

There is a major concern towards the accumulation of xenobiotics such as pollutants, drugs, carcinogens, hydrocarbons, food additives and pesticides in the environment and their toxic effects on humans. They can able to interfere with the physiological aspects and eventually causes health hazards in humans and wildlife. Though the exact mechanism of xenobiotics is not clear, they exert their effects through oxidative stress and endocrine disruption. It is believed that these foreign substances after their entry, perturb anti-oxdiant to pro-oxidant balance and interfere with endocrine system thereby target almost all compartmets of humans including liver, kidney, brain, reproductive system, circulatory system and eventually affect metabolism, development, and reproduction. In the current review, we summarize the mechanisms underlying the toxic effects of xenobiotics on liver, kidney, development and reproduction.

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Introduction

The word xenobiotics is derived from the Greek word '*xenos'* meaning foreign. The xenotiotics are chemically synthesized and do not occur in nature. Most xenobiotics do not readily biodegrade and thus are persistent in environment (Fetzner, 2002). Such chemicals are known as persistent chemicals. It was estimated that humans are exposed to about one to three million xenobiotics in their lifetime. Most of these xenobiotics gain entry to the body via food, drinking water, air, drugs, inhalation, etc and they undergo a broad range of detoxication processes which render them more polar, less toxic, making them readily excretable. In the lower animals, detoxification reactions may have impact on human health. Incomplete detoxification in lower animals down the food chain may determine the extent of human exposure to xenobiotics (Patterson et al., 2010). This review article highlights the impact of xenobiotics and their toxic mechanisms underlying health hazards.

Endocrine disruption and its impacts

Hormones play a vital role in the regulation of physiological functions associated with growth, sexual development, reproduction, movement etc. in organisms. Endocrine disruptors (ED) are the agents that interfere with this endocrine system and can able to interfere with the synthesis, secretion, transport, binding or elimination of the natural hormones in the body (Diamanti-Kandarakis et al., 2009). They can act as agonists and/or antagonists to the endogenous hormones like estrogens, androgens, thyroid hormones and/or can able to bind their cognate receptors and eventually lead to improper genomic actions that may induce harmful effects (Kabir et al., 2015).

For example, a number of chemicals that are structurally similar to thyroid hormones, have been shown to bind to the thyroid receptors and exert either agonism or antagonism effects on thyroid hormone signaling (Lyn, 2009) thereby perturb thyroid hormone mediated functions (Zoeller and Crofton, 2000). Studies have shown that the 4,4'-isopropylidenediphenol (BPA) mimic thyroid hormone and can able to bind thyroid hormone receptor (TR) and antagonizes T3 thereby inhibit(s) TR-mediated gene expression (Diamanti-Kandarakis et al., 2009). Thyroperoxidase (TPO) is a heme-containing enzyme that catalyzes the oxidation of iodine and thyroglobulin thereby thyroid hormone synthesis in the thyroid gland. Studies indicated that TPO was found to be blocked by 6-propyl-2-thiouracil (PTU, used therapeutically to treat Graves disease) (Kabir et al., 2015). It has been shown that the heavy metals, DDT, dioxins, PCBs, synthetic steroids, dithiocarbamates, phytoestrogens, some of the pesticides and herbicides (Kabir et al., 2015) directly target the nervous system thereby exert endocrine disrupting effects and modulate behavior, memory, learning, sensory function, attention, and even neuro-teratogenicity. Direct effect on steroid receptor activity is the most studied mechanism. Most of the xenoestrogens acts as ligands for estrogen receptor and may compete with estrogen for binding and are thought to exert their functions by activating estrogen receptor and regulates the target genes accordingly (Singleton and

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Khan, 2003). Some of the disorders observed during the lifetime may be sexually dimorphic and related to endocrine disruption. Sexual differentiation in males is androgen-dependent and in females it is independent of estrogens and androgens. Therefore, different disorders seen in males and females are the result of endocrine disruptors that antagonize androgens and/or mimic estrogens (Diamanti-Kandarakis et al., 2009). In females, endocrine disruptors may cause polycystic ovary syndrome, precocious puberty, premature ovarian follicle, risk of breast cancer, and unwanted increase in the endometrium growth. In males, endocrine disruptors may cause hypospadias, ectopic testes, sperm anomalies and eventually decrease in fertility (Kabir et al., 2015).

Development and Reproduction

Prenatal period is believed to be one of the critical windows wherein crucial development of vital organs including reproductive tract takes place. Xenobiotics can able to cross placenta and can interfere and causes adverse effects on the developing fetus (Barr et al., 2007). The women who were exposed *in utero* to the estrogenic chemicals are at higher risk for the incidence of cancer when compared to the unexposed individuals (Colborn et al., 1993). Diethylstilbestrol (DES) is one of the drugs given to the pregnant women to prevent abortions. However, studies indicated that DES can able to cross placenta and acts as a development toxicant as evidenced by abnormality in pregnancies, reproductive organ dysfunction, reduced fertility, disorders of immune system in the children of women who were given DES during pregnancy (Fraser and Adeoya-Osiguwa, 2006). Wide usage of PCBs causes environmental pollution. Many reprotoxic effects have been associated with spermatogram, testicular spermatogenesis and steroidogenesis and epididymal post testicular sperm maturation events. Interestingly, phthalates are a group of chemicals that affects Leydig cell function via inhibition of testosterone synthesis (Diamanti-Kandarakis et al., 2009).

Hepatotoxicity

Liver is one the metabolic organs in the body and is well appreciated for its detoxification mechanisms of chemical substances. It has been shown that xenobiotics lead to the accumulation of fat in the liver in humans. An example of this trend is the fat deposition in humans who consume alcohol and progressive liver injury can lead to fibrosis and finally to cirrhosis (Maher, 1997). A link between xenobiotics, fat accumulation in the liver and changes in nuclear receptor expression has been reported. A plastic raw material, phthalate ester Di (2-ethylhexyl) phthalate (DEHP) interact with peroxisome proliferator activator receptor (PPAR)α and causes fat accumulation, liver tumors and even changes the metabolism in liver (Klaunig et al., 2018). Liver is one of the important organs of detoxification systems. When xenobiotics enter the system, the phase I and phase II systems of liver degrade the xenobiotic and in most of the cases, the degraded metabolites are excreted, however some metabolites become toxic and such toxic products exert liver injury occurs via two types intrinsic and idiosyncratic mechanisms. Intrinsic injuries are generally predictable leading to

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zonal hepatocyte necrosis which may be accompanied by little or no signs of inflammation and on the other hand, idiosyncratic liver injuries are mediated by immune mechanisms. The predominant pattern of injury is by diffuse necrosis and cholestasis (Sturgill and Lambert, 1997).

Apart from environmental pollutants, drugs such as acetaminophen are widely studied hepatotoxic. The cell death mechanisms are initiated by the formation of its metabolite N-acetyl-p-benzoquinone imine (NAPQI), cytochrome P450 enzymes (Jaeschke et al.,2014). Under normal conditions, NAPQI is converted into harmless reduced form by conjugation with glutathione, which is then excreted via the bile. However, NAPQI toxicity occurs when the glutathione is depleted due to the overdose of acetaminophen. The increasing amount of NAPQI binds to the mitochondrial protein and forms protein adducts which could be cytotoxic in nature. Glutathione levels can be replenished by N-acetyl cysteine which helps in the clearance of this toxic metabolite (Krenkel et al., 2014). Aflatoxins are the compounds produced by Aspergillus species. Aflatoxins specifically target liver and symptoms of hepatotoxicity caused by aflatoxins may include malaise, anorexia, fever, followed by abdominal pain, hepatitis and vomiting (Kumar et al., 2017).

Nephrotoxicity

Because of the active transport capability, high rate of perfusion, concentrating function, kidney is most often exposed to higher concentrations of xenobiotics than any other organ. Some xenobiotics may exert toxicity directly on renal cells. Examples of xenobiotics that causes renal cytotoxicity are therapeutic agents (like analgesics, aminoglycoside antibiotics), heavy metals (like cadmium, mercury), or other chemicals (like carbon tetrachloride, chloroform) (Kluwe and Hook, 1980). The heavy metals in plasma exist as diffusible (complexed, ionized) and non-diffusible (protein bound) forms. It is well known that the tubular reabsorption is dependent on the form that is present in the glomerular filtrate and filtrate composition is in turn dependent on the dose and the mode of intoxication. Heavy metal ions readily bind to the most abundant protein, albumin, in plasma, however, a small amount of heavy metal may escape from this kind of binding. This eventually, lead to As a result, the plasma also contains the heavy metal in free form and glomerulus always filters a small amount of albumin. Hence, the luminal fluid delivered to the proximal tubule will contain both the albumin bound form of heavy metal and also the ionized from of heavy metal (Barbier, et al., 2005). The toxic form of heavy metal is the ionized form and the extent of severity of the renal damage depends on the mode of exposure. Cadmium causes the rupture of outer membrane, inhibition of electron transport chain resulting in the release of reactive oxygen species. Most of the heavy metals causes chronic intoxication like Fanconi syndrome which causes decrease in the glomerular filtration rate, proteinuria, increased urinary flow rate, glycosuria, loss of ions, and aminoaciduria (Barbier, et al., 2005). Chemicals such as chloroform is metabolized by cytochrome P450 to a reactive metabolite, phosgene, and this metabolite is responsible for renal toxicity

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(Bailie, et al., 1984). Hypoxia and hypotension induced by phosgene may lead to anoxic injury to the kidneys. The abnormalities in the victims who are acutely exposed to phosgene include increase in blood urea nitrogen (BUN), abnormal urinalysis with numerous RBCs and WBCs (Borak and Diller, 2001). Drugs such as amphotericin B, an antifungal drug exhibits numerous side effects including kidney failure. During treatment with amphotericin B, 30% of the patients show renal malfunctions and as a result, serum manganese and potassium are reduced, and serum creatinine are elevated. The main reason of nephrotoxicity of amphotericin B is thought to be its high affinity for cholesterol and hence, to reduce these side-effects, monotherapy with amphotericin B is avoided whenever possible (Lemke, et al., 2005).

Natural toxins

Natural toxins are produced by plants as secondary metabolites and are not harmful to the organism itself but are toxic to other living forms such as, humans. Some plants produce a wide array of chemicals as a natural defense mechanism, to protect themselves against predators, microorganisms, or insects (WHO, 2018). Cycad plants were used as a source of food and medicine. These plants have also been recognized as hazards in tropical areas. Cycasin is the main toxin found in *cycas revoluta* and also in all genera of Cycadales. Cycasin is an azoxyglucoside and causes toxic effects on liver and gastrointestinal tract. In humans, there have been many cases of gastroenteritis caused by cycasin reported (Chang, et al., 2004). Pyrrolizidine alkaloids are present geographically widespread distributed plants. More than 200 alkaloids were identified in 300 different types of plant species of up to 13 families. The commonly known pyrrolizidine alkaloids are lasiocarpine, heliotrine, senecionine, monocrotaline. The main sources of pyrrolizidine alkaloids are Compositae, Boraginaceae, Leguminosae (Prakash, et al., 1998). Many of these sources are weeds and can grow in fields and contaminate food crops. Pyrrolizidine alkaloids can cause adverse health effects. They have DNS-damaging potential and can lead to cancer (WHO, 2018). Mycotoxins are the toxic compounds produced by certain types of fungi. Fungi that produces mycotoxins can grow on many foodstuffs like nuts, dried fruits, spices, cereals(WHO, 2018). Fumonisin B1, aflatoxin B1, and ochratoxin A are toxic to mammals causing variety of adverse effects like mutagenicity, teratogenicity, hepatotoxicity, resulting in diseases like hepatic carcinoma, esophageal cancer, equine leukoencephalomalacia, toxic hepatitis, edema, hemorrhage, hepatic carcinoma, and kidney failure (Reddy, et al., 2010). Upon ingestion of mycotoxin contaminated food, the gastro-intestinal tract is affected. They can alter the normal functions of intestine like nutrient absorption and barrier function. Some of them can also affect the histomorphology of intestine (Liew and Mohd-Redzwan, 2018). Solanines and chaconine are the natural toxins found in all Solanaceae plants like potato, tomato, eggplant. They are found in potato sprouts, green parts, bitter tasting peel and also in green tomatoes (WHO, 2018). Solanines and chaconine can induce

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gastro-intestinal effects by cell membrane disruption and systemic effects by acetylcholinesterase inhibition (Mensinga, et al., 2005).

To reduce the health risk caused from the natural toxins, WHO recommends people not to assume that when something is called as 'natural' it is automatically safe; do not consume and throw away damaged, bruised, or discolored food, particularly food contaminated with moulds; throw away the food item that does not taste or smell fresh or is having an unusual taste (WHO, 2018).

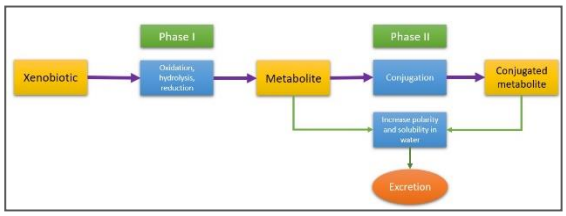

Figure 1: Biotransformation of xenobiotics

Mechanism of Action of Xenobiotics

The mechanism of action describes how a xenobiotic enters into the living organism, followed by its distribution, metabolism and its interaction with the cellular macromolecules. These events involve cellular, molecular and biochemical reactions (Park et al., 2014). In general, biotransformation of xenobiotics is believed to be one of the important underlying mechanisms of its toxicity. The mechanisms may broadly be classified into direct or indirect. Directly, xenobiotics may undergo hydrolysis and exhibit electrophilic property. This eventually targets the proteins thereby elicit toxicity. Such xenobiotics area classically known as direct acting xenobiotics. The xenobiotics with the electrophilic property, interacts with the cellular macromolecules like DNA, lipids, proteins and results in the toxicity inside the organism (Park et al., 2014). On the other hand, xenobiotics undergo biotransformation in the liver wherein and phase I and phase II systems. Phase I system includes several cytochrome P450 enzymes and xenobiotics undergoes mainly oxidation reactions which in turn elevate the electrophilicity of the xenobiotics (Yagishita et al., 2016). Phase II system include enzymes that carry out the conjugation reactions include acetylation, glucuronidation, methylation, and glutathione mediated by transferases and convert the xenobiotics into more water-soluble forms (Park et al., 2014; Omiecinski et. al., 2010). The metabolites of the parent xenobiotc compound, now elicit toxicity and hence, known as indirect acting xenobiotics (Patterson et al., 2010). The ultimate outcome of these events is toxicity to the living organism. Though the final goal of biotransformation is to convert the xenobiotics into harmless substances by converting lipophilic chemicals to more water-soluble and facilitate to excrete the same through urine. However, some substances, during their transformation become highly reactive and perturb redox-cycling system and eventually causes oxidative stress induced damage of the organs and prolonged presence of the reactive metabolites in the body may lead to adverse effects and disease conditions (Park et al., 2014). On the other hand, some substances, they interfere with the endocrine system and switch on or off genes thereby changes the molecular networks and physiological events of the

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cells. Hence, xenobiotics induced toxicity relies on oxidative stress and endocrine disrupting properties. Figure 1 illustrates the biotransformation of xenobiotics through phase I and phase II systems.

Conclusion

To summate, the concept of xenobiotics and their toxic effects is of major concern. They exert their effects via endocrine disrupting properties and oxidative stress. Xenobiotics such as heavy metals, pesticides, natural toxins and chemicals/drugs interfere with the redox system and endocrine system. This eventually damage the organs. In addition, as prolonged exposure to xenobiotics at low concentrations may be mutagenic and teratogenic. To avoid the toxic impact of xenobiotics, some measures should be taken which include effective treatment of wastewater, strict laws and regulations must be adopted which are economically, environmentally, and socially acceptable with an emphasis on the removal of xenobiotics from the environment.

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Identification of putative drug targets in *Morexalla catarrhalis* using subtractive proteomics

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Abstract

Now a days, the emergence of drug resistance developed by bacteria is one of the major concerns. Thie eventually caused a high demand to prioritize an effective drug target. In these days, the reverse vaccinology strategies to identify a drug target is well acknowledged. Subtractive proteomics is one of the reverse vaccinology strategies to identify the drug targets in bacteria. In the current study, various *in silico* tools subjected to subtractive proteomics pipeline was used to identify the drug targets in *Moraxella catarrhalis,* a gram-negative bacterium that causes respiratory tract and ear infection in infants and adults. Almost, 52 potent drug targets were identified in this study and eventually, one protein was identified as a potential new drug target and forwarded to the structure-based studies i.e. nitrate reductase. Furthermore, virtual screening of 500 compounds from the ZINC database was performed against the nitrate reductase and based on the binding energies and ADMET analysis, three compounds were shortlisted. Nitrate reductase could be one of the potential drug targets identified in *M. catarrhalis*. However, further in vivo experiments were warranted.

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Introduction

Moraxella catarrhalis is a human-restricted commensal and has evolved from an emerging to a well-established pathogen and studies have shown that this organism has become one the major pathogens of upper and lower respiratory tract infections in humans. In children, it causes about 17% of acute otitis media infections in children (Verduin et al., 2002), sinusitis, bacteremia, otitis media, pneumonia, tracheitis, bronchitis, and, less commonly, ocular infections in children. Whereas in adults, it causes laryngitis, bronchitis, pneumonia, nosocomial infections and endocarditis. Furthermore, *M. catarrhalis* was found to cause other types of infections, such as bacteremia, endocarditis and meningitis, especially in immunocompromised patients (Tan et al., 2002; Smidt et al., 2013). Hence, this organism is considered to be one of the emerging pathogens associated with respiratory tract infections (Verhaegh et al., 2008).

In general, the classical antibiotic treatment reduces the clinical burden. However, improper regimen and unrestricted antibiotic use is one of the underlying mechanisms of the development of antibiotic-resistant bacteria (Ashraf et al., 2021). This eventually reduced the viable antibiotic options. One of the cogent examples of the development of antibiotic resistance by *M. catarrhalis* against the amoxycillin. On the other hand, studies have shown that the Chronic Obstructive Pulmonary Disease (COPD) and other respiratory diseases caused by *M. catarrhalis* are difficult to treat because of the rising MICs and antimicrobial drug resistance. Though *M. catarrhalis* was thought to be a harmless commensal, the hospital outbreaks of this organism related respiratory diseases indicated that there may be shift from commensal to nosocomial pathogen. However, with the advanced molecular and analytical techniques, the pathogenic and virulence factors related to this shift (commensal to pathogenic) of *M. catarrhalis* came to limelight.

Published reports (de Vries et al., 2009; Parameswaran et al., 2009; Augustniak et al., 20118) have shown that the mechanisms of pathogenesis of *M. catarrhalis* may include a) evasion of immune surveillance such as complement system, residing in the lymphoid tissue and activation of non-specific B cells to produce polyclonal antibodies and redirecting adaptive immunity, b) biofilm formation; and c) interference at the level of protease-antiprotease balance. Other studies also suggested that the virulence factors such as periplasmic and outer membrane components released from the outer membrane vesicles to the host also favor colonization of this bacteria in the host system.

Since antibiotic resistance has been one of the major problems these days, development of antimicrobial drugs with bactericidal activities is one of the emerging areas of biomedicine. To accomplish this task, identification of suitable drug targets is a fundamental step. However, conventional studies to identify such potential drug targets is often associated with economic constraints and also not time bound. Hence, alternative strategies which can able to identify drug targets is urgently need. With the advancements in the

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molecular and genetic engineering principles, genome architecture and proteome of many pathogenic organisms are known. Thus, comparative and reverse vaccinology strategies or subtractive proteomic analysis proved to be exigent job in the identification of drug targets and design of new drugs against the intended drug targets of pathogens. The major advantage of this approach is cost-effectiveness and opened avenues to unlock the new pathways for finding vaccine or drug candidates. The other advantage of this systems biology approach sped-up the drug discovery process and also useful to screen many targets at a time against the selected drug targets and also reduced the failure rate in clinical trial process. Using this comparative and subtractive genomic approach, so far, many drugs have been prioritized and identified therapeutic targets for harmful pathogens like *Clostridium botulinum*, *Mycoplasma pneumoniae*, *Rickettsia, Neisseria gonorrhoeae*, *Salmonella typhi*, and *Shigella dysenteriae* (Sudha et al., 2018; Vilela Rodrigues et al., 2019; Tanwer et al., 2020)

The major goal of this study was to identify the drug target(s) in *Moraxella catarrhalis* BBH18 and therapeutic candidates against this pathogen. To address this aspect, systems biology approach was used wherein the genome of *M. catarrhalis* was explored. In this study, comparative and subtractive genomics analysis approach, molecular docking analysis, Protein-Protein Interaction (PPI) network analysis, essentiality, drug ability of target proteins and ADMET properties were used to identify drug targets and therapeutic candidates.

Materials and Methods

The approach of systems biology approach was employed for the drug target prioritization against the *M. catarrhalis* which holds clinical and biological importance. In this study, BBH18 strain of *M. catarrhalis* was selected and potent drug targets were identified. The flow chart represent the pipeline of in silico analysis performed in this study for the identification of dug targets and also selection of appropriate therapeutic candidate (Figure 1). The following methodology was performed to accomplish this task.

a) The proteome of *M. catarrhalis* BBH18 strain (genome size: 1.9 Mb; protein coding genes of 1636) was retrieved from NCBI database (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000092265.1/) and subjected to CD-HIT analysis (60 % identity) to remove the paralog or duplicate sequences.

b) The non duplicate protein sequences were subjected to BLASTp analysis and searched against the human proteome. This step was important to remove homolog or similar sequences.

c) The non paralog and non-homologous protein sequences were now subjected to DEG database to identify essential sequences (> 50% identity; Bit score > 100).

d) The subcellular localization of DEG proteins were analyzed using PSORTb database

e) Cytoplasmic or membrane proteins were selected and its involvement in KEGG

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pathways was evaluated using KAAS server available in KEGG database.

f) Efflux pump membrane transporter (D5VAP4) transporter was selected in the present study and its virulence factor efficacy was analyzed through VDFD database

g) D5VAP4 was searched against the drug data bank and also protein data bank to identify suitable ligands or drugs and also for template selection.

h) Further, if any drug was available in the drug data bank against the protein, the drug was selected as ligand and docked against the selected protein

i) SWISS-MODEL database was used for homology modelling

j) The predicted model was analyzed for its quality using ERRAT and Ramachandran plot analysis

k) Molecular docking was performed using EASY-DOCK pipeline

l) The protein was also screened against the zinc library for suitable ligands and redocking was performed against the step I (the coordinates of grid box used for docking analysis of zinc library compounds). Only compounds with molecular weights of 300 to 500, rotatable bonds with <10 and RMSD values at least 0.3 to 0.7 were considered as the cut-off values.

m) String database was used to analyze the protein-to-protein interactions

Results and Discussion

The protein coding genes as per the NCBI Ref Seq in NCBI database was found to be 1636 and CD-HIT analysis revealed that only six genes were paralogs (Table 1). The non paralogs were subjected to BLASTp and searched against the human proteome to remove the homologs and accordingly, 519 homologs were identified and eliminated. The remaining 1121 non homologous protein sequences were analyzed for essential genes suing DEG database (Table 1). Accordingly, 91 essential sequences were identified using DEG and were subjected to drug databank to investigate drugs if any. Out of 91 essential sequences, 38 sequences showed significant hits against drug databank. The subcellular localization of essential genes was shown in table 1.

Table 1. Non-homologous essential drug target identified out of 38 DEGs

S. No.	Uniprot Protein IDs	Protein's Name	PSORTb Results
1	D5VAP4	Efflux pump membrane transporter	Cytoplasmic Membrane

Molecular docking analysis

In the current study, efflux pump membrane transporter (Unipot ID: D5VAP4) was selected as drug target. Efflux pumps are membrane proteins responsible for transporting substances across the cell membrane. In prokaryotes, these proteins are associated with intercellular signaling, processes associated with microbial virulence, and removal of unwanted metabolites and toxic substances from the cell. Thus, they contribute to the maintenance of cell homeostasis. The activity of efflux pumps is one of the reasons for bacterial resistance to certain antibiotics and bactericides. This occurs when the substance structurally resembles the pump's natural substrate or when the selectivity of the pump is modest

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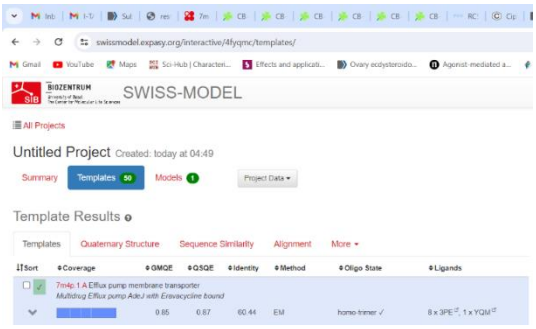
(multidrug resistance, MDR pumps). Chromosomal DNA usually encodes pumps with a broader spectrum of substrates. In contrast, mobile genetic elements, such as plasmids, typically contain genes encoding pumps with greater substrate selectivity.

Homology modelling

D5VAP4 was subjected to swiss model server to build model of this protein. Among the several templates, 7m4p.1 template (Figure 2; Zhang et al., 2021) was selected since it exhibited >60% identity with a GMSE and QSQE scores of > 80.

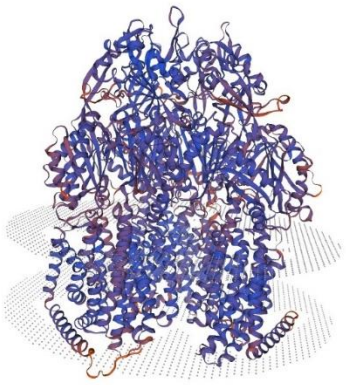
Fig 2:

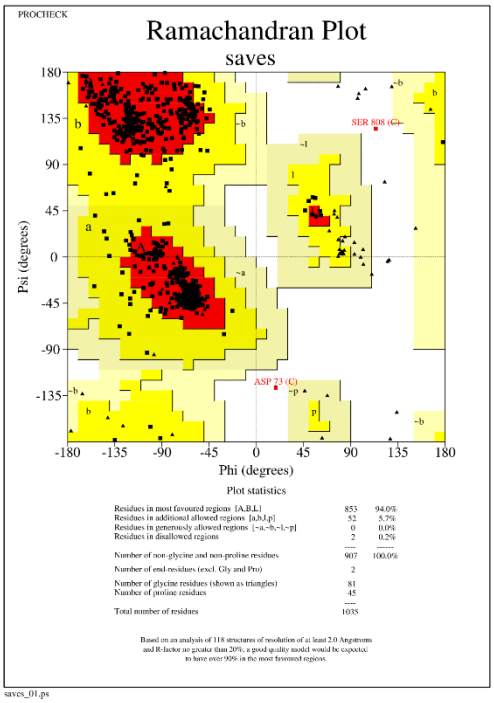


Quality of predicted model

The pdb structure of predicted model was subjected to SAVES server to assess the quality. ERRAT values indicated that the predicted model was of good quality with a score > 96.277. Ramachandran plot analysis showed that the modelled protein exhibited >94 % amino acids in favoured regions, >5% in allowed regions. Only two amino acids, ASP 73 and SER 808 were present in disallowed regions.

Homology model of D5VAP4





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Sequence of D5VAP4

```
>tr|D5VAP4|Release 2023_02/2023_02|03-May-2023
MSRFFIHRPIFAWVIAILIMLVGILAINNLPIEQYPKIAPPQVKVRAVYPGADAETVENS
VTQIIEQQMKGIDGLMYMSSNSSAAGSASVTLTFENGTDPDTAQVQVQNKLQSAMSSLPE
TVQRTGLNVEKSASDFLMVTAFVSEDGSMDQSDIADYVATSIVDPISRVEGVGGTNVFGS
SYAMRIWLDPAKLRAYNLIPSDVSSAIRSQNVQVSAGQLGTLPTNTDRVVINATISVQSY
LQTPEQFEDILLKTDTAGAQVRIKDVARVELGSENYQFRAQYNGQAASGLAIMLAPGANA
LEVREAVGVRLDELSQNFPTGLKMVVPYDTTPFVRLSITQVVYTLLEAVVLVFLVMFLFL
QNWRATIIPTLAVPVVILGTFAVLSVFGYSINILTMFAMVLAIGLLVDDAIIVVENVERI
LEENPSIQVMDATIQSMREISKVVIGIALILSVVFVPMIFFGGSSGVIYRQFAVTLMTSM
VLSAFIALVFTPALCVTILKRQAHKDINIQTGFFGWFNRFFYQTSRRYENFIGKTYASKL
AYLAVYTGIVAVMALIFMRLPSSFVPEEDQGAVMTLVQLPAGSTLDKTNAVMDKLANYYH
DKETDNIESVFTISGFGFMGSGQNSGMAFIKLKDWDERAGSENTAQSIARRAMVMNMMIP
EASLIFPIAPPPIQGFGNTSGFDLQLKDVGGVGHEALLDARNQLMGMAMQNPAIASIRPG
GQEDAPKLKVDINQAQAAAYGVPLTAINDTIAQAWGGSYVNDFIDRGRIKKVYIQGEPNS
RVVPEDINRWYVRNQSGEMVSFGAFSGSQWEYGSPSLARYNGVSSMVLTGSAALGVSTGD
AMEAMAQMASQLPAGIDFEWTGLSLEQQKSGGQAPILYALSILVVFLCLAALYESWSVPF
AVILVIPLGVIGSLLLTKIHGLANDVYLQVALLTVIGLSAKNAILIIEFAKEIQESGQTL
KASIMMAARMRLRPIIMTSLAFGVGVVPLYIATGAGSGSQNAVGTGVLGGVLTSTFLGIF
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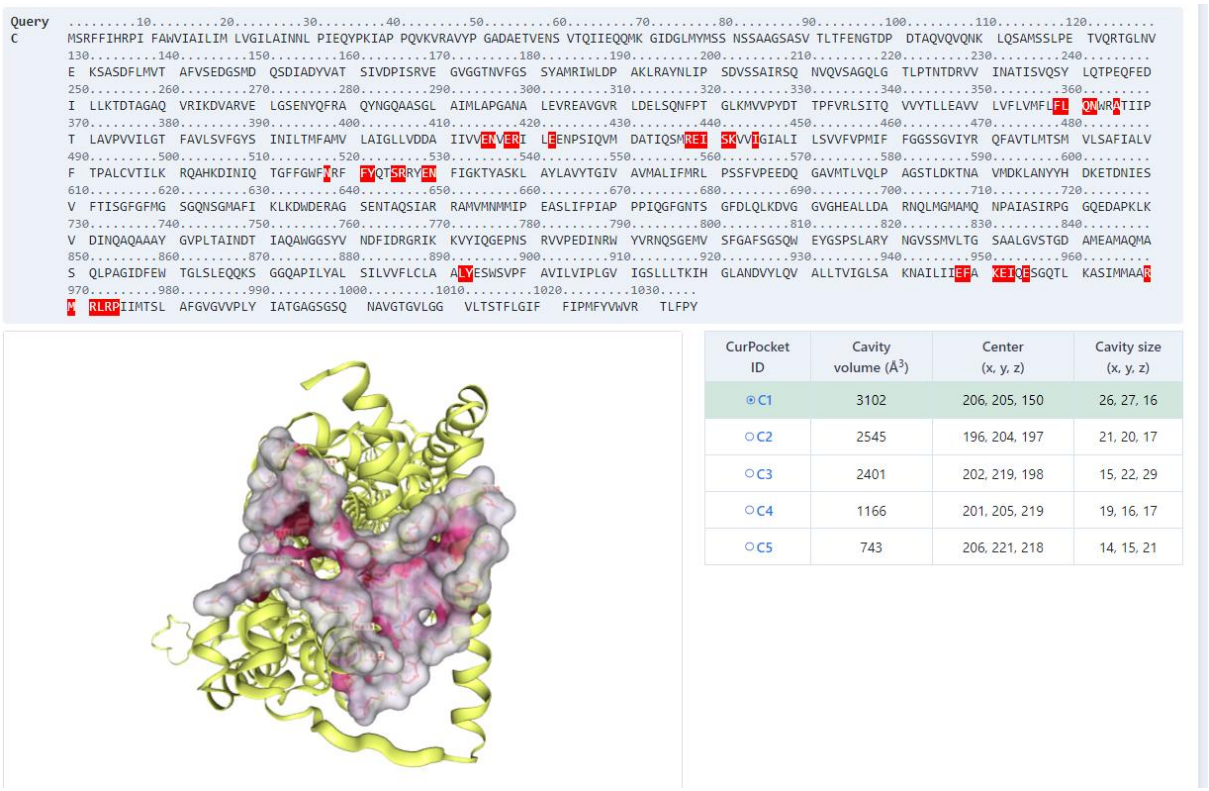

CurPocket ID	Cavity volume (Å^3)	Center (x, y, z)	Cavity size (x, y, z)
C1	3102	206, 205, 150	26, 27, 16
C2	2545	196, 204, 197	21, 20, 17
C3	2401	202, 219, 198	15, 22, 29
C4	1166	201, 205, 219	19, 16, 17
C5	743	206, 221, 218	14, 15, 21

Ligand selection

Based on the significant hits of selected protein (Table 2), dequalinium was selected as one of the ligands. Based on the literature survey, ciprofloxacin (Mikucka et al., 2000) and eravacycline (Sun et al., 2021) were also selected as ligands. Ciprofloxacin and eravacycline showed good efficacy against *M. catarrhalis*. Hence, they were selected and docked against the protein D5VAPR.

Molecular docking

Molecular docking was performed using Autodock Vina which was in built in EASY Dock tool. The x, y and z coordinates and active site prediction were performed using CB-dock server. The grid box coordinates were 206, 205, 150 from center and 25, 27 and 16 were the docking box size of x, y and z coordinates respectively.

>tr|D5VAP4|Release 2023_02/2023_02|03-May-2023

MSRFFIHRPIFAWVIAILIMLVGILAINNLPIEQYPKIAPPQVKVRAVYPGADAETVENSVTQIIEQQMKGIDGL
MYMSSNSSAAGSASVTLTFENGTDPDTAQVQVQNKLQSAMSSLPETVQRTGLNVEKSASDFLMVTAFVSED
GSMDQSDIADYVATSIVDPISRVEGVGGTNVFGSSYAMRIWLDPAKLRAYNLIPSDVSSAIRSQNVQVSAGQL
GTLPTNTDRVVINATISVQSYLQTPEQFEDILLKTDTAGAQVRIKDVARVELGSENYQFRAQYNGQAASGLAIM
LAPGANALEVREAVGVRLDELSQNFPTGLKMVVPYDTTPFVRLSITQVVYTLLEAVVLVFLVMFLFLQNWRATI
IPTLAVPVVILGTFAVLSVFGYSINILTMFAMVLAIGLLVDDAIIVVENVERILEENPSIQVMDATIQSMREISKVVI
GIALILSVVFVPMIFFGGSSGVIYRQFAVTLMTSMVLSAFIALVFTPALCVTILKRQAHKDINIQTGFFGWFNRFF
YQTSRRYENFIGKTYASKLAYLAVYTGIVAVMALIFMRLPSSFVPEEDQGAVMTLVQLPAGSTLDKTNAVMDKL
ANYYHDKETDNIESVFTISGFGFMGSGQNSGMAFIKLKDWDERAGSENTAQSIARRAMVMNMMIPEASLIF
PIAPPPIQGFGNTSGFDLQLKDVGGVGHEALLDARNQLMGMAMQNPAIASIRPGGQEDAPKLKVDINQAQ
AAAYGVPLTAINDTIAQAWGGSYVNDFIDRGRIKKVYIQGEPNSRVVPEDINRWYVRNQSGEMVSFGAFSGS
QWEYGSPSLARYNGVSSMVLTGSAALGVSTGDAMEAMAQMASQLPAGIDFEWTGLSLEQQKSGGQAPILY
ALSILVVFLCLAALYESWSVPFAVILVIPLGVIGSLLLTKIHGLANDVYLQVALLTVIGLSAKNAILIIEFAKEIQESGQT
LKASIMMAARMRLRPIIMTSLAFGVGVVPLYIATGAGSGSQNAVGTGVLGGVLTSTFLGIFFIPMFYVWVRTLF
PYKPKAQPVTHAKSHEHV

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Interacting amino acids between dequalinium and D5VAP4

	Name	Visible	Color	Parent	Distance	Category	Types	From	From Chemistry	To	To Chemistry
1	d:UNK1:...	Yes		Ligand Non-bond Monitor	2.64063	Hydrogen Bo...	Conventional Hydrogen Bond	d:UNK1:H40	H-Donor	C:GLN361:OE1	H-Acceptor
2	d:UNK1:...	Yes		Ligand Non-bond Monitor	2.02373	Hydrogen Bo...	Conventional Hydrogen Bond	d:UNK1:H38	H-Donor	C:GLU415:OE2	H-Acceptor
3	d:UNK1:...	Yes		Ligand Non-bond Monitor	2.79079	Hydrogen Bo...	Conventional Hydrogen Bond	d:UNK1:H37	H-Donor	C:MET970:O	H-Acceptor
4	C:ARG97...	Yes		Ligand Non-bond Monitor	4.14516	Electrostatic	Pi-Cation	C:ARG973:NH2	Positive	d:UNK1	Pi-Orbitals
5	C:GLU42...	Yes		Ligand Non-bond Monitor	3.29364	Electrostatic	Pi-Anion	C:GLU422:OE1	Negative	d:UNK1	Pi-Orbitals
6	C:ARG41...	Yes		Ligand Non-bond Monitor	5.29481	Hydrophobic	Alkyl	C:ARG419	Alkyl	d:UNK1	Alkyl
7	C:ARG97...	Yes		Ligand Non-bond Monitor	5.23071	Hydrophobic	Alkyl	C:ARG973	Alkyl	d:UNK1	Alkyl
8	C:TYR52...	Yes		Ligand Non-bond Monitor	5.21656	Hydrophobic	Pi-Alkyl	C:TYR522	Pi-Orbitals	d:UNK1	Alkyl
9	d:UNK1 -...	Yes		Ligand Non-bond Monitor	5.4428	Hydrophobic	Pi-Alkyl	d:UNK1	Pi-Orbitals	C:ARG973	Alkyl

Interacting amino acids between Ciprofloxacin and D5VAP4

	Name	Visible	Color	Parent	Distance	Category	Types	From	From Chemistry	To	To Chemistry
1	C:ARG41...	Yes		Ligand N...	3.53701	Hydrogen Bond;Halogen	Conventi...	C:ARG4...	H-Donor;Halogen Acceptor	d:UNK1:F1	H-Acceptor;Halogen
2	C:ARG97...	Yes		Ligand N...	3.79598	Hydrogen Bond	Carbon ...	C:ARG9...	H-Donor	d:UNK1:O2	H-Acceptor
3	d:UNK1:...	Yes		Ligand N...	3.70605	Hydrogen Bond	Carbon ...	d:UNK1:...	H-Donor	C:PHE359:O	H-Acceptor
4	d:UNK1:...	Yes		Ligand N...	3.5637	Hydrogen Bond	Carbon ...	d:UNK1:...	H-Donor	C:GLN361:OE1	H-Acceptor
5	C:GLU41...	Yes		Ligand N...	2.97091	Halogen	Halogen ...	C:GLU4...	Halogen Acceptor	d:UNK1:F1	Halogen
6	C:ARG97...	Yes		Ligand N...	4.53572	Electrostatic	Pi-Cation	C:ARG9...	Positive	d:UNK1	Pi-Orbitals
7	C:ARG97...	Yes		Ligand N...	3.71424	Electrostatic	Pi-Cation	C:ARG9...	Positive	d:UNK1	Pi-Orbitals
8	C:GLU42...	Yes		Ligand N...	4.12743	Electrostatic	Pi-Anion	C:GLU4...	Negative	d:UNK1	Pi-Orbitals
9	C:GLU42...	Yes		Ligand N...	3.36592	Electrostatic	Pi-Anion	C:GLU4...	Negative	d:UNK1	Pi-Orbitals
10	d:UNK1 -...	Yes		Ligand N...	5.26156	Hydrophobic	Pi-Alkyl	d:UNK1	Pi-Orbitals	C:ARG419	Alkyl
11	d:UNK1 -...	Yes		Ligand N...	5.00744	Hydrophobic	Pi-Alkyl	d:UNK1	Pi-Orbitals	C:ARG419	Alkyl

Interacting amino acids between Eravacycline and D5VAP4

	Name	Visible	Color	Parent	Distance	Category	Types	From	From Chemistry	To	To Chemistry
1	d:UNK1:...	Yes		Ligand Non-bond Monitor	2.90586	Hydrogen Bond	Conventional Hydrogen Bond	d:UNK1:H31	H-Donor	C:GLN361:OE1	H-Acceptor
2	d:UNK1:...	Yes		Ligand Non-bond Monitor	2.17265	Hydrogen Bond	Conventional Hydrogen Bond	d:UNK1:H28	H-Donor	d:UNK1:O1	H-Acceptor
3	d:UNK1:...	Yes		Ligand Non-bond Monitor	2.0553	Hydrogen Bond	Conventional Hydrogen Bond	d:UNK1:H26	H-Donor	C:ARG969:O	H-Acceptor
4	C:LEU36...	Yes		Ligand Non-bond Monitor	3.31537	Hydrogen Bond	Carbon Hydrogen Bond	C:LEU360:CA	H-Donor	d:UNK1:O8	H-Acceptor
5	d:UNK1:...	Yes		Ligand Non-bond Monitor	3.52272	Hydrogen Bond	Carbon Hydrogen Bond	d:UNK1:C1	H-Donor	C:GLU423:OE2	H-Acceptor
6	C:GLU41...	Yes		Ligand Non-bond Monitor	2.95201	Halogen	Halogen (Fluorine)	C:GLU415:O	Halogen Acceptor	d:UNK1:F1	Halogen
7	C:GLU41...	Yes		Ligand Non-bond Monitor	3.02319	Halogen	Halogen (Fluorine)	C:GLU415:CD	Halogen Acceptor	d:UNK1:F1	Halogen
8	C:ARG97...	Yes		Ligand Non-bond Monitor	3.9564	Electrostatic	Pi-Cation	C:ARG973:NH1	Positive	d:UNK1	Pi-Orbitals
9	C:GLU42...	Yes		Ligand Non-bond Monitor	3.20464	Electrostatic	Pi-Anion	C:GLU422:OE1	Negative	d:UNK1	Pi-Orbitals
10	d:UNK1 -...	Yes		Ligand Non-bond Monitor	4.73275	Hydrophobic	Pi-Alkyl	d:UNK1	Pi-Orbitals	C:ARG419	Alkyl

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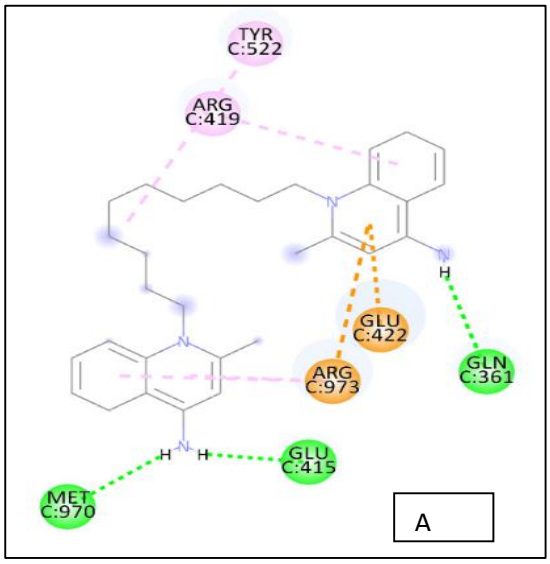
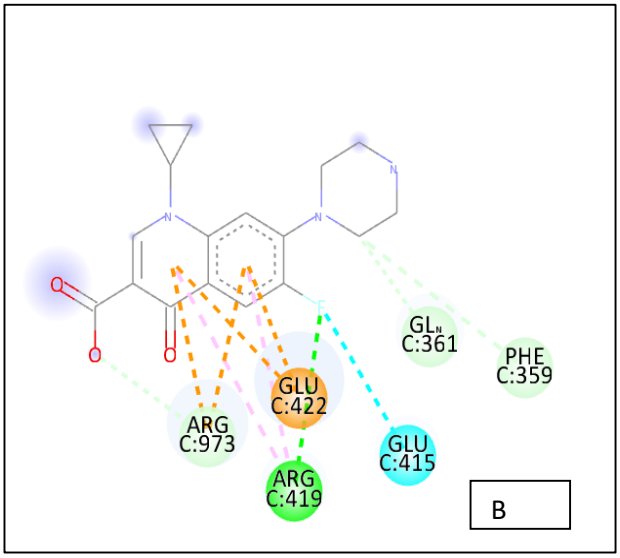
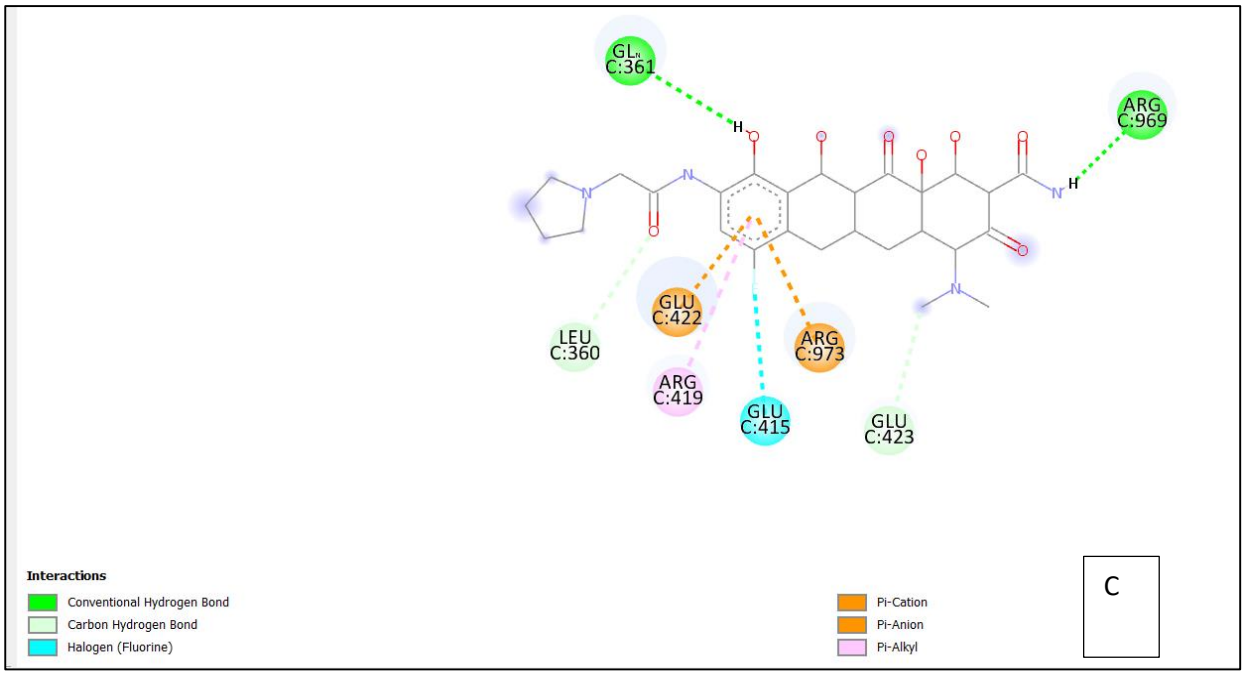
Interacting amino acids between selected ligands (dequalinium: A; Ciprofloxacin: B; Eravacycline: C) and D5VAP4







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The binding affinities were analyzed and as per the molecular docking analysis, dequalinium exhibited a binding affinity of -7 kcal/mol while ciprofloxacin and eravacycline against the D5VAP4 exhibited binding affinities of -7.6 kcal/mol and -7.5 kcal/mol respectively. The magnitude of binding affinities exhibited by the ligands against the D5VAP4 was in the order of eravacycline = ciprofloxacin>dequalinium. Ciprofloxacin (Pubchem ID: 2764) and the D5VAP4 of *M. catarrhalis* BBH18 were both used to screen the Zinc library with the defined parameters such as M.W: >300 to <350, RMSD: 0.7 A^3; rotational bonds: >2 and <8. This screening yielded almost 1200 significant hits against the zinc library. All the compounds were downloaded and molecular docking was performed using the zinc ligands and D5VAP4. Among the zinc ligands, the binding affinity between D5VAP4 and pipemidic acid (Drug databank id: DB13823; pubchem id: 4831; zinc ligand id: ZINC29345454) was equivalent to binding energy of ciprofloxacin and D5VAP4.

Compound	Binding affinity	Interacting amino acids	Hydrogen bond
Ciproflaxacin	-7.6 kcal/mol	Arg419, Arg973, Glu415 Phe359, Gln361, Glu422	Arg419, Arg973 Phe359, Gln361
Pipemedic acid	-7.2 kcal/mol	Gln361, Phe359, Glu418, Arg419, Glu422, Arg973	Phe359, Gln361

Conclusion:

In this study, new target i.e. efflux pump membrane transporter was identified from *M. catarrhalis* BBH18 strain. In addition, screening of zinc library and drug databank indicated two therapeutic targets such as pipemidic acid (quinole based compound) and dequalinium (bisquinoline) respectively. However, further in vivo and in vitro studies are warranted.

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BHAII-MS-5

Reverse vaccinology strategies to identify potential drug targets in *Candida albicans* sc5314

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Abstract

Fungal infections are one of the serious threats to humans. Among the fungal infections, candidiasis caused by *Candida albicans* affect oral cavity, skin, gastro-urinary tract and mucosa- membranes of humans. *C. albicans* is a polymorphic and opportunistic fungus which escape the normal surveillance of immune system and causes candidiasis. The development of resistance by this pathogen against the antifungal drugs is of prime concern and should be immediately addressed. With the advent of advanced molecular and analytical technologies, the genome architecture of this pathogen is known which was explored in this study to identify potential targets using in silico analysis for the development of alternative drug design strategies. In this study, the proteome of *C. albicans* wild strain SC 5314 was retrieved followed by the removal of duplicate sequences, and human homologous sequences. A total of 357 sequences were found to be human non homologous and a total of 20 essential proteins involved in fungal unique metabolic pathways were identified. Among the pathways, genes related to isoprenoid pathway were selected as drug targets for this study. Both pharmaceutical and plant-based drugs were screened against ERG8 and based on molecular docking analysis. Among plant-based drugs, one of the plant-based antifungal agents that exhibited high binding affinity as compared to fluconazole and posaconazole was presented in this study. Lipinski 'Rule of 5' and ADME properties revealed that the selected antifungal agent was predicted to be safe and hence, could be used for *in vivo* experimental analysis.

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1. Introduction

The development of antibiotic resistance or multidrug resistance during fungal infections is one of the alarming factors worldwide and should be immediately addressed (Fisher et al., 2022). However, discovery of drugs is not an easy process because of economic constraints, and most strikingly development of drugs against specific fungal protein targets is time consuming (Ismail et al., 2018). Therefore, approaches that speed up the drug discovery process is of paramount importance. With the advent of advanced molecular techniques, the complete genome of several fungi is now known and offered several insights into the structural and functional organization which in turn led to the identification of proteins associated with fungal metabolic pathways (Basenko et al., 2018). Systems biology approach using various computational methods and tools allowed the biologists to isolate and identify proteins that are specific to fungal metabolism which consequently sped up and revolutionized the drug discovery process through a paradigm shift from ligand-based approach to target-based design of drugs (Mdluli and Spigelman, 2006; Karahalil, 2016). Systems-biology approach through 'omics' based technologies encompasses the exploration of comparative, subtractive, and functional methods to identify the proteins that are actively involved in pathogen metabolism (Bappy et al., 2023). This trend is particularly helpful to design antifungal drugs especially for drug resistant pathogens (Lin and Qian, 2007; Fischbach and Walsh, 2010).

Candida albicans is an archetypical opportunistic pathogen and published reports have shown that this fungus is carried on mucosal membranes of humans without causing any damage (Richardson and Rautemaa, 2009). In general, *C. albicans* is a diploid fungal pathogen and harmless in nearly 70% of human population, while in immunocompromised situations, the transition of *C. albicans* from a commensal to pathogen occurs and leads to candidiasis (Meiller et al., 2009; Schulze and Sonnenborn, 2009). Candidiasis is characterized by the formation of "thrush", a spotty white infected membrane in gastrointestinal tract epithelial cells, vaginal region, and oropharyngeal mucosa and even causes mortality in severely ill patients (Niemeic et al., 2017). Estimates have shown that almost 50 % of mortality rate in US occurs due to cardiovascular and blood stream infections caused by *Candida* species (Kabir et al., 2012). Furthermore, the risk factors for invasive candidiasis which include antibiotic treatment, indwelling catheters and prolonged stay in ICU presents a persistent problem in patients and despite of the use of modern antifungal agents, the overall mortality is increasing day by day (Laupland et al., 2005; Eggimann et al., 2011). Studies in murine models have shown that the *C. albicans* negatively affect kidneys and causes sepsis (Lüttich et al., 2013).

Though, the exact pathogenesis of *C. albicans* induced mucosal and invasive infections is not clear, it has been shown that the adaptation of *C. albicans* in various niches of anatomical sites require versatile nutrient acquisition, metabolic status and stress resistance (Morgan et al., 2013;

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Niemeic et al., 2017). To compliment these adaptation requirements, several changes such as phenotypic transition and genetic modifications play a pivotal role (Shin et al., 2004; Bougnoux et al., 2006; Yang et al., 2023). It has been shown that the phenotypic transition from yeast to hyphae could be one of the underlined mechanisms and the hyphal formation eventually establish biofilm network and ascribed to the colonization of fungi thereby candidiasis (Ramage et al., 2023; Do et al., 2023). On the other hand, genetic modifications such as gene expression, translation and post translation modifications, chromosomal rearrangements and genetic alterations like insertions, deletions and exchange of nucleotides also sustain the growth of fungi under stressful conditions (Selmecki et al., 2010; Nobile et al., 2012).Thus, the changes in the phenotype and genotype within the *C. albicans* populations promote their ability to adapt in host micro-environments which is at least in part defined by the properties of the local mucosa and also its ability to establish within the complex microbiota of that anatomical location (Morgan et al., 2013). Once established, they can able to produce various proteins including aspartic proteases which prevent opsonization process via cleaving a complement mediated pathway protein, a convertase C3b (which acts as a protein that activate a membrane attack complex proteins) and escape the immune surveillance (Reisbeck, 2013). Studies also indicated that the dissemination of increased risk of chronic mucocutaneous candidiasis is often associated with the impairment of T17 cells due to genetic mutations of *C. albicans* (Okada et al., 2016). These events at least in part underlie antifungal drug resistance and/or tolerance. Antifungal drug resistance of *C. albicans* could be attributed to the mutations at the level of genes encoding drug targets (Fisher et al., 2018). For example, mutation of genes encoding lanosterol demthylase and beta-glucan synthase develop resistance against azole and echinocandids, respectively (Fisher et al., 2023). On the other hand, antifungal tolerance seems to be overexpression of genes encoding proteins in drug susceptible cells and sustain their growth at drug concentrations above the minimal inhibitory concentrations of fungistatic drugs like fluconazole (Berman and Krysan, 2020).

Considering the aforementioned data, the central objective of this study was twofold: identification of drug targets and screening of antifungal agents of plant origin against the selected drug targets of *C. albicans*. To accomplish this task, we took the advantage of genome of *C. albicans*and using systems biology approach, possible drug targets were identified. Further, molecular docking analysis was performed using plant based metabolities and modelled proteins of *C. albicans*. The current study might be helpful for scientists to develop *in vivo* and *in vitro* experiments and the computational strategy shown in this study may be useful for the scientists to screen the drug targets in various pathogenic strains of *Candida* species.

2. Materials and Methods

The pipeline of systems biology approach using several online bioinformatics tools in the identification

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and prioritization of unique drug targets from the wild strain,*C. albicans* SC5314 was shown in Figure 1.

2.1. Retrieval of *Candida albicans* SC5314 proteome

The genome size of *C. albicans* SC5314 was about 14.3 Mb (Assembly: ASM18296v3 ; NCBI RefSeq assembly: GCF_000182965.3) with a total number of 6263 genes (https://www.ncbi.nlm.nih.gov/datasets/genome/GCF_000182965.3/). The representative *C. albicans SC5314*proteome was retrieved from the Uniprot Universal Protein Knowledgebase (Uniprot: https://www.uniprot.org/; The Uniprot Consortium, 2021).

2.2. Elimination of paralogs and mini proteins

The reviewed proteins were submitted to CD-HIT tool (http://cd-hit.org/) with parameters such as "sequence identity cut-off" was set to 0.6 (60% identity) [Huang et al., 2010]. This resulted in the elimination of paralog sequences among the reviewed proteins. In general, protein sequences with<100 amino acids are actively involved in cellular biological and regulatory activities. However, they are less likely to be drug targets. Hence, non-paralog protein sequences with less than 100 amino acids were removed and proteins with >100 amino acids were further analyzed.

2.3. Identification of orthologs in host

The non-paralog protein sequences (with > 100 amino acids) were submitted to BLASTp(threshold e-value: 0.0001) in the NCBI database and searched against *Homo sapiens* (taxid: 9606) to separate the orthologs. The queries that resulted in no significant hits were considered as non-homologous sequences which were specific to the pathogen and used for further investigation with the objective of developing a pathogen-specific drugs [Pourhajibagher and Bahador, 2016].

2.4. Identification of essential genes

The non-orthologous sequences, thus obtained from the previous step were submitted to BLASTp in the DEG database (www.essentialgene.org/) against eukaryotes to identify essential proteins (threshold e-value: 1e-100 and percent identity was set to >25 %).

2.5. Selection of unique metabolic pathways specific to fungus

KEGG pathway analysis (http://www.genome.jp/kegg/pathway.html) was used to identify metabolic pathways for both species i.e. *C. albicans* and *Homo sapiens* by entering the respective three-letter KEGG organism codes in the organism box -"cal" for *C. albicans* and "hsa" for *H. sapiens*. The fungus's unique pathways were then screened against the humans by manual comparison. The host non homologous sequences were then submitted to the KAAS server using BLASTp(https://www.genome.jp/kaas-bin/kaas_main) to identify KO numbers accordingly and the KO number was then used to search the KO server (KEGG ORTHOLOGY) (https://www.genome.jp/kegg/ko.html) for protein pathways that are unique to pathogen's metabolism. On the other hand, proteins which were found to be involved in common pathways were excluded from the study.

2.6. Identification of drug targets

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The selected proteins that were unique to the fugal metabolic pathways were examined to know their distinctiveness as pharmacological targets by submitting the protein sequences toDrugBank server database (https://www.drugbank.ca/structures/search/bonds/sequence)with default parameters. If significant results were obtained against the submitted proteins, it demonstrates the presence of druggability of targets and absence of significant hits indicates the distinctive of proteins as new targets.

2.7. Analysis of cellular localization

The unique protein drug targets obtained from the previous step were submitted to the CELLO v.2.5 (http://cello.life.nctu.edu.tw/) server to forecast the subcellular localization.

2.8 Interactome analysis

Protein-protein interactions are key for the sustainability of the pathogen in the host system. To examine the probable interactions between the query protein and other interacting proteins, unique drug targets was submitted to the STRING 11.5 (https://string-db.org/) server with high confidence score of ≥0.700.

2.8. Identification of plant-based antifungal drugs

Based on the available literature, twenty-nine plant based metabolites with antifungal activity were selected. The chemical structure of these plant based antifungal agents in SDF (3D) format were retrieved from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/) and the SDF format was converted into protein databank format using the Open Babel v2.3 program [44].

2.9. Molecular docking analysis

Homology modelling

In the current study, the molecular models of selected unique protein targets were modelled using a homology modelling tool Swiss model server (https://swissmodel.expasy.org/), an online resource for automated protein structure prediction based on the identification of structural templates, alignment of target sequence and template structure, model building and evaluation of model quality. To assess the quality of the modelled proteins were submitted to ERRAT and analyzed via Ramachandran plot available in the SAVES server (https://saves.mbi.ucla.edu/). The binding pockets of modelled proteins were determined using CASTp server (http://sts.bioe.uic.edu/castp/), which analyze the pockets based on recent theoretical and algorithmic results of computational geometry wherein all calculated parameters are rotationally invariant, and do not involve discretization and they make no use of dot surface or grid points.

Molecular docking

Molecular docking is one of the efficient procedures to understand the interaction between the ligand and protein and protein and protein interactions. This approach is widely used in drug development process and in reverse vaccinology. In the current study, the interactions between the ligand (antifungal agent) and receptor (target proteins) was performed by employing Server HDock (http://hdock.phys.hust.edu.cn/) as it allows for connection within therapeutic targets and potential treatments.Fluconazole, a

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synthetic triazole is the currently employed antifungal drug and hence, selected as reference antifungal agent and docked against the selected modelled proteins. The binding affinity between the plant based antifungal agent and the target proteins were compared against the fluconazole and the target proteins. The docked complexes were visualized and analyzed the binding sites of the metabolites using discovery studio.

Results and Discussion

Candida albicans SC5314 is a pathogenic yeast that has developed as a multidrug-resistant organism. In many countries, community health experts have identified that *C. albicans* has affected hospitalized patients with serious illness. Diseases commenced by *C. albicans* frequently do not react to regularly consuming antifungal medications, renderi ng them tough to cure effectively. Hence, we investigated the proteome of this yeast to anticipate the prospective drug targets and drugs to deal with this pathogenic yeast.

Since most therapeutics tends to bind with essential gene products, essential proteins are considered to be among the most effective drug targets. A prospective drug target has to be an essential protein as it has a vital attribute for the existence of the pathogen and our target pathogen yeast contains such essential proteins specifying that drugs will act out in opposition to those proteins will lead the pathogen to death.The proteome of *C. albicans* SC5314 showed 6110 proteins and out of which 1111 proteins were reviewed proteins. The reviewed proteins (UniprotKB/Swiss-Prot) were used for further experimental studies. Reviewed proteins represent the high quality manually annotated and non-redundant proteins based on experimental results and scientific conclusions and hence, they were selected and used in this study. After eliminating the paralogous sequences, 1063 non-duplicate large proteins were left since the CD-HIT service found at 60% identity. The non paralogous sequences were subjected to BLASTp analysis and searched against humans to eliminate homologous sequences. A total of 357 non-paralogous and non-homologous sequences were subjected to database of essential genes server under defined parameters i.e. > or equal to 25 % identity and bit score > 100 and also which exhibited significant hits against the fungal species (*Saccharomyces cerevisiae*: DEG2001; *Schizosaccharomyces pombe 972hr*: DEG2009; *Aspergillus fumigatus*: DEG2008 and *Komagataella phaffi* GS115: DEG2027). This step resulted in 102 essential sequences with defined parameters (Table 1).

Table 1: Retrieval of proteins from *Candida albicans* SC5314 and identification of non paralogs and non homologs

Number of reviewed proteins analyzed	1111
Number of non paralogs identified based on CD HIT	1063
Number of non-homologous sequences identified using BLASTp against humans	357
Number of essential proteins identified using DEG	102

The KEGG server had 127 *C. albicans* metabolic pathways (https://www.kegg.jp/kegg-

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bin/search_pathway_text?map=cal&keyword=&mode=1&viewImage=true) and 346 human metabolic pathways (https://www.kegg.jp/kegg-bin/search_pathway_text?map=hsa&keyword=&mode=1&viewImage=true), with 11 metabolic pathways being specific only for *C. albicans*. Proteins involved in these unique pathways can be used as therapeutic targets. Following a BLAST at the KAAS server, 20 out of 47 non-homolog essential proteins were discovered to have both KO orthology and metabolic pathway involvement. These 20 proteins (Table 2) are essential for the proper functioning of metabolism, and solely associated with *C. albicans* unique pathways (Table 4).

Table 2: KEGG pathway and KAAS analysis of non paralogous and non homologous sequences of *C. albicans*

Number of KEGG pathways identified from *C. albicans* SC5314	127
Number of KEGG pathways identified from *Homo sapiens*	356
Number of unique pathways identified from *C. albicans*	11
Number of essential genes related to the metabolic pathways of *C. albicans*	20

Among the 20 proteins of metabolic importance, a cytoplasmic enzyme phosphomevalonate kinase (sp|A0A1D8PLH0|ERG8_CANAL; K00938 mvaK2; phosphomevalonate kinase [EC:2.7.4.2]) was selected for further experimental study because of its metabolic importance in terpenoid pathway.

Table 3: Unique metabolic pathways associated with C. albicans when compared against Homo sapiens.

KEGG Pathway	Metabolism
cal00261	Monobactam biosynthesis
cal00300	Lysine biosynthesis
cal00332	Carbapenem biosynthesis
cal00460	Cyanoamino acid metabolism
cal00660	C5-Branched dibasic acid metabolism
cal00680	Methane metabolism
cal00791	Atrazine degradation
cal00909	Sesquiterpenoid and triterpenoid biosynthesis
cal00999	Biosynthesis of various plant secondary metabolites
cal01110	Biosynthesis of secondary metabolites
cal01501	beta-Lactam resistance

The protein-protein interactions revealed that the selected enzyme ERG8 showed interactions with different metabolically active proteins involved in pathways like terpenoid backbone biosynthesis, biosynthesis of secondary metabolites, steroid biosynthesis, synthesis and biodegradation of ketone bodies, butanoate metabolism and valine, leucine and isoleucine degradation.

Molecular docking was performed between the ERG8 and selected antifungal plant-based candidates. Based on the SWISS-MODEL analysis, the structure of ERG8 was modelled. The modelled protein showed good quality as indicated by ERRAT value and Ramachandran plot analysis. Based on the literature survey, thirty-eight (38) plant based antifungal agents were collected from the NCBI database and docked against the ERG8.

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Table 4: Unique proteins involved in metabolism of *C. albicans*

Polysaccharide
Storage polysaccharide
▼Structural polysaccharide
sp|O13318|PHR2_CANAL; K22832 GAS1; 1,3-beta-glucanosyltransferase GAS1 [EC:2.4.1.-] sp|P43076|PHR1_CANAL; K22832 GAS1; 1,3-beta-glucanosyltransferase GAS1 [EC:2.4.1.-] sp|Q59VW6|PGA5_CANAL; K22833 GAS2; 1,3-beta-glucanosyltransferase GAS2 [EC:2.4.1.-] sp|Q5AJY5|PGA4_CANAL; K22836 GAS5; 1,3-beta-glucanosyltransferase GAS5 [EC:2.4.1.-]
Histidine kinases
sp|Q5A872|SLN1_CANAL; K11231 SLN1; osomolarity two-component system, sensor histidine kinase SLN1 [EC:2.7.13.3]
▼1.1.1.25 shikimate dehydrogenase (NADP+)
sp|Q5AME2|ARO1_CANAL; K13830 ARO1; pentafunctional AROM polypeptide [EC:4.2.3.44.2.1.101.1.1.252.7.1.712.5.1.19]
▼1.1.1.270 3beta-hydroxysteroid 3-dehydrogenase
sp|Q5A888|ERG27_CANAL; K09827 ERG27; 3-keto steroid reductase [EC:1.1.1.270]
▼2.5.1.19 3-phosphoshikimate 1-carboxyvinyltransferase
sp|Q5AME2|ARO1_CANAL; K13830 ARO1; pentafunctional AROM polypeptide [EC:4.2.3.44.2.1.101.1.1.252.7.1.712.5.1.19]
▼2.7.1.71 shikimate kinase
sp|Q5AME2|ARO1_CANAL; K13830 ARO1; pentafunctional AROM polypeptide [EC:4.2.3.44.2.1.101.1.1.252.7.1.712.5.1.19]
▼2.7.1.39 homoserine kinase
sp|Q92209|KHSE_CANAL; K00872thrB; homoserine kinase [EC:2.7.1.39]
▼2.7.4.2 phosphomevalonate kinase
sp|A0A1D8PLH0|ERG8_CANAL; K00938 E2.7.4.2, mvaK2; phosphomevalonate kinase [EC:2.7.4.2]
▼2.7.13.3 histidine kinase
sp|Q5A872|SLN1_CANAL; K11231 SLN1; osomolarity two-component system, sensor histidine kinase SLN1 [EC:2.7.13.3]
▼4.1.2.13 fructose-bisphosphate aldolase
sp|Q9URB4|ALF_CANAL; K01624 FBA, fbaA; fructose-bisphosphate aldolase, class II [EC:4.1.2.13]
▼4.1.99.12 3,4-dihydroxy-2-butanone-4-phosphate synthase
sp|Q5A3V6|RIB3_CANAL; K02858ribB, RIB3; 3,4-dihydroxy 2-butanone 4-phosphate synthase [EC:4.1.99.12]
▼4.2.1.1 carbonic anhydrase
sp|Q5AJ71|CAN_CANAL; K01673cynT, can; carbonic anhydrase [EC:4.2.1.1]
▼4.2.1.10 3-dehydroquinate dehydratase
sp|Q5AME2|ARO1_CANAL; K13830 ARO1; pentafunctional AROM polypeptide [EC:4.2.3.44.2.1.101.1.1.252.7.1.712.5.1.19]
▼4.2.3.4 3-dehydroquinate synthase
sp|Q5AME2|ARO1_CANAL; K13830 ARO1; pentafunctional AROM polypeptide [EC:4.2.3.44.2.1.101.1.1.252.7.1.712.5.1.19]
▼5.3.1.24phosphoribosylanthranilate isomerase
sp|P43073|TRPF_CANAL; K01817trpF; phosphoribosylanthranilate isomerase [EC:5.3.1.24]
▼3.6.1.74 mRNA 5'-phosphatase
sp|O93803|CET1_CANAL; K01098 CET1; polynucleotide 5'-triphosphatase [EC:3.6.1.74]
▼6.5.1.3 RNA ligase (ATP)
sp|P43075|TRNL_CANAL; K14679 TRL1; tRNA ligase [EC:6.5.1.3]

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Based on the binding affinities (Autodock Vina Program; Grid box: a) centre coordinates (x, y and z: 5, 4 and 1); b) size (x, y and z= 35, 31, 31); Cavity volume: 2783 A^3), the docking analysis revealed that almost 18 plant based antifungal agents exhibited lower binding energies than fluconazole (a reference drug). Among the 18 compounds, protopine exhibited binding affinities of -8.9 kcal/mol, respectively with ERG8 as compared to the fluconazole and ERG8 (-7.2 kcal/mol).

The interactions between the protopine and ERG8 and fluconazole and ERG8 was visualized using Discovery studio to predict the interactive bonds and type of amino acids involved. Inadequate ADME data is frequently linked with the malfunction of clinical trials in the course of several drug development projects. ADME analysis also revealed that the selected compound did not violate the Lipinski rule of five and also exhibited good gastrointestinal property.

Figure 1: Involvement of *C. albicans* ERG8 (2.7.4.2) in the terpenoid pathway

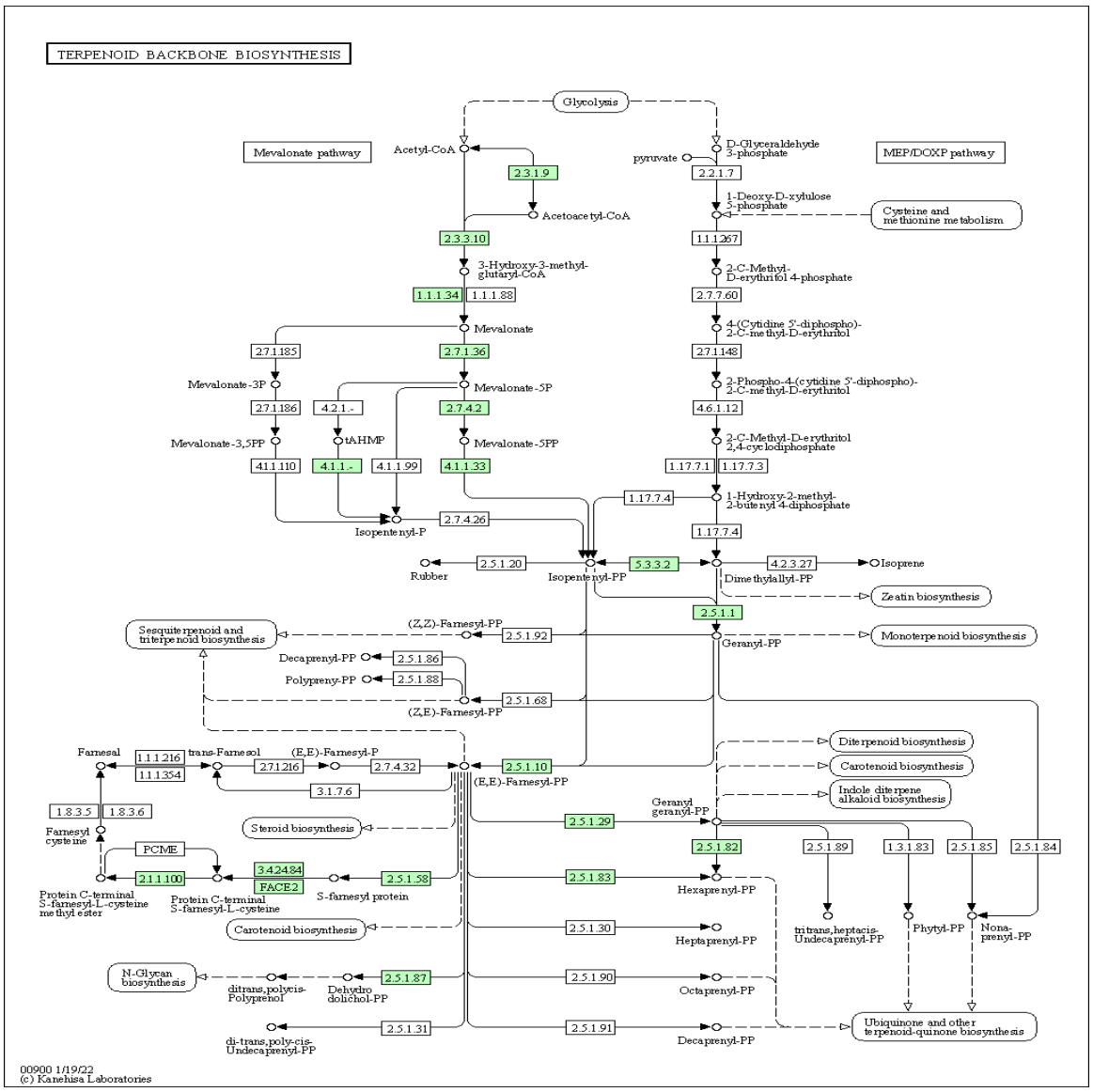

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Figure 2: PPI network of ERG8 with other proteins

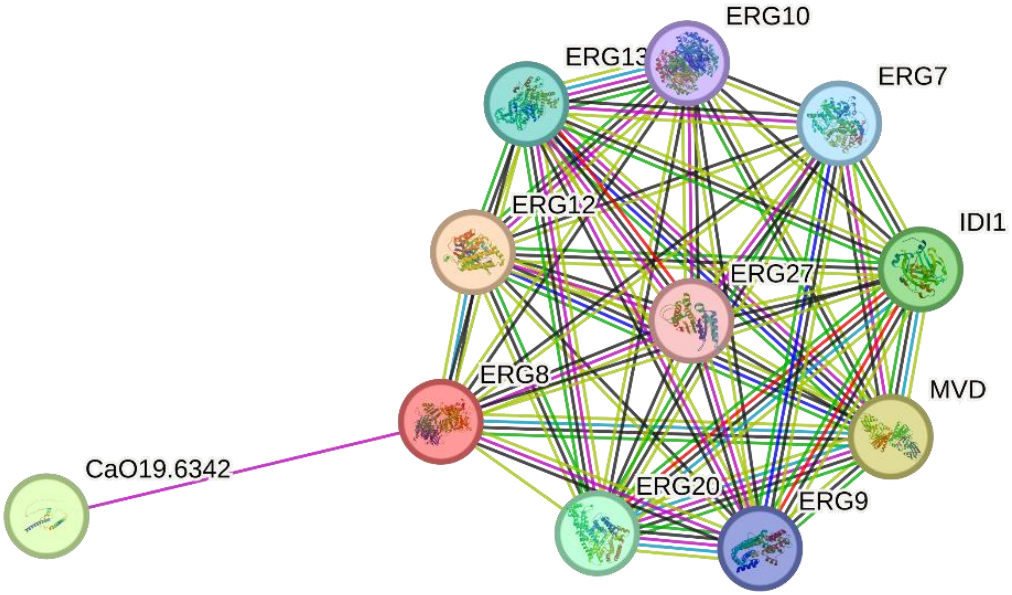
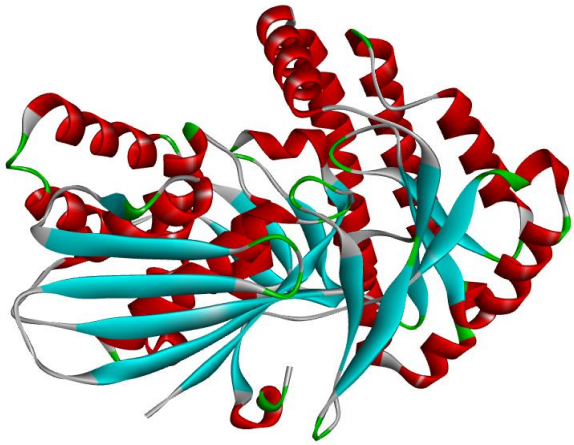


Figure 3: Amino acid sequence of *C. albicans* ERG8

```
.....10.....20.....30.....40.....50.....60.....70.....80.....90.....100.....110.....120.....
MSKAFSAPGK AFLAGGYLVL EPIYDAYVTA LSSRMHAVIT PKGTSLKESR IKISSPQFAN GEWEYHISSN TEKPKEVQSR INPFLEATIF IVLAYIQPTE AFDLEIIIYS DPGYHSQEDT ETKTSSNGE
130.....140.....150.....160.....170.....180.....190.....200.....210.....220.....230.....240.....
K TFLYHSRAIT EVEKTGLGSS AGLVSVVATS LLSHFIPNVI STNKDILHNV AQIAFCYAQK KIGSGFDVAT AIYGSIVYRR FQPALINDVF QVLESDPEKF PTELKKLIAS NWEFKHERC
250.....260.....270.....280.....290.....300.....310.....320.....330.....340.....350.....360.....
T LPHGIKLLYG DVKGGSETPK LVSRVLQWKK EKPEESSVVY DQLNSANLQF MKELREMREK YDSDPETYIK ELDHSIEPLT VAIKNIRKGL QALTQKSEVP IEPDVQTQLL DRCQEIPGC
370.....380.....390.....400.....410.....420.....430..
V GGVVPGAGGY DAIAVLVLEN QVGNFKQKTL ENPDYFHNVY WVDLEEQTEG VLEEKPEDYI GL
```

Figure 4: Homology model of *C. albicans* ERG8 using SWISS-MODEL and Ramachandran plot analysis



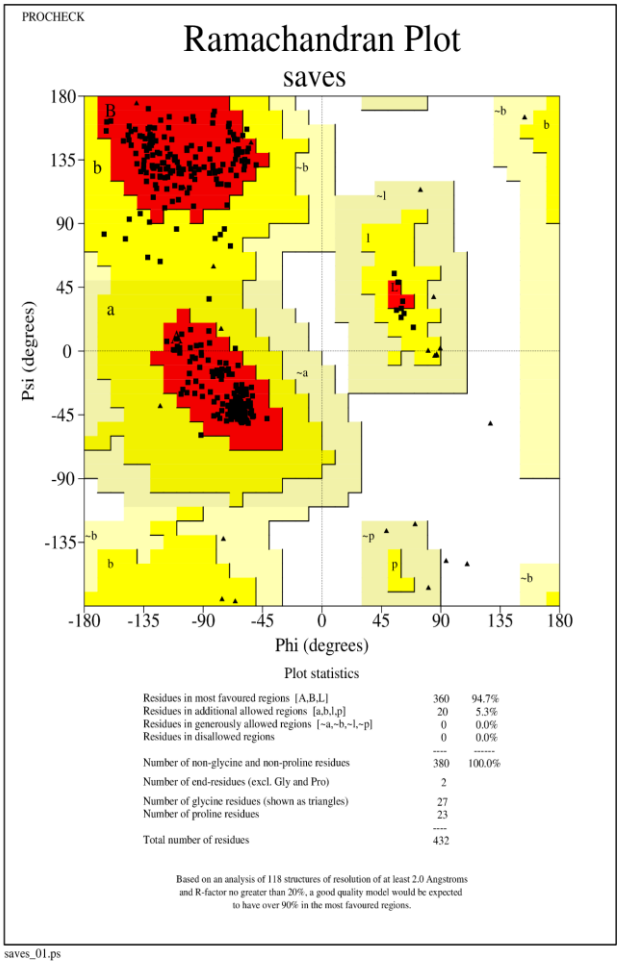


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Table 6: Molecular docking analysis of ERG8 and different plant based antifungal agents using Autodock Vina in built in EASY-DOCK server

Compound	Pubchem	Kcal/mol
Protopine	4970	-8.9
Dicentrine	101300	-8.5
Caledonixanthone E	5464634	-8.4
Viniferin	5315232	-8.4
Chlorogenic acid	1794427	-8.2
Betulinic acid	64971	-8.2
Alpha-allocryptopin	98570	-8.1
Spirostanol	12304444	-8.1
Usnic acid	5646	-7.9
3methoxysampangine	122683	-7.8
Clausenidin	5315947	-7.8
Glaucine	16754	-7.8
Quercetin	5280343	-7.8
Jatrorrhizine	72323	-7.7
Eriobofuran	178939	-7.4
Flavones	10680	-7.4
Resveratrol	445154	-7.3
Fluconazole	3365	-7.2
Pterostilbene	5281727	-7
Acetoxychavicol acetate	166872	-6.9
Cyclocolorenone	160491	-6.4
abscisic acid	5375199	-6.3
Cerbinal	13786166	-6.2
4-Methyl-7-hydrox	5280567	-6.2
Agropyrene/ Capillen	3083613	-6
Carvacrol	10364	-5.9
Eugenol (Clove oil)	3314	-5.9
Gallic acid	370	-5.9
N-methyl-N-formyl-4-hydroxy-beta-phenylethylamine	11446673	-5.8
Caffeic acid	689043	-5.7
Thymol	6989	-5.7
Eucalyptol	2758	-5.5
Capric acid	2969	-5
Linalool	6549	-5
Ajoene	5386591	-4.6
Cycleanine	121313	-8.5

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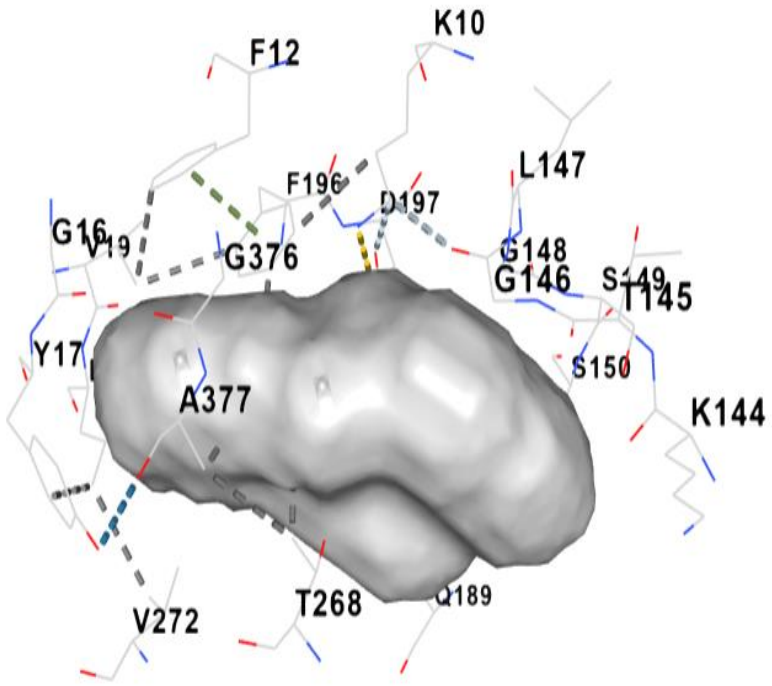
Figure 5: Molecular docking analysis of protopine and ERG8

Interactive amino acids

LYS10 PHE12 GLY16 TYR17 VAL19 LEU20 LYS144 THR145 GLY146 LEU147 GLY148 SER149 SER150 GLN189 SER194 PHE196 ASP197 THR268 VAL272 GLY376 ALA377



Conclusion

This study of finding suitable drug targets and screening potential drugs will speed up the process to get remedies with fewer trials and error repeats of assays against *C. albicans*. Pathogen protein ERG8 were found as suitable drug target and the metabolites protopine can be grater alternative to get rid of this deadly pathogen. Finally selected protein and metabolite to block those proteins may be greater foresteps of drug discovery against the pathogen. However, *in vivo* and clinical trials are highly recommended to validate the outcome of the study.

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List of anti-fungal plant metabolites used for docking studies

Metabolites	Pub-chem ID	Source	References
Ajoene	5386591	*Allium sativum*	Meena et al.(2013); Ledezma & Apitz-Castro (2006)
Thymol	6989	*Thymus vulgaris*	Meena et al.(2013); Alagawany et al. (2021).
Carvacrol	10364	*Origanum vulgare*	Meena et al.(2013); Rathod et al.(2021).
Linalool	6549	*Ocimumbasilicum*	Meena et al.(2013); An et al. (2021).
Caffeic acid	689043	*Eucalyptus globulus*	Meena et al.(2013); Zielińska et al. (2021).
Chlorogenic acid	1794427	*Malus pumila,*	Meena et al.(2013); Chen et al. (2021); Navarro-Orcajada et al. (2021)
Pterostilbene	5281727	*Prunus amygdalus*	Meena et al.(2013); Wawszczyk et al. (2022).
Resveratrol	445154	*Vitis vinifera*	Meena et al.(2013); CAO, et al. (2021)
Viniferin	5315232	*Dryobalanopsaromatica.*	Meena et al.(2013); Shang et al. (2021).
2-(3,4-dimethyl-2,5-dihydro-1H-pyrrol-2-yl)-1-methylethyl pentanoate	5277433	*Datura metel*	Meena et al.(2013); Raghuwanshi(2019).
N-methyl-N-formyl-4-hydroxy-beta-phenylethylamine	11446673	*Cyathobasisfruticulosa*	Meena et al.(2013); Thawabteh et al. (2019)
Acetoxychavicol acetate	166872	*Alpinia galanga*	Latha et al. (2009); Sok et al. (2021)
Eugenol(Clove oil)	3314	*Eugenia caryophyllus*	Latha et al. (2009); Rojas et al. (2021).
Spirostanol	129674828	*Smilax medica*	Sauton et al. (2006); Jastrzebska et al. (2021)
Cocsoline	21579624	*Epinetrumvillosum*	Meena et al.(2013); Purwayantie et al. (2018)
Dicentrine	101300	*Glauciumoxylobum*	Meena et al.(2013); Perewalo et al. (2021).
Glaucine	16754	*Glauciumoxylobum*	Meena et al.(2013); Akaberi et al. (2021).
Clausenidin	5315947	*Clausena exca*	Sunthiktikawinsakul et al. (2003); Al-Abboodi et al. (2021)
Agropyrene/Capillen	3083613	*Agropyron repens Beauv*	Khare(2007); Grummer (1961).
Eriobofuran	178939	*Eriobotrya japonica Lindl*	Khare(2007); Li et al.(2016).
Cyclocolorenone	160491	*Magnolia grandiflora Linn*	Khare(2007); Jena et al. (2021).
Cerbinal	13786166	*Gardenia jasminoides Ellis*	Khare(2007); Li et al. (2019).
Flavones	10680	*Alpinia officinarum Hance*	Khare(2007); Zaragozá et al. (2020).
Capric acid	2969	*Arachis hypogaea Linn*	Khare(2007); Kim et al. (2020).
Usnicacid	5646	*Parmeliaperlata (Huds.) Ach*	Khare(2007); Araújo et al. (2015).
Protopine	4970	*Fumaria vaillantii*	Meena et al.(2013); Huang et al. (2022).
Alpha-allocryptopin	98570	*Glauciumoxylobum*	Meena et al.(2013)
3-methoxysampangine	122683	*Cleistopholis patens*	Meena et al.(2013); Liu et al. (1990)
Jatrorrhizine	72323	*Mahonia aquifol*	Jung et al. (2006); Li et al. (2014); Jiang et al. (2011); Wang et al. (2018); Wang et al. (2019)
Caledonixanthone E	5464634	*Calophyllum caledonicum*	Larcher et al. (2004)

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Protective action of *Moringa oleifera* on glyphosate-induced hepatotoxicity in albino rats

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Abstract

The present study was aimed to evaluate the protective efficacy of *Moringa oleifera* (MO), against glyphosate-induced hepatotoxicity in rats. Healthy male rats (n = 24) were randomly divided into four groups with six rats each. Rats in group 1 served as controls and did not receive any treatment. Animals in group II and III received glyphosate at a dose of 500 mg/Kg body weight and MO leaf extract at a dose of 500 mg/Kg body weight, respectively. Rats in group IV were maintained similar to that of group II but also received MO leaf extract at a dose of 500 mg/Kg body weight. The experimental duration was about 55 days. After completion of the experimental period, the rats were sacrificed and liver was isolated immediately and subjected to biochemical analysis. No significant changes in the body weights were observed between the control and experimental rats, however, the tissue somatic index of liver was reduced in glyphosate administered rats over controls. Significant reduction in the activity levels of superoxide dismutase, and catalase with a concomitant increase in the lipid peroxidation levels were observed in the liver tissue of glyphosate treated rats over controls. Whereas co-administration of glyphosate with MO significantly increased the levels of antioxidant enzymes and inhibited the levels of lipid peroxidation in liver tissue of rats over glyphosate treated rats. The results of present study indicating the protective role of *Moringa oleifera* to combat glyphosate-induced hepatotoxicity in rats at least in part mediated through antioxidant capacity.

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Introduction

Organophosphates are most commonly used insecticides in agriculture & pest control and studies indicated that the accumulated majority share in global insecticides usage (De Bleecker et al., 1994). Though promising, the wide usage of the OPs often causes side effects in humans which is of major concern (Robb et al., 2024). Published reports also suggest the deleterious effects in experimental animal models (Peter et al., 2014). Glyphosate [N-(phosphonometyl)glycine] is one of the widely used OPs and a broad-spectrum herbicide to control weeds (Mahendrakar et al., 2014). Glyphosate also known as Roundup, and consists of isopropyl amine salt of N-(phosphonomethyl) glycine and surfactant. Roundup is a target specific herbicide, inhibits the shikimic acid pathway, which is important for plant protein synthesis (Schonbrunn*et al.*,2001). It is a water-soluble and its half-life period >35 day's therefore it has been applied in the field 2 or 3 times, depending upon the growth of weeds. Glyphosate herbicides are among the world's most widely used herbicides (Monsanto, 2002) and the Glyphosate is the world's leading agrochemical (Baylis, 2000). Even though glyphosate is considered a low-toxic herbicide, recent studies have revealed toxic effects resulting from even low-dose commercial formulations. Roundup showed significant decrease in the Hb% and TLC and highly significant increase in alkaline phosphatase (Dhanarajam*et al*., 2010). According to Adam (2002) effect of Round-up show respiratory effect, blood-stained weeping from noses and diarrhea. In case of chronic exposure many reported carcinogenicity. Farmers in fields repeatedly use Roundup/Glyphosate with the recurrence of weeds. Since herbicides are intended to kill plants, accumulation of herbicides may take place in the soil and it may percolate in nearby water. They get fall on crops may not killing or destroying but accumulate inside the leaves, grains or fruits, by consuming such product by birds or mammals indirectly or accidentally herbicides enter in body. As farmers are not aware about the bad effects of herbicides therefore unusual and repeated use of herbicides may cause adverse effect on human beings and animals (EPA.1996). The liver plays vital functions in vertebrates, including nutrient metabolism and detoxification processes (Elufioye and Habtemariam, 2019). It is widely recognized that the liver is the major organ affected by xenobiotic exposure, and, experimental studies have demonstrated that exposure to glyphosate itself may have a hepatotoxic potential and may cause liver injury (Soudani et al. 2019; Milić et al. 2018; Mesnage et al. 2017; Tizhe et al. 2020).

Plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal applications. *Moringa oleifera* (Moringaceae family) is considered as a highly nutritious plant with its medicinal values (Talreja,2011). The leaves are an excellent source of vitamins (A, B, C), calcium, proteins, potassium, carotenoids - leutin, alpha- and beta-carotene, xanthine, chlorophyll and other phytochemicals with known powerful antioxidant property (Fuglie et al., 1998; Kasolo et al., 2010; Pandey, A et al., 2012).

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Hence extracts from different parts of this plant have been used for the treatment of fever, bronchitis, eye/ear infections, inflammation of mucous membrane, diarrhea, gastric ulcer including anti-cancer, anti-inflammatory and hepatoprotective agents (Pareek et al., 2023). Recently, novel derivatives of thiocarbamates and nitriles which stimulate insulin release in animals have been found in the plant, explaining its anti-diabetic actions also (Fahey, 2005). This study was designed to determine the leaf extract of *M. oleifera* could restore Glyphosate induced Hepato toxicity in rats.

Materials and Methods

Preparation of Moringa leaf extract

Plant materials

Samples of *M. oleifera* leaves were collected from plantations in Tirupati, Andhra Pradesh, India and were also identified and authenticated by a plant taxonomist.

Preparation of extract

The leaves of the plant were cleaned thoroughly, dried in room temperature and crushed into coarse powder, from which 20 g was taken and soaked separately in 100 ml of water and chloroform by keeping it in a Shaker for 3 days. It was filtered through cheese cloth and reduced to 10% of its original volume (organic solvent). The filtrate was concentrated in vacuum using a rotary evaporator while aqueous extract was dried using water bath.

Procurement and maintenance of experimental animals

Healthy male rats were housed in polypropylene cages (18" x 10" x 8") lined with sterilized paddy husk, and provided filtered tap water and rat food (purchased from HLL Animal Feed, Bangalore, India) ad libitum in an air-conditioned environment (25 ± 2°C) with a 12-hour light and 12-hour dark cycle. All the animals were maintained at the animal facility available at Department of Biotechnology, Sri Padmavati Mahila Visvavidyalam, and Tirupati.

Experimental Design

Rats were divided into 4 groups with 6 rats in each group. Animals in the group 1 served as control, and rats in groups II administered with glyphosate alone at a dose of 500 mg/kg body wt. Group III administered with MO leaf extract alone at a dose of 500mg/kg body wt and group IV treated with both glyphosate and MO leaf extract orally for 55 days.

Necropsy

After completion of the experimental period (55 days), the rats from control and experimental groups were fasted overnight, weighed and humanely euthanized via cervical dislocation. Blood was drawn immediately via cardiac puncture using a heparinized syringe and serum was separated by centrifugation at 2000g for 15 min after overnight storage at 4°C. Serum was stored at - 20° C until further hormone analysis. Rats were dissected, liver was collected and weighed to the nearest milligram by using Shimadzu electronic balance after clearing off the adhering tissues. Organ indices were determined by using the formula:

TSI = [weight of the tissue (g) / Body weight of the animal (g)] × 100

Estimation of lipid peroxidation in Liver

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Lipid peroxidation in Liver was estimated spectrophotometrically by measuring a break down product of lipid peroxidation, thiobarbituric acid reactive substances (TBARS) was determined by the method of Ohkawa et al. (1979). Briefly, the tissues were homogenized (10% W/V) in 1.15% KCL solution. 0.5 ml of saline (0.9% sodium chloride), 1.0 ml of (20% W/V) trichloro acetic acid (TCA) was added to 2.5 ml of homogenate. Samples were centrifuged for 20 minutes at 4000 x rpm. 0.25 ml of TBA reagent was added to 1.0 ml of supernatant, and samples were then incubated at 95°C for 1 hour. Equal volumes of n-butanol were added to the supernatant and mixed, the contents were centrifuged for 15 minutes at 4000 rpm. The organic layer was transferred into a clear tube and its absorbance was measured at 532 nm. The rate of lipid peroxidation was expressed as µ moles of malondialdehyde formed/gram wet weight of Liver tissue.

Assay of antioxidant enzymes in Liver

Superoxide dismutase (SOD) (EC 1.15.1.1)

The activity of SOD was determined by the method of Mishra and Fridovich, (1972). SOD was assayed in the microsomal fraction according to its ability to inhibit the autooxidation of epinephrine at alkaline medium. Briefly, the Liverwere homogenized (10% W/V) in 50 mM ice-cold sodium phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenate was centrifuged at 10,500 rpm for 60 min. The supernatant (cytosol) fraction was used for the assay of the enzyme activity. The reaction mixture contained 0.05 M carbonate buffer (pH 10.2), 30 mM epinephrine (freshly prepared) and the enzyme source. Changes in absorbance were recorded at 480 nm, measured at 10 seconds intervals for 1 minute in a spectrophotometer. The enzyme activity was expressed as Units/mg protein/min.

Catalase (CAT) (EC 1.11.1.6)

The activity of CAT was determined by based on its ability to decompose H_2O_2, from the method of Chance and Maehly (1955). The reaction mixture contained (2.5 ml, vol) mixer of 2.4 ml of 50 mM phosphate buffer (pH 7.0), 0.1 ml of Livertissue homogenate and 10 µl of 19 mM H_2O_2. Then the absorbance was read at 240 nm. CAT activity was expressed as µM of H_2O_2 consumed/min/mg protein.

Statistical analysis

All data were expressed as means ± S.D. and analyzed by analysis of variance (ANOVA) and Duncan's multiple range test using SPSS unpaired student's t-test. All data were reported as mean ± standard deviation (SD). $p<0.05$ was considered significantly different.

Results and Discussion

The body weights of rats did not show any significant change in all the groups (Table 1). The liver tissue indices of control and experimental rats were presented in Table 1. No significant changes were observed in the indices of liver tissue of control and MO alone administrated rats; significant decrease was observed in glyphosate administrated rats. Whereas in co-treatment of MO along with glyphosate, significant changes were not observed in the Liver tissue of rats over controls. The present findings i.e. no changes in the body weights and liver

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Table1: Protective Action of MO on the body weights and liver TSI of glyphosate-treated rats

Parameter	Control	Glyphosate	MO	Glyphosate +MO
Body weights	223.65 ± 1.13	204.05^{ns} ± 3.68 (-8.78)	225.92^{ns} ± 3.46 (1.01)	214.38^{ns} ± 3.55 (-4.14)
Liver	7.54 ± 0.91	4.19^{ns} ± 0.63 (-44.4)	7.82^{ns} ± 0.7 (3.71)	7.23^{ns} ± 0.47 (-4.11)

Values are mean ± S.D. of 6 individuals for each group
Values in parenthesis are percent change from that of control.
Mean values with different superscripts in a row differ significantly at $p < 0.05$.

Table 2: Protective Action of MO on the Lipid peroxidation and SOD, CAT of glyphosate-treated rats

Parameters	Control	MO	Glyphosate	Glyphosate + MO
LPX	4.56 ± 0.20	2.65* ± 0.28 (-41.88)	6.10* ± 0.18 (33.77)	3.60* ± 0.12 (-21.05)
SOD	0.25 ± 0.04	0.56* ± 0.03 (49.6)	0.33* ± 0.02 (32.00)	0.45* ± 0.01 (80.00)
CAT	22.67 ± 0.28	27.44* ± 0.4 (21.04)	17.98* ± 0.43 (20.68)	23.39* ± 0.33 (3.17)

indices indicated that the general metabolism of the animals in glyphosate treated appears to be normal. The levels of malondialdehyde were significantly ($p<0.001$) increased in the liver tissue of glyphosate treated rats when compared to control rats (Table 2). A significant decrease ($p<0.001$) in the activity levels of SOD, and CAT were observed in liver tissue of glyphosate treated rats when compared with control rats (Table 2). Co-administration of MO and Glyphosate significantly ($p<0.001$) reduced levels the malondialdehyde with a significant increase in the activity levels of enzymatic antioxidants ($p<0.001$); however, no significant increase was observed in MO alone received rats (Table 2).

The present findings suggest that the glyphosate intoxication perturb antioxidant to prooxidant system thereby accelerate oxidative stress in the liver of rats. These events eventually cause hepatotoxicity in glyphosate treated rats. Studies of Djaber et al. (2022) suggested that exposure to glyphosate deteriorated the hepatic antioxidant system as evidenced by an increase of lipid peroxidation (malondialdehyde) and advanced oxidation protein product (AOPP). Also, the antioxidant defense system such as reduced

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glutathione (GSH), glutathione *S*-transferase (GST), catalase (CAT), and superoxide dismutase (SOD) and also raised the levels of serum hepatic marker enzymes as evidenced by an increase in aminotransferases (ALT, AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), and total bilirubin (TB), along with reduced total protein content and albumin in both the brain and liver of rats. The present study also supports the findings of Bali et al. (2019), Cattani et al. (2017), de Souza et al. (2019) and Qi et al. (2023). Interestingly, the present results also suggest that the co treatment of rats with glyphosate and MO leaf extract exhibited deteriorated lipid peroxidation levels with a concomitant increase in the antioxidant enzymes in the liver of rats suggesting negation of oxidative damage induced by glyphosate by MO. The results are in agreement with Reda et al. (2023). The antioxidant potential of MO leaf extract could be due to the desirable nutritional contents, including vitamins, amino acids, minerals, and fatty acids, besides various types of antioxidants including flavonoids, ascorbic acid, glucsinolates, carotenoids, and phenolics.

To summate, as MO leaf extract possess antioxidant potential and could be used as a tool to mitigate the OP induced oxidative stress in rats. The rat model system used in the current study might be used by researchers and toxicologists to authenticate the antioxidant potential of plants and their products.

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