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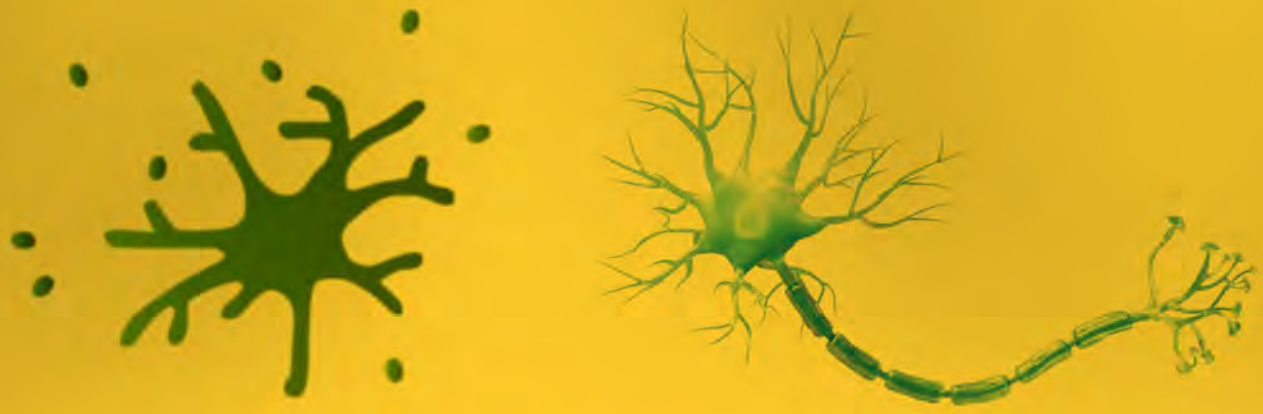

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RESEARCH ARTICLES

Bioscience Biotechnology Research Communications

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Have we tamed the coronavirus? May be yes,
as pandemics do not die, they can only be faded !

Science and technology has made it possible, in the shortest span of time, it has shown that with firm determination and international cooperation, we can win over the onslaughts of even the worst of the pandemics. COVID-19 is perhaps fading over now, due to our coordinated efforts worldwide. Though we have lost millions, in the two year period, partly due to the mishandling of the viral attacks and somewhat by our own follies and carelessness. Anyway lessons learnt from the past, always make us more stronger and determined. Let us now not relax and work on a better mode, as all is still not well yet. The almost taming of the virus and its cousins have indicated some of the concealed failures, on which we have to focus now. We have to be more vigilant, and even a bit of laxity can spoil the good work done. On societal and governmental parts, utmost care and caution is required on a long term basis.

On behalf of Bioscience Biotechnology Research Communications, we falter at words to express our deep sense of solitude and grief on the catastrophic events of the world wide pandemic, spanning over two years now. We pray for the strength to bear this universal calamity and come up with long lasting fortitude to eradicate it soon.

Biosc Biotech Res Comm is an open-access international platform for publication of original research articles, exciting meta-reviews, case histories, novel perspectives and opinions in applied areas of biomedical sciences. It aims to promote global scientific research and development, via interactive and productive communications in these areas, helping scholars to present their cherished fruits of research grown on toiled and tilled trees of hard work in life sciences. Being the publication of a non-profit academic Society for Science and Nature, Bhopal India, since 2008, *Biosc Biotech Res Comm* strongly believes in maintaining high standards of ethical and quality publication.

Quality publication is one of the ways to keep science alive, and good journals have a leading role to play in shaping science for humanity! As teachers, we have great responsibilities, we have to advocate our students to accomplish and show them the path to test their mettle in hard times to excel, especially in the post COVID 19 era. Science and its advocates will rise more to the occasion and will soon provide succor to the already grief stricken humanity.

Sharique A. Ali, PhD
Editor-in-Chief

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A Report on the Scientific and Teaching Activities at Ataturk University Supported by the European Higher Education Mobility Agreement Program Under a Memorandum of Understanding Between Ohio State University USA and Ataturk University, Turkey

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ABSTRACT

This report presents the scientific and teaching activities at Ataturk University (ATA), Erzurum, Turkey, supported by the European Higher Education Mobility Agreement, ERASMUS. program and under a Memorandum of Understanding between Ohio State University, Ohio, USA, and Ata Turk University, Turkey. Among the objectives for planned exchanges is the discussion and planning for future research and academic cooperation, including ERASMUS-supported activities such as a new OSU study abroad initiative. The academic exchanges will allow insights into the host country's university teaching and research system and curriculum comparison of general biology, environmental sciences, fisheries, and aquaculture programs. Graduate education philosophy will be encouraged with a new impetus for curriculum development that will include a better understanding of the host university's teaching methodologies (basic and applied science balance) to improve teaching outcomes. The ERASMUS program office located at ATA not only encourages but, most importantly, finances career advancement opportunities of university academic staff worldwide. Additionally, the program regularly invites foreign scholars to visit ATA for a week to establish research, teaching, and collaboration programs that support both graduate and undergraduate educational and research experiences. Some of the recommendations of the scientific joint meeting were to encourage student exchanges between the two institutions, along with the recruitment of faculty members at both institutions who envision strong participation in international education and research. It was also recommended that there must be promotion of an international research collaboration through joint research proposals, and academic networking should include the National Science Foundation, U.S., and TUBITAC (Turkish partner institution).

KEY WORDS: SCIENTIFIC, COLLABORATION, RESEARCH, MOU ATATURK UNIVERSITY, TURKEY OHIO STATE UNIVERSITY USA.

INTRODUCTION

Ataturk University (ATA) was established in 1957 with the Faculty of Agriculture and the Faculty of Arts being among the first departments to offer educational courses. During its establishment, an agreement was signed with the University of Nebraska, which resulted in the academic institution profile of ATA being similar to the mission of American Land-Grant Universities. Faculty of Fisheries is one of 23 faculties that include Faculties of Medicine, Pharmacy, Nursing, Dentistry and in more general areas such as Law, Architecture, Engineering, Health Sciences,

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and Education. The undergraduate student population of ATA is spread across the main campus in Erzurum, and 12 vocational schools. The university additionally hosts 6,749 international students which amount to 2% of total student population.

The ERASMUS program office located at ATA not only encourages but most importantly finances career advancement opportunities of university academic staff worldwide. Additionally, the program regularly invites foreign scholars to visit ATA for a week to establish research, teaching, and collaboration programs which support both graduate and undergraduate educational and research experiences. Our Memorandum of Understanding with ATA addresses a multidisciplinary strategy that will be implemented by forming relationships and exchanges between both institutions, faculty, teachers, and staff from SENR and other OSU departments, such as Food Sciences, Public Health, EEOB, etc.

Among the objectives for planned exchanges is the discussion and planning for future research and academic cooperation, including ERASMUS+ supported activities such as a new OSU study abroad initiative. The exchanges will allow insights into the host country's university system and curriculum comparison of general biology, environmental sciences, fisheries, and aquaculture programs. Graduate education philosophy should encourage new impetus for curriculum development that will include better understanding of the host university's teaching methodologies (basic and applied science balance) to improve teaching outcomes. Some graduate programs at ATA are offered in English, such as food engineering, horticulture, animal science, and agricultural economics, to name a few. An important aspect of the agreement to encourage student exchanges between the two institutions must be the recruitment of faculty members at both institutions who envision strong participation in international education and research. The promotion of an international research collaboration through joint research proposals and academic networking should include National Science Foundation, U.S. and TUBITAC (Turkish partner institution).

Scientific Visit and Recommendations: On the first day of the visit, I met Dr. Omer Comakli, President of ATA who declared both logistic and financial (competitive) support for our program. Specifically, we addressed during the meeting the intent of collecting preliminary data on polyploidy induction in rainbow trout and arctic char, two species of fish that ATA scientists have enormous experience and excellent environmental conditions on the university fish farm located in the heart of the campus. This will involve two Departments (Engineering, Dr. Eren, and Fisheries, Drs. Telat and Arslan).

Prof. Saltuk Ceyhun is current Chair of the Graduate School of the Natural and Applied Sciences. His laboratory in the Department of Fisheries studies a suite of biomedical aspects of zebrafish as a model species using morphological, behavioral, physiological and molecular methods to understand mechanisms of action of environmental toxicants, polystyrene plastic particles, simulated climate changes and nutritional, diet induced pathologies (for instance related to obesity). This is followed by antioxidant and protective action of phytochemicals extracted from local Turkish plants historically used in folk-medicine, such as carob (*Carotonia siliqua*). His papers are published in "Environmental Health Perspective", "Human and Experimental Toxicology" or "Science of Total Environment" among other 1st tier journals.

Figure 1: Visit with the President of Ataturk University, Dr. Omer Comakli (Photo 1 Prof.Dr. Konrad Dabrowski the second to the left and 2 Prof.Dabrowski the second to the right).

Dr. Arslan Murat, who spent several years in the OSU aquaculture laboratory, is utilizing techniques he learnt in Columbus, fatty acids and vitamins analysis, and continues to study the effect of tocopherol and synergistic antioxidant compounds on reproduction and gamete quality of salmonid fishes. He and his associates are interested in the possible use of plant extracts in extended shelf-life storage of fish fillets, and providing a label of "functional food" to fish farms producing trout or salmon with diet supplements containing such phytochemicals. This aspect attracted interest from OSU faculty of Department of Food Science. Prof. Telat and Arslan collaborate on several aspects of reproduction and gamete quality in salmonids, including novel antioxidant compounds, diluidine, which was proven to extend the viability of trout eggs during in vitro storage and this result was associated with preventing tocopherol (vitamin E) degradation.

Facilities available in the experimental fish farm (Photo) allows to extend of research of salmonid fish nutrition for practical aspects of vacuum-packed fillet storage and consumer acceptance.

Figure 2: Presentation of the aquaculture research and teaching program at OSU to general public at Ataturk University (Photo 3).

Figure3: In the laboratory of Dr. Saltuk Ceyhun (zebrafish as model animal in medicine, toxicology, behavioral and molecular biology sciences) (Photo 3-5).

Figure 4: In the laboratory of Prof. Arslan Murat (fish physiology, nutrition, reproduction) (Photo 6).

Figure 5: Meeting with faculty in the Department of Fisheries (Prof. Dr. Yanik Telat, Dean (OSU alumni) (Photo 7)

Figure 6: Laboratory, fish farm and fish processing facilities managed by Prof. Yanik Telat during scientific visit of Prof. Konrad Dabrowski(Photo 8-14).

Figure 7: Laboratory of Prof. Dr. Eren Zeynep, Department of Engineering, ultrasonic equipment utilized in structural chemistry of microcystin degradation and has the potential to be used in many aspects of fish biology (Photo 15).

Figure 8: Hatching jars for salmonids, vacuum-packed fillets of Salvelinus (left) and rainbow trout (right) fillets.

Figure 9: Laboratory, fish farm and fish processing facilities managed by Prof. Yanik Telat during scientific visit of Prof. Konrad Dabrowski(Photo 8-14).

Dr. Zeynep Eren from Department of the Chemical and Environmental Engineering is working on the major toxicant of algal blooms, microcystin, degradation by the use of high frequency ultrasound technology. Dr. Eren spent already one week visiting SENR July 2022 and we continued discussion of research project on monitoring in in situ experiments regarding biocontrol of cyanobacteria blooms by phytoplanktivorous fishes that are considered invasive in North America but can address cyanobacteria blooms in natural water bodies.

In her laboratory in ATA she demonstrated her equipment (Photo 15) that allows to explore many potential uses of ultrasound in many aspects of aquatic animals' biology. These include in vivo studies of increased cellular membrane penetration of cryoprotectants such as methanol in live fish embryo or immersion protocols that would increase penetration of steroid hormones to enhance sterilization of fish.

I have met in ATA Dr. Emre Harorli, Department of Management and Organization from Vocational College in Erzurum, who is working on COVID-related risk factors. He evaluated by online survey in 2020 the effectiveness of some protection measures to prevent the spread of the pandemic and designed predictive models to avoid COVID-19 infections. He was not able to visit OSU in July due to the extended duration for visa application; however, our Department of Public Health, College of Medicine, has expressed great interest in hosting him at OSU and establishing a collaborative program.

Erzurum, Turkey June 12-16, 2023 Visit with the President of Ataturk University, Dr. Omer Comakli (Photo 1 Prof. Dr. Konrad Dabrowski the second to the left and 2 Prof. Dabrowski the second to the right). Atatürk University offers a variety of facilities and services to support academic and student life. The university operates one of the largest training and research hospitals in Turkey, serving as a key healthcare provider for the Eastern Anatolia and Eastern Black Sea regions. Healthcare services at the university hospital are available 24 hours a day, with free access for students under the age of 25.

The campus includes diverse social and recreational facilities, such as movie theaters, fitness centers, Olympic swimming pools, and a bowling center. The university also provides cultural courses in areas such as traditional folk dances, Turkish folk music, Western music, and theater. Additionally, the campus hosts cultural festivals, concerts, ceremonies, exhibitions, and other events throughout the academic year (Ataturk University, 2011 2021, 2024 and Ataturk University QS Ranking 2023).

CONCLUSION

The present report describes the scientific and teaching activities at Ataturk University (ATA), Erzurum, Turkey, supported by the European Higher Education Mobility Agreement, ERASMUS program under a Memorandum of Understanding between Ohio State University, Ohio USA, and Ataturk University, Turkey. Among the objectives were planned exchanges for future research and academic cooperation, including ERASMUS-supported activities such as a new OSU study abroad initiative. The exchanges will allow insights into the host country's university system and curriculum comparison of general biology, environmental sciences, fisheries, and aquaculture programs. The program regularly invites foreign scholars to visit ATA for a week

to establish research, teaching, and collaboration programs that support both graduate and undergraduate educational and research experiences. Some of the recommendations of the scientific joint meeting were to encourage student exchanges between the two institutions, along with the recruitment of faculty members at both institutions who envision strong participation in international education and research. It was also recommended that there must be promotion of an international research collaboration through joint research proposals and academic networking.

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Reforming the Earth Through Bioremediation: A Systematic Review of Approaches to Reverse Anthropogenic Environmental Damage

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ABSTRACT

In the age of rapid industrialization and modernization, Mother Earth is enduring escalating environmental harm driven by anthropogenic activities. Uncontrolled population growth and increasing resource demands are leading to the overexploitation of natural resources. Exploiting Mother Nature without realizing the consequences may lead to serious environmental degradation, including climate change, biodiversity loss, and pollution of terrestrial and aquatic ecosystems. This systematic review study examines the role of bioremediation as a sustainable and eco-friendly approach to addressing these environmental challenges. Recent studies show that bioremediation methods such as biostimulation, bioaugmentation, bioaccumulation, biosorption, phytoremediation, and rhizoremediation are effective in reducing pollutant concentrations in soil and water. Specifically, microorganisms have demonstrated the ability to either degrade or transform toxic pollutants into less harmful forms, thereby preventing bioaccumulation and reducing ecological risks. Bioremediation represents a promising, low-cost, and sustainable approach for restoring polluted environments. It plays a vital role in reducing environmental hazards and improving ecosystem health, particularly in areas impacted by industrial waste and heavy metal contamination. Future research should focus on enhancing the efficiency of microbial strains, integrating multi-omics technologies, and applying bioremediation at a larger scale to address complex and mixed pollutant scenarios effectively.

KEYWORDS: ANTHROPOGENIC DAMAGE, BIOREMEDIATION, PHYTOREMEDIATION, RHIZOREMEDIATION, XENOBIOTIC.

INTRODUCTION

Due to industrialization and urban growth, a huge amount of chemicals are released into the air, water, and soil. According to reports by Third World Network, more than 400 million kilograms of toxins are released globally. These pollutants, ranging from heavy metals to synthetic chemicals, pose severe threats to ecosystems and human health. And to clean up these toxic pollutants using traditional methods needs a lot of money; the cost of removal of 1 meter cube soil from a 1-acre contaminated site is estimated as US$0.6–2.5 million (Chen et al., 2018 Singh et al 2024).

Also, the traditional techniques such as excavation, soil washing, and thermal desorption only transfer the contaminants rather than cleaning, thus creating new contaminated sites, which are not only cost-intensive but also unsustainable. But the bioremediation is sustainable; it has emerged cost cost-effective and eco-friendly technologies. It utilizes biological systems, microorganisms, plants, or enzymes to detoxify or remove environmental pollutants. It has the potential to remove or make the toxic substance harmless. The basic principles of bioremediation were established in early 2000s research. For instance, Dua et al. (2002) emphasized key factors influencing successful bioremediation:

1. The nature of pollutants (organic or inorganic)
2. The soil structure, pH, Moisture contents, and hydrogeology,
3. The nutritional state, microbial diversity of the site. Temperature and oxidation-reduction (redox Potential).

Bioremediation activity through microorganisms is stimulated by supplementing nutrients, electron acceptors,

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and substrates, or by introducing microorganisms with desired catalytic capabilities (Ma et al., 2007; Baldwin et al., 2008). Cell surface expression of specific proteins allows the engineered microorganisms to transport, bioaccumulate, and/or detoxify heavy metals as well as to degrade xenobiotics (Muhammad et al., 2007 Singh et al, 2024).

Another problem comes with the underground storage tanks, which contaminate the groundwater, the most important source of drinking water. In addition to this, the plastics that are thrown in soil also get leached deep underground, causing contamination of the underground water. A study published in Environmental International in 2022 revealed the presence of microplastics in human breast milk. The researchers found that 75% of breast milk samples tested contained microplastics, particularly polyethylene (used in plastic bottles and food packaging) and polypropylene (used in bottle caps).

Bioremediation can be done using several methods involving microbes, plants, and enzymes. Microbial bioremediation uses bacteria, fungi, and algae to degrade or transform pollutants. Phytoremediation employs plants to absorb or degrade contaminants, particularly heavy metals and organic pollutants, through processes like phytoextraction and phytodegradation. Enzyme-mediated bioremediation utilizes specific enzymes to break down pollutants more directly. Bioaugmentation introduces specialized microorganisms to accelerate pollutant degradation.

Bioremediation: According to van Dillewijn et al. (2009), Bioremediation is defined as the process by which various biological agents, primarily microorganisms, degrade environmental contaminants into less toxic forms. Types of bioremediations:

In Situ Bioremediation: This type of bioremediation occurs on-site, meaning it takes place at the location of contamination without moving the contaminated material. The goal is to treat pollutants directly where they are found, thereby minimizing the disruption to the surrounding environment: Biosparging, Bioventing, and Bioaugmentation.

Ex Situ Bioremediation: Ex situ Bioremediation is the type of Bioremediation where the process of decontamination occurs off-site, often in specialized treatment systems such as bioreactors or land farms. But soil or water contamination cannot be done off-site, as we cannot take all the soil and all the water from nature to the laboratory and process it. Thus, there comes the In situ Bioremediation, where the treatment occurs on site, meaning on the site of contamination, for e.g., soil and groundwater.

Land farming (Solid-phase Treatment system), Composting (Anaerobic, converts solid organic wastes into humus-like material), Biopiles

Phytoremediation: The use of plants to remove toxicants is becoming an interesting topic for research, as it has great potential for cleaning up the environment with high sustainability. One of the major advantages of phytoremediation is the low cost. In addition to this, it preserves the environment by not altering the natural state. Plant roots and shoots can be used to absorb and concentrate hazardous compounds, particularly heavy metals, from aqueous solutions, known as rhizofiltration. It has been accepted and utilized widely as an effective and environmentally friendly green technology for the permanent removal of pollutants (Chen et al., 2018).

Advantages of Phytoremediation (Huang et al., 2004):

1. Preserve the natural properties of soil
2. Energy efficient, as sunlight is the source of energy for plants
3. Maintains microbial diversity in soil

In addition to these, Plants also act as accumulators of metals like Se, Co, Cu, Cd/Zn and Ni, which can also be toxic for the soil health. These include crops like *Astragalus racemosus, Haumaniastrum robertii, Ipomea alpina, Thlaspi caerulescens, and Sebertia (Lasat, 2002) (Wuana and Okieimen*, 2010).

In Situ Bioremediation of Oil Spills in the Antarctic: Ever since the beginning of Industrial Revolution, toxic substances, including anthropogenically generated hydrocarbons, have been released into the environment without the proper treatment. Successive oil spills in the ocean and its coast is becoming a major issue since the use of fossil fuels. However, the Antarctic ecosystem is one of the few last spots to remain uncontaminated by the anthropogenic hydrocarbons but the probability of its contamination is increasing due to human intervention (Berkman, 1992) (Platt and Mackie, 1980) (Reinhardt and Van, 1986).

Biological degradation by natural occurring microorganism to remove pollutants from the environment is the major mechanism to remove the petroleum products from the surroundings but very little is known about the same in the coldest regions. (Atlas, 1981), (Leahy and Colwell, 1990) (Bragg et al., 1994). Bioremediation can be defined as the procedure to enhance the rate of natural degradation through human intervention. For doing so, procedures like biostimulation (the addition of chemicals such as nutrients or surfactants to stimulate the natural flora) and bioaugmentation (the addition of an exogenous organism) can be referred to make an impact. However, the effectiveness of bioaugmentation is very low due to extreme conditions.

Sea water: Several studies were carried out at Morbihan Bay, Kerguelen archipelago, between different timelines to evaluate the benefit of a slow-release fertilizer (Inipol EAP 22, Elf Atochem) addition in sub-Antarctic seawater. In situ studies were conducted in a small sheltered bay using psychrophilic saprophytic and hydrocarbon-degrading bacteria in which the changes in bacterial community were studied during a particular time period after the addition of the contaminant. A decrease in the ratios of C17/pristane and C18/phytane over time was reported to reveal the bacterial degradation of oil (Blumer et al., 1973; Berkman, 1992).

All results revealed a clear response of microbial seawater communities to hydrocarbon contamination. The percentage of hydrocarbon-degrading bacteria ranged from 0.001% of the total community before contamination to more than 80% after 2 weeks of contamination. However, a two orders of magnitude increase in total bacterial abundance was observed after contamination with crude oil with added fertilizer. In these surveys, it was observed that the bacterial population was enhanced by the addition of minerals and nutrients released by INIPOL EAP 22. Thus, this increase in the bacterial population clearly indicates the possibility of degradation after the contamination.

Table 1. Plants and the pollutants they remove

Plants	Pollutants	References
Ambrosia artemisifolia, *Apocynum cannabinum*	Pb	(Wang et al., 2002)
Brassica juncea	Pb, Se, Cu	(Wang et al., 2002) (de Souza et al., 1999) (Watanabe et al., 1998)
Helianthus annus, *Pteridium esculentum*	As	(Wang et al., 2002) (Brown et al., 1995)
Medicago sativa	Benzopyrene, PAE, PAH	(Brown et al., 1995)
Melastoma malabathricum	Al	(Watanabe et al., 1998)
Nephrolepis exaltata	Hg	(Chen et al., 2009)
Pteris vitata	As, Hg, Cs, Sr	(Wang et al., 2002) (Chen et al., 2009)
Salix viminlais	Cd, Zn, Cu	(Salt et al., 1995)
Raphanus sativus *Silene vulgaris*,	Cu	(Choudhary et al., 2009)
Thlaspi caerulescens	Zn, Cd	(Robinson et al., 2006) (McCutcheon et al., 2003)

Sea-ice: Similar studies were conducted in the northern hemisphere ("Geologie Archipelago") (Delille et al., 1997) using a similar protocol to observe the population growth of bacteria after contamination. A concomitant enrichment in oil-degrading bacteria was generally observed: from less to 0.001% of the community in uncontaminated samples to up to 10% after 30 weeks of contamination. It was also observed that the addition of Inipol EAP 22 (fertilizer) enhanced the both saprophytic and hydrocarbon-utilizing microflora. Also, bacterial population was larger in sea-ice as compared to the population tin the sea water which may be reflection of generally higher nutrient concentrations in the ice (Marra et al., 1982) (Sullivan, 1985), lower temperature or lower grazing pressure (Turley et al., 1986) (Gonzales, et al., 1990) (Grossmann and Dieckmann, 1994).

Soils: In antarctica, a study was conducted from February to December in 1985 in the Geologie Archipelago. Four study stations were chosen on Petrel Island and were studied in four different time periods: winter, snowmelt, summer, and freeze-up. An initial increase in the order of magnitude of total bacterial Abundance was observed in all contaminated soils, but they were always limited in time. However, these differences between the contaminated and uncontaminated soils completely disappeared after July. A very low level of degradation of the contaminant was suggested by the chemical analysis of the soil residues at the end of the study. The rate of microbial degradation of hydrocarbons in soil is affected by several physiochemical and biological factors such as concentration of pH, nutrients, oxygen, quality, quantity, etc. (Margesin and Schinner, 1997).

This observation can indicate the presence of hydrocarbon-utilizing microbes in the Antarctic soils and their potential for bioremediation. However, their sudden low level was surprising which can indicate that petroleum contaminants can exert toxic effects on the active microbial community as reported by Long et al 1995. Although it has been demonstrated that microbes can affect chemical pollutants, the presence of chemical pollutants can also affect the microbial community by altering their structure or through acute toxicity (Delille and Siron, 1993). In the studied areas, temperatures close to 0 °C have been shown to allow the biodegradation in seawater and sea-ice, whereas Antarctic soils are thermally unstable, experiencing large temperature fluctuations with temperatures dropping well below 0°C at night and reaching much more than 20°C during sunny afternoons (Harris and Tibbles, 1997). Thus, they suffer freeze freeze-thaw cycles. Antarctic soils also experience other extreme environmental stress (Wynn-Williams, 1990). All these fluctuations may seriously affect bacterial activity, so they must acclimate continuously and be able to switch on and off rapidly.

Microbial Bioremediation: Hazardous subs. such as nuclear waste, heavy metals, pesticides, hydrocarbons, etc. pose a serious risk to both nature and human wellbeing. Microbial bioremediation offers a sustainable and affordable solution to mitigate their harmful effects. The US Environmental Protection Agency (USEPA) recognizes bioremediation as a safe and effective method for restoring polluted environments. Many pollutants come from Industries, such as effluents from the paper and pulp

industry and heavy metals from their iron and steel and many related industries, which contribute to pollution. Xenobiotic components are also among them. Human activities like using chemicals, fertilizers in agriculture, paint, and plastic also produce these components. The persistence of these pollutants in the environment poses serious ecological and health risks. Traditional treatment methods are often expensive and may generate secondary pollutants. In contrast, bioremediation harnesses the natural ability of microorganisms to degrade these toxic compounds efficiently and sustainably.

Bioremediation: a natural way to remove heavy metals: Heavy metals are metals with higher densities that are released into the environment primarily through human activities like mining, industrial processes, and agricultural practices. They caused soil, air, and water pollution that hurts humans, plants, and even growing microorganisms. Traditional methods cannot permanently detoxify heavy metals. Additionally, they are costly and may produce 2° pollutants (Hazardous byproducts). Bioremediation, on the other hand, uses microbes that can tolerate high levels of heavy metals for effective treatment. Bioremediation of heavy metals can be done ex-situ by removing the contaminated material for treatment elsewhere or in-situ by applying biological agents directly to the polluted site. Some technologies include (either metabolism-dependent or metabolism-independent) oxidation-reduction mechanisms, bio transformation, methylation, and plant microbial remediation.

From Waste to Worth: Bioremediation of Rubber: Rubber waste is a growing environmental concern due to its non-biodegradable nature wide spread use in various Industries (rubber effluent). Each year, vast quantities of used rubber waste, primarily from discarded tires, accumulate and are thrown away. Bioremediation offers a sustainable alternative by utilising microorganisms capable of breaking down sulphur bonds (rubber compounds) into harmless byproducts. Some bacteria break the rubber's polyisoprene structure into simpler compounds. According to some experiments, the rubber waste is first mixed with organic matter and inoculated with rubber-degrading microbes, promoting biodegradation in controlled composting conditions. Introducing specialized rubber-degrading microbial strains into contaminated sites enhances the natural breakdown process.

Protozoa: Nature's Tiny Purifiers in Industrial Wastewater Treatment: Protozoa are single-celled microorganisms found abundantly in aquatic environments with unique physiological adaptations that allow them to survive in contaminated conditions. Protozoa, once considered a hindrance in activated sludge systems, are now recognised for their vital role in wastewater management by breaking down pollutants like copper, hydrocarbons, and even uranium. The increasing concentration of wastewater, driven by industrial effluents, excessive pesticide use, and other human activities, has made effective treatment essential. Protozoa improve effluent quality by consuming dispersed bacterial pollution and maintaining microbial balance. Beyond contaminated removal, Protozoa like ciliates, flagellates, amoebae, etc. play a key role in maintaining ecological balance by regulating bacterial populations through predation.

They enhance bacterial carbon mineralisation, improving effluent quality and microbial balance. Protozoa also contribute to heavy metal bioremediation, particularly in removing excess copper from industrial effluents and contaminated water. Additionally, they aid in hydrocarbon degradation, where free-living ciliates like Paramecium play a key role in the breakdown of polycyclic aromatic hydrocarbons (PAH). In Uranium bioremediation, protozoa influence the bacterial community involved in uranium reduction, facilitating detoxification in contaminated groundwater. Overall, Protozoa contribute significantly to environmental restoration by improving wastewater treatment efficiency, reducing pollutants and supporting microbial ecosystems. (Rehman et al., 2007)

Bioremediation of Xenobiotics: harnessing microbes to combat xenobiotic pollution in petroleum: Xenobiotics are chemical substances that are foreign to animal and plant life. The extensive use of Petroleum (a fossil fuel formed over millions of years) and industrial activities has introduced xenobiotic pollutants like PAHs into the environment. Factors like rapid industrialisation, urban expansion, and increased chemical usage in agriculture, medicine and personal care have intensified contamination. Bioremediation offers a sustainable approach to degrading these pollutants by harnessing microorganisms capable of breaking down xenobiotics. Methods like biostimulation enhance native microbial activity, while bioaugmentation introduces specialized microbes for effective degradation. Bacteria such as *Pseudomonas bacillus* and alcaligenes play a crucial role in utilising these compounds as energy sources. Advanced techniques like Bio leaching are also being explored to improve efficiency.

Enzyme based Bioremediation: Nature's catalysts for a cleaner Planet: Microbial enzymes (oxidoreductases, hydrogenases, deoxygenases and dehalogenases) produced by various microbes such as bacteria and fungi, play a crucial role in accelerating biochemical reactions by lowering activation energy. They are highly stable and versatile and essential for breaking down environmental pollutants into non- toxic forms making them vital for bioremediation. The effectiveness of degradation depends on enzyme -substrate interaction where enzymes bind to specific substrates through their active site, initiating the catalytic process. These enzymes offer advantages over traditional methods due to their specificity and Eco friendliness. Various microbial products including biofilms, surfactants, pigments and extracellular compounds have shown significant effectiveness in bioremediation. However, because of enzyme production downstream processing and microbial strain selection poses changes. To address this researcher are using enzyme immobilization technologies for efficient production. Additionally genetic engineering is being employed to develop modified microorganisms offering a cost-effective alternative to traditional enzyme production methods.

Xenobiotics: water and soil bioremediation: Cyanobacteria, also known as blue green algae, are the first oxygen producing organisms. They play a vital role in bioremediation due to their ability to degrade complex organic substances and thrive in polluted environments without external nutrients. In wastewater remediation, they are widely used for removing phosphorus and inorganic pollutants. Their ability to degrade toxic substances improves water quality. In soil remediation, cyanobacteria improve fertility and reduce salinity and alkalinity caused by excessive pesticide and fertilizer use making them valuable for restoring degraded agricultural lands.

Microalgae:Nature's tiny Warriors for pollution clean up: Microalgae are microscopic photosynthetic organisms belonging to the diverse group of cyanobacteria (blue green algae), diatoms, green algae and dinoflagellates. Microalgae have ability to remove pollutants such as heavy metals pesticides pharmaceuticals and excess nutrients making them a promising tool for wastewater treatment and soil the contamination. They contribute to carbon sequestration offering a dual benefit of pollution reduction and greenhouse gas mitigation. Microalgae employ several strategies to remove contaminants, including biosorption, biodegradation, and phytoremediation. Strains like *Chlorella* and *Spirulina* show high affinity for metals like lead, cadmium, and mercury. They can absorb nitrates, phosphates and organic matter, reducing eutrophication risks. Some species like *Ulva, Chlorella*, and *Scenedesmus* can break down antibiotics, endocrine disruptors, and herbicides preventing their accumulation in the ecosystems.

One of the most promising aspects of microalgae bioremediation is its potential for biofuel generation. After pollutant removal, the algal biomass can be processed into bioethanol, biodiesel and biogas, creating a circular economy approach that links environmental clean-up with sustainable energy production. Despite its potential, microalgae-based bioremediation face challenges like high operational cost and in strain selection and optimisation (to enhance pollutant uptake). There are also problems in harvesting and the recovery of the biomass. Future research focuses on designing and improving bio-reactors for the growth of microalgae and also of genetic engineering.

Bioremediation of Pesticides Using Actinobacteria: Pesticides are compounds that are used to protect the plant from unwanted organisms called pests. They can be both natural and synthetic. They are of different kinds depending upon the target organisms- fungicides, insecticides, herbicides, etc. Even after its harmful effects on humans and the environment, the usage of pesticides becomes necessary due to the increasing demand of agricultural produce, due to the growing population and overconsumption. Chemicals present in pesticides can be very toxic and are prone to bioaccumulate in the environment. Therefore, it becomes a necessity to remove these toxins from the environment. One of the ways through which it can be done is through the process of bioremediation, in which microbes can break down the toxic substances into their less toxic forms. Various microbes have been found to be effective in degrading pesticides into their less toxic metabolites using their metabolic activities (Gupta et al., 2017) (Hussaini et al., 2013).

Table 2. Microorganisms and the pollutants they remove

Microorganism	Pollutant	Reference
Citrobacter sp.	U	(Renninger et al., 2001)
Cupriavidus metallidurans, Escherichia coli	Zn and Cu Zn and V	(Grass et al., 2002)
Escherichia hermannii, Enterobacter cloacae	V and Zn Pb, Cu, V, Cr	(Hernandez et al., 1998)
Saccharomyces cerevisiae	Cu, Zn, Cd, Pb, Fe, Ni, Ag, Th, Ra, U, Hg	(Machado et al., 2008) (Ghosh et al., 2006)

Due to advancements in the agricultural sector, huge amounts of chemical pesticides are easily available in the market, which are eventually released in the environment through different sources (Briceno et al., 2007). There are two types of sources- point sources, i.e., distinguishable sources, and non-point sources i.e., from a widespread area. The washing of spraying containers is one of the main reasons of the point source contamination of the soil (Neumann et al., 2002) (Spanoghe et al., 2004).

Upon being released from the source, they eventually spread in the water, soil, air, and even in the food we eat, which leads to neuropsychiatric defects such as depression and anxiety in humans and can also be lethal for many organisms.

Bioremediation is known as an environment friendly method to remove pollutants from the environment. The microbial biomass can metabolize various chlorinated and non-chlorinated pesticides. The microbes - bacteria, fungi, algae, and actinobacteria can be used to remove toxic compounds from the soil (Megharaj & Naidu, 2017). They can completely metabolize toxic compounds effectively and thus, eliminate pollutants from the environment (Abatenh et al., 2017) (Gupta et al., 2019) (Rathour et al., 2018). The on-site removal of pollutants is considered as in situ

remediation, whereas off-site management of pollutants is considered ex-situ remediation.

Role of actinobacteria: Actinobacteria are Gram-positive saprophytes having higher Guanine + Cytosine (>55%) content in their DNA. They have the ability to grow in harsh environments and are known for actively participating in the biodegradation process. They consume pesticide residues for energy requirements, and their degradation is usually accomplished through a synergistic group activity. In a mixed microbial population, they remove hazardous compounds either directly or accelerate the biotransformation efficiency of other organisms. The synergistic activity between actinobacterial species and Pleurotus ostreatus was observed during bioremediation (Byss et al., 2008). Chlorpyrifos is a broad-spectrum chlorinated organophosphorus insecticide used to increase crop productivity. It was found to cause disruption in biogeochemical cycles (Chishti et al., 2013).

Highly efficient actinobacteria, Streptomyces strains, were isolated, which can degrade up to 90% of chlorpyrifos within 24 hours and convert it to 3,5,6-trichloro-2-pyridinol (TCP) (Kao et al., 2004). But TCP had greater solubility than its parental compound, creating another problem. Its antimicrobial activity also inhibited the multiplication of chlorpyrifos-degrading microbes (Singh & Walker, 2006). An actinobacterium, Gordonia sp. JAASI, isolated from rice field, actively degrade chlorpyrifos and its intermediates such as TCP into diethylthiophosphoric acid (DETP) (Abraham et al., 2013).

Diazinon is effectively degraded by Arthrobacter species, but requires co-metabolism process by Streptomyces species to initiate degradation (Gunner & Zuckerman, 1968). Other species of Actinobacteria have also been observed to degrade toxic chemical compounds. The mono-, di-, and trichlorinated pesticides are readily degraded by several species of actinobacteria, but polychlorinated pesticides, such as pentachlorophenol (PCP), have higher stability, extensively utilized as biocides, wood, and leather preservatives (Briceno et al., 2006). The actinobacteria species are also able to metabolize other synthetic pyrethroids i.e., cypermethrin, fenvalerate, fenpropathrin and permethrin.

Pesticide degradation includes several metabolic pathways, which depend on the pesticide properties, environmental conditions and on the microbe's nature. This comprises of: (a) transformation facilitated by oxidative enzymes; (b) hydrolysis mediated by hydrolases; (c) Reduction by reductive enzymes; (d) Conjugation reaction includes xyloxylation, alkylation, acylation and nitrosylation; (e) Reductive dehalogenation facilitated by dehydrohalogenase enzyme. Various enzymes such as carboxylesterases, laccases, phosphotriesterases, peroxidases, haloalkane dehalogenases, lipases, oxygenases, cellulases, etc., are involved in the degradation pathways of different compounds (Sharma et al., 2018). The application of enzymes is preferred as compared to microbial degradation as they do have need to be acclimatized and can be easily used in harsh environmental conditions (Choi et al., 2015).

The mechanism involves degrading organic compounds into inorganic components and the usage of energy obtained from degraded metabolites to detoxify the pollutants and also for their growth and development. In a natural environment, biodegradation comprises transfer of substrates in well-coordinated microbial biomass, called metabolic cooperation (Abraham et al., 2002). Microbes act together physically or chemically with the compounds and provide structural alterations for complete degradation. In this approach, the microbes such as actinobacteria play as a main converters and pesticide degradation agents (Morillo & Villaverde, 2017).

Factors such as microbial species, chemical composition and concentration of pesticides, environmental conditions, etc. are known to affect the biodegradation process. Despite all the conditions, actinobacterial-assisted pesticide degradation is found as an ideal, efficient and sustainable approach for bioremediation of pesticides. They have great potential in the decomposition of pesticides, nutrient cycling, and biodegradation to repair polluted environments.

CONCLUSION

Bioremediation is not solely the responsibility of nature or a select group of environmental stewards, it is a collective duty of everyone to protect it by not misusing the resources, least use of harmful substances, use of sustainable alternatives to harmful daily life items. The alarming presence of microplastics in human breast milk (Environmental International, 2022) and the bioaccumulation of harmful chemicals from pesticides in food chains highlights the urgent need for sustainable actions, can you imagine the life of a baby who started its life by drinking plastic indirectly. Traditional remediation methods are often expensive and inefficient, as noted by Chen et al.

(2018), transferring rather than resolving contaminants. In contrast, bioremediation presents an eco-friendly and cost-effective solution, utilizing microbes, plants, and enzymes to detoxify pollutants. Key factors such as soil pH, pollutant type, and microbial diversity (Dua et al., 2002) must be optimized to enhance effectiveness. Techniques such as bioaugmentation and genetic engineering (Ma et al., 2007; Muhammad et al., 2007) further extend the scope of remediation, offering novel approaches like recombinant DNA technology to enhance the pollutant-degrading capabilities of organisms. Molecular tools such as PCR and FISH enable precise identification of functional microbes, aiding targeted cleanup strategies.

Future research should focus on developing genetically engineered organisms tailored to specific pollutants and scalable application systems. Public awareness, policy support, and interdisciplinary collaboration are essential to integrate bioremediation into mainstream environmental management. Sustainable living practices and reduced chemical use must complement technological approaches to ensure long-term ecological balance.

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Correlational Study of Physical Activity Levels and Nutritional Behaviors Among High School Children in Kathmandu, Nepal

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ABSTRACT
Adolescents' health is increasingly influenced by behavioral risk factors such as poor diet and physical inactivity. This study aimed to explore the correlation between physical activity and dietary behaviors among high school students in Kathmandu, Nepal. A school-based cross-sectional study was conducted among 150 students aged 13–18 years using multistage random sampling. Data were collected through a validated self-administered questionnaire, and anthropometric measurements were recorded. Statistical analysis was performed using SPSS. Approximately 68.7% consumed fruits and vegetables regularly, 59.3% consumed fast food more than once per week, and 90.6% consumed snacks frequently. Physical activity participation was reported by 75.4% of students, with walking and school sports being the most common. A significant positive correlation was found between physical activity frequency and healthy dietary choices ($p < 0.05$). Despite moderate levels of physical activity, the high consumption of fast food and snacks raises concern. Health education and school-based interventions are essential to promote healthier lifestyle choices. This study found that while many high school students in Kathmandu eat fruits and vegetables and are physically active, unhealthy habits like frequent fast food and snack consumption are still common. A positive link between physical activity and healthy eating suggests that encouraging one may improve the other. These results highlight the need for school-based programs that promote better nutrition and regular physical activity to support adolescent health. Future research should include larger and more diverse samples from both urban and rural areas to improve generalizability. Studies using objective tools to measure physical activity and detailed dietary intake, including portion size and timing, are recommended. Longitudinal designs could help establish cause-and-effect relationships and track behavior changes over time. Exploring additional factors like screen time, sleep patterns, and family lifestyle would also provide a more complete picture of adolescent health.

KEY WORDS: ADOLESCENT HEALTH, DIETARY BEHAVIOR, PHYSICAL ACTIVITY,

INTRODUCTION

Adolescence is a critical developmental stage where health-related behaviors are established, many of which extend into adulthood. Globally, non-communicable diseases (NCDs) linked to physical inactivity and poor dietary patterns are increasingly affecting adolescent populations (World Health Organization, 2023). Rapid urbanization, academic pressure, digital entertainment, and the availability of fast foods have reshaped the lifestyle of adolescents, particularly in urban South Asia (Popkin et al., 2020, Ahmed 2025).

Physical activity has been positively associated with improved body mass index (BMI), reduced stress, and enhanced academic outcomes (Janssen & LeBlanc, 2010). Recent global estimates suggest that more than 80% of adolescents are not sufficiently physically active (WHO, 2023). Conversely, sedentary behavior and late-night eating habits are linked with obesity, metabolic syndrome, and poor sleep quality (Chaput et al., 2016). While physical education programs exist in many schools, a lack of structured

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curricula and infrastructure often limits their effectiveness (Bhandari et al., 2020; Melendez-Torres et al.,2023).

Despite growing evidence on adolescent health risks in South Asia, there is limited empirical research from countries in South East Asia, exploring the interaction of physical activity and dietary behaviors. For instance, In Nepal, high school students in urban centers like Kathmandu face multiple lifestyle-related risk factors due to environmental and socio-economic shifts. The Global School-based Student Health Survey (GSHS) 2015, conducted again in Nepal, indicated that 30.9% of students consumed carbonated soft drinks daily and only 28.4% consumed fruits daily (MoHP, 2017). Similarly, in yet another study conducted in Bangladesh, Rahman et al. (2020) reported that only 18% of school children engaged in daily physical activity, with a marked increase in fast food consumption among urban adolescents.

Therefore, the present study aims to assess the correlation between physical activity and dietary habits among high school students in Kathmandu, Nepal. Understanding these behaviors in a regional context is critical for designing culturally and demographically tailored interventions to improve adolescent health outcomes in South Asia. This type of study is crucial as it might provide localized data needed for evidence-based health policies tailored to Nepal's unique socio-cultural context. To the best of our knowledge, very few studies were done earlier in Nepal to highlight this very important issue. Early identification of at-risk adolescents allows timely prevention of chronic diseases. Additionally, comparing findings with countries like India, Bangladesh, and Sri Lanka will offer valuable regional insights for collaborative public health strategies across South Asia.

METHODOLOGY

A cross-sectional quantitative study design was employed to explore the correlation between dietary habits and physical activity levels among adolescents in Kathmandu. The study was conducted from March to May 2025 in six purposively selected high schools (three public and three private) located within Kathmandu Metropolitan City, ensuring diversity in socio-economic representation.

The study used a multistage stratified random sampling technique, ensuring balanced representation across different school types and student demographics. In the first stage, stratification by School Type where schools in Kathmandu were first stratified into public and private categories. From each stratum, three schools were randomly selected using a lottery method. In the second Stage 2, Class-wise Stratification i.e within each selected school, students were further stratified by grade level (classes 8 to 12), corresponding to the target age range of 13–18 years. In the third stage, simple random sampling of students was done using a computer-generated random number list. A total of 150 students were selected proportionately from each school, ensuring representation across all age groups and both sexes.

Data Collection Source: Primary data were collected on-site at the respective schools. Before data collection, the study protocol received approval from the Institutional Review Board (IRB) of SM Hospital, Katmandu and written informed consent was obtained from both the students and their legal guardians.

A structured, pre-tested, and pre-validated questionnaire was used for data collection. The instrument was adapted from the World Health Organization's Global School-based Student Health Survey and localized to suit the Nepali sociocultural context through expert review and pilot testing(WHO-GSHS,2013).

Key features of the questionnaire included four sections primarly divided into Section A: Demographics – Age, gender, class level, and parental education/occupation, Section B which included measuring the dietary behaviors by means of frequency of daily/weekly consumption of fruits, vegetables, snacks, fast foods, and sugary beverages, meal-skipping habits (e.g., breakfast skipping). The third section was related to measuring the physical activity patterns by means of evaluating the types of activity (moderate-to-vigorous physical activity,MVPA) such as school sports, recreational play, walking/cycling to school, and household chores, their frequency (days/week) and duration (minutes/day) of Sedentary behavior (screen time, sitting hours). Last but not the least, the final section was related to health and lifestyle indicators which measured self-perceived health status and sleep duration.

The questionnaire was translated into Nepali, back-translated into English, and reviewed for content and construct validity by a panel of public health experts and educators. The anthropometric measurements were done by trained field investigators who collected anthropometric data following standard WHO protocols such as height measured using a stadiometer (to the nearest 0.1 cm), weight measured using a calibrated digital scale (to the nearest 0.1 kg) and BMI calculated and categorized using WHO adolescent growth reference charts.

Data Analysis: The data were entered and cleaned using IBM SPSS version 26. Descriptive statistics (mean, standard deviation, frequencies) were calculated for demographic and behavioral variables with results were presented as percentages (%). Chi-square tests were used to identify associations between categorical variables (e.g., school type and dietary pattern).Pearson's correlation coefficient was used to assess the strength and direction of association between physical activity and dietary habits. Statistical significance was set at $p < 0.05$.

Results in this school-based cross-sectional study, data were collected from 150 high school students aged between 13 and 18 years in Kathmandu. The mean age of the students was 15.5 ± 1.6 years. Gender distribution showed that 54.9% of the respondents were girls while 45.1% were boys. Anthropometric measurements indicated that the average height was 158.2 ± 12.5 cm and the average weight was 53.4 ± 11.8 kg. The mean Body Mass Index (BMI) among

participants was 22.6 ± 3.4 kg/m², reflecting an overall moderate range for this adolescent population.

Approximately 12.3% of the students reported having health conditions including asthma, eczema, allergies, cramps, epilepsy, and anemia. Parental literacy was universal with a 100% literacy rate. Furthermore, 86.1% of the responses to the structured questionnaire were recorded by mothers, while the remaining 13.9% were documented by other family members. Regarding BMI distribution, 45% of students were classified as underweight, 23.8% as normal weight, 18.8% as overweight, and 12.3% as obese, with no cases of morbid obesity. These figures call attention to the dual burden of undernutrition and overnutrition among high school adolescents in urban Nepal.

RESULTS AND DISCUSSION

In this school-based cross-sectional study, data were collected from 150 high school students aged between 13 and 18 years in Kathmandu. The mean age of the students was 15.5 ± 2.6 years. Gender distribution showed that 55% of the respondents were girls, while 45% were boys. Anthropometric measurements indicated that the average height was 158.2 ± 12.5 cm and the average weight was 53.4 ± 11.8 kg. The mean Body Mass Index (BMI) among participants was 22.6 ± 3.4 kg/m², reflecting an overall moderate range for this adolescent population(Table 1).

Approximately 12.3% of the students reported having health conditions, including asthma, eczema, allergies, cramps, epilepsy, and anemia. Parental literacy was universal, with a 100% literacy rate. Furthermore, 86.1% of the responses to the structured questionnaire were recorded by mothers, while the remaining 13.9% were documented by other family members.

Table 1. Basic and Anthropometric Details of High School Students

Parameter	Value
Total number of students	150
Age range (years)	13–18
Mean age (years)	15.5 ± 2.6
Gender distribution	55% girls, 45% boys
Mean height (cm)	158.2 ± 12.5
Mean weight (kg)	53.4 ± 11.8
Mean BMI (kg/m²)	22.6 ± 3.4
Children with medical conditions	12.3%
Parental literacy rate	66%
Questionnaire filled by mother	86.1%

Regarding BMI distribution, 45% of students were classified as underweight, 23.8% as normal weight, 18.8% as overweight, and 12.3% as obese, with no cases of morbid obesity (Table 2). These figures call attention to the dual burden of undernutrition and overnutrition among high school adolescents in urban Nepal.

The dietary behaviors of high school students in Kathmandu revealed both encouraging and concerning trends. While 68.7% of students reported regular consumption of fruits and vegetables, a significant portion exhibited poor dietary habits. Approximately 59.3% consumed fast food more than once per week, and an overwhelming 90.6% frequently consumed snacks, indicating a shift towards high-calorie, low-nutrient foods. This pattern reflects an urgent need for school-based nutrition education and policy interventions to curb unhealthy eating practices among adolescents (Table 3).

Table 2. Categorization of children's body mass index (BMI)

Types of BMI	Frequencies	Percentages
Underweight	55	45.1%
Normal	29	23.8%
Overweight	23	18.8%
Obesity	15	12.3%
Morbid-Obesity	00	0%

Table 3. Shows the percentage of adolescents following various dietary habits

Dietary Habits	Frequency (%)
Regular fruit and vegetable intake	68.7
Fast food consumption (>1/week)	59.3
Frequent snack consumption	90.6

On the physical activity front, 75.4% of students reported participating in some form of activity, with walking and school sports being most common (Table 4 and 5). Although promising, this statistic may encompass low-intensity or irregular activity. Most notably, a significant positive correlation ($p < 0.05$) was identified between physical activity and healthy dietary behaviors. This finding suggests that physically active students are more likely to make healthier food choices, emphasizing the importance of promoting physical activity not only for fitness but also as a catalyst for broader lifestyle improvements.

Table 4. Shows the Physical Activity Participation

Physical Activity Parameter	Details
Participated in any physical activity	75.40%
Most common types of Physical activity performed by students	Walking, school sports

Table 5. List of physical activities for school children

Activities	Frequencies	Percentages
Physical activity	115	94.3%
Physical effort	105	86.1%
Gym	14	11.5%
Running	115	94.3%
Walking	115	94.3%
Physical activity classes	88	72.1%
Irregular physical activity	34	27.9%

The present study provides critical insights into the dietary behaviors and physical activity patterns among high school adolescents in Kathmandu, Nepal. The findings reveal a complex interplay of moderate healthy habits coexisting with prominent unhealthy behaviors, necessitating urgent public health attention.

Dietary Behaviors: The dietary profile of students in this study presents a dual scenario. While 68.7% reported regular consumption of fruits and vegetables, suggesting a moderate awareness and availability of healthy options, nearly one-third still fail to meet the minimum dietary guidelines. Compared to similar studies, this figure is notably higher than the 24% fruit and vegetable intake reported among adolescents in India (Patel et al., 2019) and 18% in Bangladesh (Rahman et al., 2020), indicating a relatively better nutritional trend in Kathmandu. This might be attributed to cultural dietary practices and local food availability, but gaps still persist.

Alarmingly, 59.3% of students consumed fast food more than once per week, mirroring patterns found in urban Delhi (Verma et al., 2020) and Sri Lanka (Perera et al., 2021) where fast food consumption among adolescents is steadily rising due to urbanization, commercialization, and peer influence. Furthermore, 90.6% of students reported frequent snack consumption, reinforcing concerns raised in earlier Nepali studies (Paudel et al., 2021) about the increasing preference for energy-dense, nutrient-poor processed foods. The unquantified but implied high intake of sugar-sweetened beverages aligns with WHO GSHS (2015) data that found 30.9% of Nepali adolescents consumed soft drinks daily. These trends contribute cumulatively to heightened risks of obesity, metabolic disorders, and poor academic performance.

Physical Activity: Encouragingly, 75.4% of students reported engaging in some form of physical activity, significantly higher than the 60–70% participation rates typically observed in similar South Asian contexts (UNICEF, 2022). The predominance of walking and school sports indicates a mix of informal and structured exercise habits. Compared to the Global School-based Student Health Survey (GSHS, 2015) for Nepal, which indicated low structured physical activity, this study shows a slight improvement—possibly due to increased health awareness campaigns and inclusion of physical education in the curriculum.

However, this figure must be interpreted cautiously. Since intensity and duration were not explicitly measured, students may not necessarily meet the WHO recommendation of 60 minutes/day of moderate-to-vigorous physical activity (MVPA). Research by Bhandari et al. (2020) has shown that Nepali schools often lack the infrastructure and trained personnel to conduct regular physical activity sessions, which may affect the consistency and quality of engagement.

Correlation Between Physical Activity and Diet: A significant positive correlation ($p < 0.05$) between physical activity and healthy dietary habits observed in this study aligns with findings from global literature. For instance, Janssen & LeBlanc (2010) noted that adolescents who are physically active tend to exhibit other favorable health behaviors, including better diet quality, reduced sedentary time, and improved psychosocial functioning.

The current findings strengthen the argument that physical activity may act as a behavioral gateway, encouraging adolescents to adopt a cluster of health-enhancing behaviors. This holistic perspective is vital in adolescent health interventions, especially in school settings where such patterns can be positively reinforced.

When contextualized within the broader South Asian region, Nepalese adolescents in this study appear to fare better in terms of fruit and vegetable intake than their peers in India, Bangladesh, and Sri Lanka. However, the convergence of high fast food and snack consumption rates across the region signifies a shared public health challenge driven by globalization, poor dietary literacy, and aggressive marketing of unhealthy foods.

The high physical activity participation rate is a unique strength observed in this cohort, yet structural limitations such as absence of dedicated sports periods, lack of equipment, and academic pressure remain persistent barriers in the region (Perera et al., 2021; Bhandari et al., 2020).

This study has a few limitations. Since the data were self-reported, students might not have remembered everything accurately or may have answered in a socially acceptable way. The study was cross-sectional, so it shows associations but not cause-and-effect. Physical activity was not measured by time or intensity, which limits understanding of its real impact. Also, the study was done only in Kathmandu, so the results may not apply to students in other parts of Nepal. Lastly, important factors like screen time, sleep habits, and family income were not included.

This study found that while many high school students in Kathmandu eat fruits and vegetables and are physically active, unhealthy habits like frequent fast food and snack consumption are still common. A positive link between physical activity and healthy eating suggests that encouraging one may improve the other. These results highlight the need for school-based programs that promote

better nutrition and regular physical activity to support adolescent health.

Future research should include larger and more diverse samples from both urban and rural areas to improve generalizability. Studies using objective tools to measure physical activity and detailed dietary intake, including portion size and timing, are recommended. Longitudinal designs could help establish cause-and-effect relationships and track behavior changes over time. Exploring additional factors like screen time, sleep patterns, and family lifestyle would also provide a more complete picture of adolescent health.

Conflict of Interest: None

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Effects of Glycerol and Yeast on the Biosynthesis of Chlorophyll in Mixed Algal Cultures

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ABSTRACT

The present study examined the effect of varying glycerol concentrations on chlorophyll production in a mixed culture of green algae *Chlorella vulgaris* and yeast *Saccharomyces cerevisiae*. Five treatments, including control, yeast-only, and yeast with 2, 5, and 10 g/L glycerol, were assessed over 14 days. Over two weeks, we measured the levels of chlorophyll a, b, and c. Here found that adding yeast and a moderate amount of glycerol (especially 5 g/L) led to a marked increase in chlorophyll production. However, too much glycerol (10 g/L) reduced chlorophyll levels, probably because it stressed the cells. Overall, the results show that under the right conditions, combining algae and yeast with a balanced amount of glycerol can boost chlorophyll production—useful for making biofuels or health supplements. This study gives us the insight that co-cultivation of *Chlorella vulgaris* with S*accharomyces cerevisiae* under mixotrophic conditions can significantly enhance the chlorophyll biosynthesis, particularly when it is supplemented with moderate glycerol concentrations. The combination of yeast with 5 g/L glycerol yielded the highest chlorophyll a, b, and c levels, indicating a synergistic effect that optimizes pigment production. The significance of yeast in this process is that it has contributed essential micronutrients and CO2, while glycerol served as a bioavailable carbon source that complemented photosynthetic metabolism. Future work should explore the strategies related to dynamic feeding and metabolic profiling to optimize the balance of nutrients further and assess the scalability of this approach in photobioreactor systems.

KEY WORDS: CHLOROPHYLL BIOSYNTHESIS, *CHLORELLA VULGARIS*, GLYCEROL SUPPLEMENTATION, MIXOTROPHIC CULTIVATION, *SACCHAROMYCES CEREVISIAE*, SYNERGISTIC INTERACTIONS

INTRODUCTION

Microalgae are gaining attention in biotechnology because they grow quickly, use sunlight efficiently, and can produce valuable substances like proteins, fats, and natural pigments (Li et al, 2008). One way to boost their growth and pigment production is by using mixotrophic cultivation. This method combines regular photosynthesis (using sunlight) with an additional carbon source—like a sugar or alcohol—to help algae grow even when light isn't ideal (Cheirsilp and Torpee, 2012). Chlorophyll, the green pigment algae use for photosynthesis, is a key indicator of how healthy and productive the algae are. The more chlorophyll they produce, the better their photosynthetic activity and overall biomass. This is important for industries like biofuel production, wastewater treatment, and pharmaceuticals, which rely on efficient microalgae systems (Becker, 2007 Rincon et al 2024).

Glycerol, a non-toxic and affordable compound, is often used in these systems because it works well with both algae and yeast (Yoo et al, 2010).Co-cultivating yeast and microalgae has been investigated recently as a way to increase biomass output and nutrient utilisation. By releasing CO_2 and organic compounds, yeast species like *Saccharomyces cerevisiae* and *Rhodotorula glutinis* can produce a favourable microenvironment that promotes algal growth and pigment production (Yan et al., 2020 Ori et al 2024).

By boosting photosynthetic activity and decreasing photoinhibition, this mutualistic interaction in mixotrophic systems can increase chlorophyll content, particularly when combined with glycerol supplementation (Gupta et al.,

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2016). Despite these benefits, few studies have thoroughly examined how different glycerol concentrations affect the estimate of chlorophyll in mixed cultures of yeast and microalgae grown under mixotrophic conditions. Optimising the metabolic pathways linked to chlorophyll production and raising algal-yeast consortia productivity requires an understanding of this interaction.

Thus, the goal of this work is to examine how varying glycerol concentrations affect the amount of chlorophyll in a mixotrophic co-culture of *Saccharomyces cerevisiae* and *Chlorella vulgaris* in order to provide light on their potential for industrial synergy.

MATERIAL AND METHODS

Microorganisms and Culture Conditions: The green microalga *Chlorella vulgaris* was collected from a local freshwater pond. To isolate it, the water sample was diluted several times and then spread on a nutrient-rich N-11 agar plate, following a method described by Stanier and colleagues. The identity of the algae was confirmed by looking at its unique colony shape and structure under a microscope.

At the same time, *Saccharomyces cerevisiae* (a type of yeast) was isolated from naturally fermented fruits using YPD agar, based on the methods of Fleet and others. Both the algae and yeast were kept in pure form and regularly transferred to fresh nutrient slants every two weeks to keep them healthy and free from contamination.

Mixotrophic Cultivation Setup: To study how glycerol affects growth and chlorophyll production, *Saccharomyces cerevisiae* (yeast) and *Chlorella vulgaris* (algae) were grown together in a lab experiment. They were cultured in clean 250 mL glass flasks, each containing 100 mL of a nutrient solution called BG-11. Glycerol was added in three different amounts: 2, 5, and 10 grams per liter. Each flask was started with the same amount of cells, adjusted to an optical density (OD_{680}) of 0.5, which measures how concentrated the culture is. The flasks were then kept under controlled conditions for 14 days—at a temperature of about 25°C, with constant light, and shaking at 120 rpm to keep everything well-mixed and oxygenated.

Biomass Measurement: To monitor how much the algae and yeast were growing, the optical density (OD_{680}) was measured every 48 hours. This reading gives an idea of how dense the culture is. Additionally, to measure actual biomass, 10 mL samples were taken, filtered through special pre-weighed filters (Whatman GF/C), and then dried at 60°C until they reached a constant weight. This helped determine how much solid material (biomass) was present..

Chlorophyll Estimation: Chlorophyll extraction and quantification were performed following the method described by Lichtenthaler (1987). A 10 mL aliquot of the algal culture was centrifuged at 5,000 rpm for 10 minutes. The resulting pellet was resuspended in 5 mL of 80% acetone and incubated in the dark at 4°C for 24 hours to ensure complete pigment extraction. The absorbance of the chlorophyll extract was then measured at wavelengths of 645 nm and 663 nm using a spectrophotometer. Chlorophyll concentrations were calculated using the following formulas:

Chlorophyll a (µg/mL) = 12.7 × A663 – 2.69 × A645
Chlorophyll b (µg/mL) = 22.9 × A645 – 4.68 × A663 Total chlorophyll (µg/mL) = Chlorophyll a + Chlorophyll b

Statistical Analysis: All experiments were conducted in triplicate, and the resulting data were statistically evaluated using one-way ANOVA followed by Tukey's post-hoc test. Graph Pad Prism version 9 was used for the analysis, and differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

This study investigated the influence of yeast and glycerol supplementation on the accumulation of chlorophyll a, b, and c in *Chlorella vulgaris* over 14 days. Pigment accumulation was measured under five treatments: a control (photoautotrophic), yeast-supplemented, and yeast combined with glycerol at 2 g/L, 5 g/L, and 10 g/L. Results were analyzed across seven sampling days (0, 2, 4, 6, 8, 10, 12, and 14), revealing treatment-dependent and time-dependent variations in pigment biosynthesis.

Trends in Chlorophyll a, b, and c Accumulation: In the control group, chlorophyll a increased steadily from 0 µg/L on Day 0 to 21.5 µg/L on Day 14, while chlorophyll b and c rose to 13.1 µg/L and 8.9 µg/L, respectively. These values align with those reported by Xiong et al. (2008), who found chlorophyll a content ranging from 20–22 µg/L in *Chlorella protothecoides* under photoautotrophic conditions. The typical sigmoidal curve observed in all pigments reflects distinct growth phases—lag (Day 0–2), exponential (Day 2–10), and saturation (Day 10–14).

Impact of Yeast Supplementation: When yeast extract was introduced, chlorophyll a rose to 23.8 µg/L, while b and c increased to 14.5 µg/L and 11.3 µg/L by Day 14. Yeast is rich in amino acids, peptides, and B-vitamins, which promote enzymatic pathways critical for chlorophyll biosynthesis (Song et al., 2011). It likely enhanced glutamate and magnesium availability, feeding into the tetrapyrrole pathway. This nutrient stimulation enhances chloroplast development and thylakoid density, which is consistent with findings from mixotrophic experiments using brewer's yeast as a co-substrate (Clemson University, 2020).

Glycerol + Yeast: Enhancing Pigment Accumulation

2 g/L Glycerol + Yeast: At 2 g/L glycerol, chlorophyll a reached 25.6 µg/L, with b at 16.0 µg/L and c at 12.7 µg/L. These values reflect the efficiency of low-level mixotrophy. Glycerol acts as a reduced carbon source that enters glycolysis and promotes ATP and NADPH generation, supplementing phototrophic energy needs (Lin, 2017). Song et al. (2011) showed similar improvements in pigment levels when Chlorella pyrenoidosa was supplemented with 1.5–2.5 mg/mL glycerol and glucose.

Figure 1: Chlorophyll a accumulation in *Chlorella vulgaris* under mixotrophic treatments over 14 days.

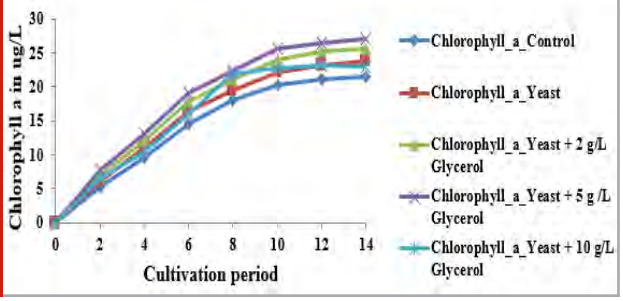


Figure 2: Chlorophyll b accumulation in *Chlorella vulgaris* under mixotrophic treatments over 14 days.

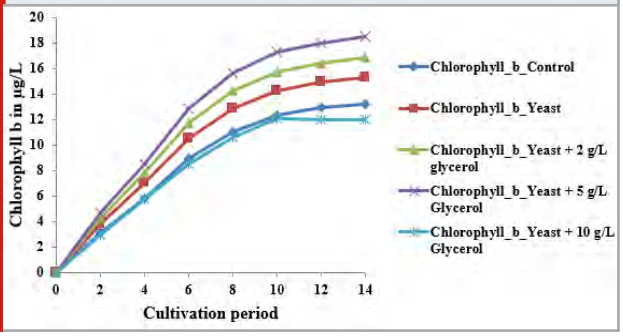
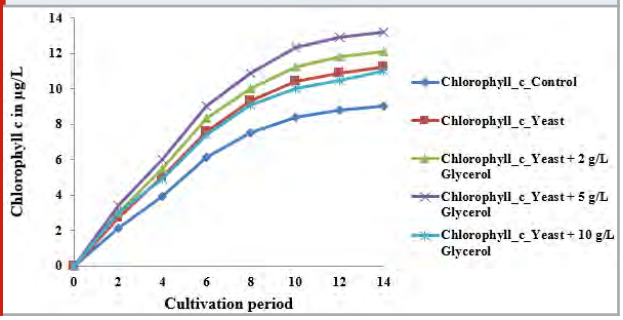


Figure 3: Chlorophyll c accumulation in *Chlorella vulgaris* under mixotrophic treatments over 14 days.



2.5 g/L Glycerol + Yeast: The maximum pigment concentrations were recorded under this treatment: chlorophyll a at 27.0 µg/L, b at 17.8 µg/L, and cat 13.8 µg/L. According to Rincon et al. (2024), mixotrophic cultivation using optimized glycerol concentrations around 5 mg/mL yields the highest biomass and pigment productivity. This condition maintains osmotic balance, enhances redox cycling, and prolongs exponential growth. The co-availability of organic and inorganic carbon also supports sustained chloroplast proliferation and pigment biosynthesis.

10 g/L Glycerol + Yeast: A slight decline in pigment concentration was observed at 10 g/L glycerol, chlorophyll a was 22.9 µg/L, b 13.0 µg/L, and c 11.2 µg/L. The reduced pigment biosynthesis at this level is likely due to substrate inhibition or osmotic stress. According to Xiong et al. (2008), glycerol concentrations above 8 mg/mL may suppress pigment gene expression and cause redirection of metabolic flux toward storage lipids rather than pigments. These findings highlight that while low to moderate glycerol concentrations enhance pigment biosynthesis under mixotrophy, high concentrations may lead to oxidative stress and metabolic diversion.

The results of one-way ANOVA for chlorophyll a across all treatment groups—including control, yeast-supplemented, and glycerol-amended cultures (2 g/L, 5 g/L, and 10 g/L)—revealed F-values ranging from 0.00007 to 0.002, with corresponding p-values exceeding 0.99. These exceptionally high p-values indicate that there were no statistically significant differences among the triplicate measurements within each treatment, demonstrating a high degree of repeatability and precision in chlorophyll a quantification.

Similarly, chlorophyll b analysis yielded F-values between 0.00081 and 0.0066, accompanied by p-values consistently greater than 0.993. This uniformity among replicates across all groups further supports the reproducibility and accuracy of the chlorophyll b extraction and measurement procedures.

In the case of chlorophyll c, although the F-values were slightly higher—ranging from 0.0008 to 0.0732—the p-values remained above 0.92 for all treatments. Despite chlorophyll c being generally more sensitive due to its typically lower concentrations and susceptibility to extraction inconsistencies, the results still confirm acceptable consistency and statistical robustness in its estimation.

These findings corroborate well with multiple peer-reviewed studies (Lv et al., 2010; Safi et al., 2014; Parmar et al., 2011), which emphasize that statistically insignificant replicate variance is an essential prerequisite for deriving biologically meaningful differences between treatment conditions.However, excessive glycerol (10 g/L) posed a hindrance to the process, possibly due to osmotic stress or metabolic imbalance, aligning with previous findings on substrate inhibition in algal cultures (Xiong et al., 2008; Rincon et al.,2024).

The statistical correctness and high repeatability of chlorophyll measurements show that the experimental setup is reliable, and it also reinforces the potential scalability of this cultivation strategy. These results show the importance of carefully balancing nutrient inputs in mixotrophic systems to avoid metabolic trade-offs. The results not only help in the advanced understanding of algal-yeast interactions but also highlight a promising approach for enhancing pigment yield in microbial yield in microalgal biotechnology, with implications for biofuel, food, and nutraceutical applications (Li et al., 2008; Gupta et al., 2016; Lv et al., 2010, Ori et al 2024).

CONCLUSION

This study gives us the insight that co-cultivation of *Chlorella*

vulgaris with *Saccharomyces cerevisiae* under mixotrophic conditions can significantly enhance the chlorophyll biosynthesis, particularly when it is supplemented with moderate glycerol concentrations. The combination of yeast with 5 g/L glycerol yielded the highest chlorophyll a, b, and c levels, indicating a synergistic effect that optimizes pigment production. The significance of yeast in this process is that it has contributed essential micronutrients and CO2, while glycerol served as a bioavailable carbon source that complemented photosynthetic metabolism. Future work should explore the strategies related to dynamic feeding and metabolic profiling to optimize the balance of nutrients further and assess the scalability of this approach in photobioreactor systems.

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Conflict of interest: The authors declare no conflict of interest

Data Availability: Data are available with the corresponding author

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Kinetic Analysis of Immobilized L-asparaginase from *Aspergillus flavus*

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ABSTRACT
The search for other asparaginase sources like eukaryotic microorganisms can lead to an enzyme with less adverse effects. It has been observed that eukaryotic microorganisms like yeast and filamentous fungi have a potential for asparaginase production. L-asparaginase is generally used as a therapeutic agent in the treatment of cancer. It is commonly produced by bacteria. Analysis of Immobilized L-asparaginase kinetics prepared from fungi *Aspergillus flavus*. In this study, L-asparaginase from the fungus *Aspergillus flavus* was partially purified by a step purification method and then immobilized. The enzyme showed about 7.57-fold purification and 19.55% recovery. The kinetic analysis of the immobilized enzyme showed optimum activity at 7.0 pH and 40 °C. Km value for free L-asparaginase was 5×10-6 M, while that for the immobilized one was 3.33×10^{-6}M. The enzyme is more stable at neutral pH than at acidic and retains 85% of its activity after 53 days of storage. The findings of the present study also extend the idea of finding L-asparaginase potential in fungi and their application in translational medicine. As mentioned earlier, fungal asparaginase may be superior in comparison to bacterial enzyme, due to reduced adverse reactions associated with fungal enzyme, so the present study demands legitimate appraisal to unravel the biotechnological potential of fungal L-asparaginase and its immobilization.
The immobilized enzyme can be an attractive candidate for biotechnological applications, including the management of cancer.

KEY WORDS: *ASPERGILLUS FLAVUS,* L-ASPARAGINASE, ANTICANCER, IMMOBILIZATION, KINETICS.

INTRODUCTION

Anti-tumor enzyme L-asparaginase (L-asparagine aminohydrolase EC 3.5.1.1) produces L-aspartate and ammonia by hydrolysis of L-asparagine (Asselin et al., 1991). L-asparaginase is generally used as therapeutic agent in treatment of acute lymphoblastic lymphoma and other related malignancies (Capizzi et al., 1970). This enzyme reduces the concentration of intracellular L-asparagine, since tumor cells are unable to synthesize this amino acid and are selectively killed due to L-asparagine deprivation. The enzyme has been isolated from variety of microorganisms like bacteria, fungi, algae, plants and animals (Wriston and Yellin, 1973). The enzymes isolated from *E. coli* and *Erwinia carotovora* are being commercially used in treatment of acute lymphoblastic leukaemia (Capizzi, 1993), despite the fact that bacteria are also positively associated with cancer (Khan and Shrivastava, 2010). Prolong administration of bacterial L-asparaginase can cause hypersensitivity in the patients, leading to allergic reactions and anaphylaxis (Reynolds and Taylor, 1993, Earl, 2009, Shrivastava et al., 2010, Shrivastava et al., 2012a Duarte et al 2024). The corresponding antibodies produced cause an anaphylactic shock or neutralization of the drug effect.

The search for other asparaginase sources like eukaryotic microorganisms can lead to an enzyme with less adverse effects. It has been observed that eukaryotic microorganisms like yeast and filamentous fungi have a potential for asparaginase production (Wade et al., 1971, Wiame et al., 1985, Pinheiro et al., 2001, Sarquis et al., 2004 Duarte 2024). The mitosporic fungal genera such as *Aspergillus, Penicillium* and *Fusarium* are commonly reported in scientific literature to produce asparaginase (Shrivastava et al., 2012b, Curran et al., 1985 Duarte et al 2024) and

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require legitimate appraisal for their asparaginase potential. The present paper reports kinetic analysis of immobilized L-asparaginase from *Aspergillus flavus.*

MATERIAL AND METHODS

Microorganism: *Aspergillus flavus* was obtained from the Department of Biotechnology, College of Life Science, Cancer Hospital and Research Institute, Gwalior (M.P.), India. It was maintained on Potato Dextrose Agar (PDA) [containing (gL-1) potato starch 200, glucose 20, agar 17, distilled water; pH 7.2] at 40C.

Production of L-asparaginase: Fungus was cultured on PDA petri plates for 96 hours at 37^{0}C and its newly growing mycelia was transferred on modified Czapek-Dox's medium [Composition (gL^{-1}): glucose 2, L-asparagine 10, KH2PO4 1.52, KCl 0.52, $MgS0_4.7H_2O$ 0.52, $CuN0_3.3H_2O$ trace, $ZnS0_4.7H_2O$ trace, $FeS0_4.7H_2O$ trace, agar 20; pH 6.2] and incubated in shaker incubator for 96 hours at 37^{0}C. The culture was filtered by Whatmann no.1 in line filter holder (pore size – 45 mm) and the supernatant (i.e. mycelia free broth) was used as a crude extract for assaying L-asparaginase activity.

Assay of L-asparaginase: Enzyme activity was estimated by Nesslerization method (Bilimoria, 1969). The reaction mixture contained 0.2 mM of Sodium acetate buffer (pH 5.2), 20 µM of L-asparagine and suitable dilution of enzyme extract. The mixture was incubated for the 15 minute at 37^{0}C. the reaction was stopped by addition of 0.1 ml of 15% trichloroacetic acid (TCA) and finally was centrifuged at 6000g for 10 minute. The resulting supernatant (0.5 ml) was dissolved in 4 ml ammonia-free distilled water and treated with 1 ml Nessler's reagent (1N). The liberation of ammonia was determined at 450 nm in spectrophotometer. The ammonia content was estimated by a standard curve prepared from various dilutions of 4 mM Ammonium chloride solution.

$$\text{Relative activity (\%)} = \frac{\text{L-asparatinase activity}}{\text{Maximum L-asparatinase activity}} \times 100$$

Total protein content was estimated according to Lowry's method with BSA as standard (Lowry et al., 1951).

Purification of L-asparaginase: The purification was carried out using crude enzyme extract at 0-4^{0}C, unless otherwise mentioned. Finely powdered ammonium sulphate was added to the crude extract at the rate of 1 g minute-1. This solution was kept for overnight at 4^{0}C. L-asparaginase activity was associated with the fraction precipitated at 70-80 % saturation. The protein precipitate was collected by centrifugation at 15,000 g for 15 minutes. The supernatant was discarded and the pellet was dissolved in 500 µl Phosphate Buffer Saline (PBS). The resulting purified asparaginase preparation was stored in refrigerator and used for further experiments. Desalting of precipitated protein was performed by gel filtration chromatography with Sephadex G-25 in 0.05 M PBS buffer at pH 7.4. This desalted protein was further passed through Sephadex G-100 columns (1×10 cm) in the same buffer. The protein was eluted with the same buffer at a flow rate of 5 ml 5 min-1. L-asparaginase activity and total protein concentration was estimated at each step of purification process.

Immobilization of L-asparaginase in Calcium alginate complex: Immobilization of enzyme was performed as per the method of Yousuff and Al-Omair, 2008. Combination of Calcium and Sodium alginate mixture were prepared by adding 3 gm sodium alginate in 100 ml distilled water and autoclaved for 15 minute at 120^{0}C. Then 3 ml of purified enzyme was mixed with 8 ml sodium alginate and stirred for 15 minutes for proper mixing. 1 ml glutaraldehyde was mixed in this solution and the resultant slurry was transferred to a dropping funnel with plastic tip to fall on 7% cold $CaCl_2$ dropwise. This chilled $CaCl_2$ solution was used for enzyme immobilization and led to formation of enzyme beads. These beads were left in $CaCl_2$ solution for 20 minutes for hardening. Beads were collected after decanting supernatant and washed with distilled water and stored in refrigerator until further use.

Kinetic study of immobilized L-asparaginase

***Effect of substrate concentration on the free and immobilized enzyme*:** Varying substrate concentration ranging from 1.25, 2.5, 5.0, 10 and 20 µM were used and effect on activity of immobilized and free enzyme was observed through Nesslerization reaction (Bilimoria, 1969).

Effect of temperature on free and immobilized L-asparaginase: Temperature profile of L-asparaginase was determined by performing enzymatic assay by previous method at various temperatures 5, 10, 20, 30, 40, 50 and 600C.

Effect of incubation time on free and immobilized L-asparaginase: Incubation periods of routine enzymatic assay were altered for 5, 10, 15, 20, 25 minutes.

Effect of pH on free and immobilized enzyme: The optimum pH for activity of free and Immobilized L-asparaginase was determined by 0.2 mM Sodium acetate buffer at pH ranging from 4, 5, 6, 7, 8, 9.

Thermodynamic Properties of immobilized L-asparaginase: Thermodynamic parameters of immobilized L-asparaginase including energy of activation (Ea), enthalpy of activation (ΔH), entropy of activation (ΔS) were calculated by standard methods ADDIN EN.CITE (. The activation energy of the reaction catalyzed by enzyme was determined from equation no.1,

$$Ea = - RT \ln K \qquad (1)$$

Where R is gas constant and T is absolute temperature in kelvin.

The Gibbs free energy (ΔG) was estimated using the equation no.2,

$$\Delta G = \Delta H - T\Delta S \qquad (2)$$

Where, ΔH and ΔS are the enthalpy and entropy, respectively, which were determined using equation no.3,

$$\ln K = \Delta H/RT - \Delta S/R \qquad (3)$$

Plot of lnK against 1/T gave a straight line whose slope was ΔH/RT, and the intercept was ΔS/R.

RESULTS

Purification of L- asparaginase from *A. flavus* was performed. The percentage yield and enzyme activity was observed at every step of purification. The data for purification of L-asparaginase is mentioned in following manner (Table 1).

Effect of different parameters on kinetic activity of free and immobilized enzyme is presented in Figure 1-4. Functional stability of L-asparaginase was also measured during the study. L-asparaginase activity was found 14.98 IU/ ml at the time of bead preparation, while the reproducibility of average activity was maintained despite significant storage with 12.84 IU/ ml after 53 days of bead preparation. Thus, immobilized enzyme maintained about 86% of its activity in comparison to its initial status.

Table 1. Purification of L-asparaginase from *Aspergillus flavus*

Fraction	Volume	Total Enzyme Activity ($IU.ml^{-1}$)*	Total Protein concentration ($mg.ml^{-1}$)	Specific Activity (IU.mg-1)**	Purification Fold §	¶Yield (%)
Crude	28 ml	105.84	2.03	52.13	1	100
Ammonium Sulphate	23 ml	40.04	15.07	2.65	0.05	37.80
G-25	1 ml	31.70	0.02	14.80	0.28	29.95
G-100	1 ml	20.70	0.05	394.66	7.57	19.55

* Enzyme Activity: Enzyme activity is defined as 1µM ammonia released per ml in one minute.
** Specific Activity: 1µM ammonia released per minute per mg of protein.
§ Purification fold: Specific activity of fraction/specific activity of homogenate
¶ Yield (%): Total activity of fraction/Total activity of homogenate X 100

Figure 1: LineweaverBurk plot for L-asparaginase from Aspergillus flavus. Km values were found as 5×10-6 M and 3.33×10-6 M for free and immobilized L-asparaginase, respectively.

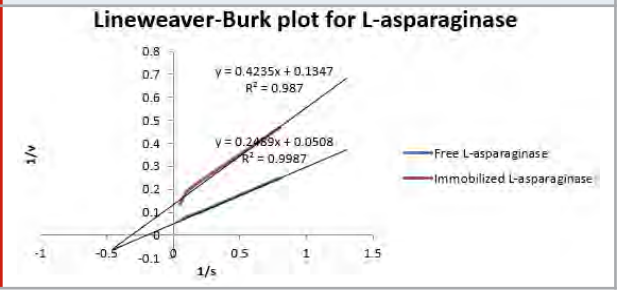

Figure 2: Effect of temperature on relative activity of free and immobilized L-asparaginase from *Aspergillus flavus*.

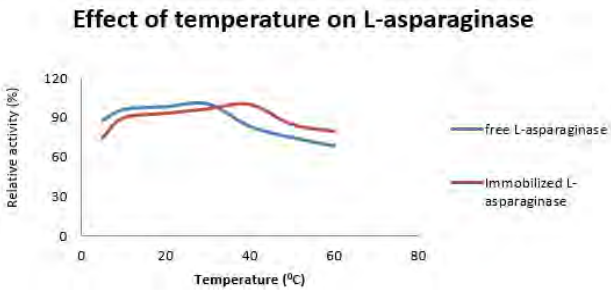

Figure 3: Effect of pH on relative activity of immobilized and free L-asparaginase from *Aspergillus flavus*.

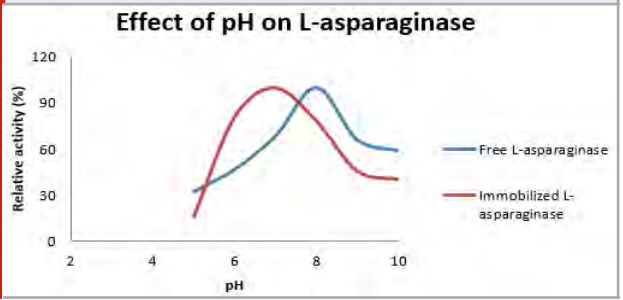

Figure 4: Effect of time on relative activity of free and immobilized L-asparaginase from *Aspergillus flavus*.

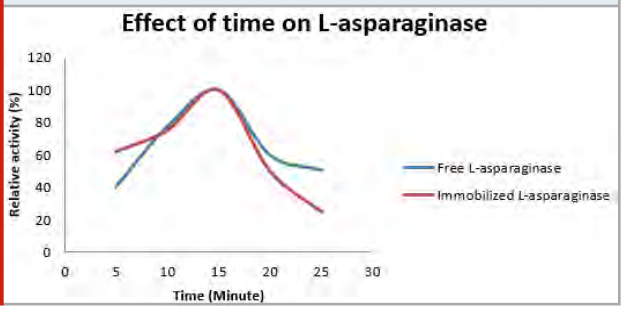

Table 2. Thermodynamic properties of immobilized L-asparaginase

Sr. No.	Thermodynamic parameter	Value
1	Activation energy (Ea)	5.13 KJ/ M
2	Enthalpy of activation (ΔH)	2.52 KJ/ M
3	Entropy of activation (ΔS)	5.09 KJ/ K/M

Thermodynamic parameters: Thermodynamic parameters of immobilized enzyme were estimated (Zeng et al., 2009, Pogaku et al., 2012).

DISCUSSION

Bacterial L-asparaginases show many side effects and allergic reactions. An attempt was made to partially purify L-asparaginase from A. flavus which is eukaryotic in nature. L-asparaginase production from A. flavus was already reported by researchers (Soniamby et al., 2011). Two-step purification through ammonium sulphate precipitation followed by Sephadex G-100 gel filtration chromatography was performed (Table 1). Purification fold was 7.57 and total yield was 19.55%. This purification fold was lower in comparison to some previous studies (Oza et al., 2009).

Purification was followed by immobilization and kinetic studies of immobilized and free enzyme. Immobilization of the enzyme increased several beneficial kinetic parameters, including thermal stability, reduced Km, and increased specific activity. Surprisingly, the kinetic study indicated that Km value of the immobilized bead was 1.5 times lower compared to the free enzyme. Low Km value is related to its degree of effectiveness against tumors (Schwartz et al., 1966). Therefore, it can be assumed that immobilized A. flavus asparaginase will be superior in efficacy against tumors in comparison to free enzyme (Figure 1). Although, some other studies also showed that Km value decreases with immobilization in some enzymes (Ertan et al., 2006).

During analysis of optimum temperature for enzyme activity, it was observed that optimum temperature was 40 0C and 20 0C for immobilized and free enzyme respectively. Another important serendipitous observation was 4.8 fold increased specific activity of immobilized enzyme at 40 0C. Effect of temperature also showed that immobilization increases the thermal stability of asparaginase (Figure 2). These results are in accordance with other studies performed on different enzymes where immobilization increases stability of enzyme (Mateo et al., 2000, Grazu et al., 2005). Furthermore, study of pH indicated that pH 7 was optimum for immobilized enzyme in compare to free enzyme which showed maximum activity at pH 8. Similar to increase in L-asparaginase specific activity with temperature after immobilization, immobilization also increased specific activity with pH by 1.73 fold (Figure 3).

Increase in optimum pH, temperature for maximum activity was also observed in other study analyzing immobilization of oxalate oxidase enzyme (Pundir et al., 1999). Increase in specific activity of immobilized asparaginase suggests favorable confirmation changes after immobilization leading to increase in specific activity of enzyme as evidenced in other studies (Chen et al., 2009). Alteration in kinetic parameters of immobilized enzyme in comparison to free enzymes are reported probably due to structural modifications in active sites resulted through this process (Liburdi et al., 2012) Although immobilization affects kinetic parameters of enzymes, but we did not find any difference in optimum incubation temperature for enzyme activity. During the study, 15 minute was the optimum incubation time for both free and immobilized enzyme (Figure 4).

An important objective of enzyme immobilization is to make it as a reusable biocatalyst. This situation demands long term stability of immobilized enzyme. Our immobilized enzyme maintained 85% of initial enzyme activity despite 53 days of storage. This observation further appraises superiority of A. flavus immobilized enzyme in comparison to free L-asparaginase. Although this study gives significant insights towards potential of A. flavus asparaginase and its immobilized derivative, but proper and careful studies are required for application of this enzyme.

The thermodynamic parameters of immobilized L-asparaginase were evaluated to understand catalytic activity of enzyme. This study of asparaginase catalyzed reactions was a favorable reaction in direction of product formation and could be helpful in its industrial scale production (Table 2). This study also extends the idea of finding L-asparaginase potential in fungi and their application in translational medicine. As mentioned earlier, that fungal asparaginase may be superior in comparison to bacterial enzyme, due to reduced adverse reactions associated with fungal enzyme, so present study demand legitimate appraisal to unravel biotechnological potential of fungal L-asparaginase and its immobilization.

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Production, Characterization Purification, and Antitumor Activity of L-Asparaginase from *Aspergillus niger*

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Evaluating KIM-1 as a Biomarker for Arsenic-Induced Nephrotoxicity: A Cytotoxic and Molecular Approach

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ABSTRACT

Arsenic contamination is a major global health concern, primarily due to its presence in drinking water from natural and industrial sources. Chronic arsenic exposure is associated with severe toxicity, particularly affecting the kidneys, which serve as a major site for arsenic accumulation and excretion. While the nephrotoxic effects of heavy metals such as cadmium, lead, and mercury are well-documented, arsenic-induced kidney toxicity remains less understood. Kidney Injury Molecule-1 (KIM-1) is a highly specific and non-invasive biomarker for renal injury, making it a valuable tool for assessing arsenic-induced nephrotoxicity. This study investigates the toxic effects of sodium meta-arsenate on human kidney ACHN cells by evaluating cytotoxicity and relative KIM-1 gene expression. ACHN cells were treated with 20 µM and 30 µM sodium meta-arsenate for 24 hours. Cytotoxicity was assessed microscopically and spectrophotometrically using the Crystal Violet assay, while KIM-1 gene expression was analyzed to determine arsenic-induced renal injury. The results demonstrated cytopathic effects in arsenic-treated cells. Gene expression analysis revealed that arsenic exerts a dose-dependent toxic effect on human kidney cells, as reflected by cytotoxicity and alterations in KIM-1 expression. This study highlights the significance of KIM-1 as a potential biomarker for arsenic-related kidney damage and underscores the need for further research into the molecular mechanisms underlying arsenic toxicity.

KEY WORDS: EVALUATING KIM-1; ARSENIC CONTAMINATION; KIDNEY INJURY; CRYSTAL VIOLET ASSAY.

INTRODUCTION

Arsenic contamination remains a critical global health concern, primarily due to its widespread presence in groundwater used for drinking and agriculture (Levin et al., 2025; Rai et al., 2023). Chronic exposure to inorganic arsenic, particularly through contaminated water sources, has been linked to a spectrum of adverse health outcomes, including carcinogenesis, cardiovascular disease, neurotoxicity, and nephrotoxicity (Ganie et al., 2023; Joardar et al., 2021). The kidneys, as primary organs for arsenic accumulation and excretion, are especially vulnerable to its toxic effects (Khaleda et al., 2025; Liu et al., 2021). While the nephrotoxic profiles of heavy metals such as cadmium, lead, and mercury have been extensively characterized, the molecular mechanisms underlying arsenic-induced renal injury remain comparatively underexplored (Mishra et al., 2022).

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Previous studies also suggests that arsenic compounds disrupt mitochondrial function, induce oxidative stress, and impair autophagic and mitophagic pathways in renal tubular cells (Wan et al., 2021; Li et al., 2022). However, the identification of sensitive and specific biomarkers for early detection of arsenic-induced nephrotoxicity is still evolving. Traditional markers such as serum creatinine and blood urea nitrogen (BUN) often fail to detect subclinical or early-stage renal damage (Griffin et al., 2019; Vaidya et al., 2008).

Kidney Injury Molecule-1 (KIM-1) has emerged as a promising biomarker for proximal tubular injury due to its high specificity and early upregulation in response to nephrotoxic insults (Ichimura et al., 2004; Jana et al., 2022). KIM-1 is minimally expressed in healthy renal tissue but is markedly elevated in both urine and tissue following toxic or ischemic injury. Elevated urinary KIM-1 levels have been reported in populations exposed to environmental arsenic, suggesting its potential utility in detecting early, subclinical renal damage (Karmakova et al., 2021; Baro et al., 2025). Although several in vivo studies have demonstrated arsenic-induced nephrotoxicity and the involvement of

mitochondrial and oxidative stress pathways, there is a lack of in vitro data specifically evaluating the dose-dependent cytotoxicity of sodium meta-arsenate and its impact on KIM-1 gene expression in human renal epithelial cells (Ramadan et al., 2022; Song et al., 2025). Moreover, the mechanistic link between arsenic-induced cellular injury and KIM-1 upregulation remains insufficiently defined in human cell models.

To address this gap, the present study investigates the cytotoxic and molecular effects of sodium meta-arsenate on human renal ACHN cells. By employing both microscopic and spectrophotometric cytotoxicity assays and quantitative gene expression analysis of KIM-1, this study aims to elucidate the dose-dependent relationship between arsenic exposure and renal epithelial injury. The findings are expected to enhance our understanding of arsenic-induced nephrotoxicity and support the validation of KIM-1 as a sensitive and early biomarker for arsenic-related kidney damage. This work may also contribute to the development of improved screening tools for environmental nephrotoxins and inform public health strategies in arsenic-endemic regions.

MATERIAL AND METHODS

Cell Culture: Human renal adenocarcinoma (ACHN) cells, originally derived from a metastatic renal carcinoma, were obtained from the National Centre for Cell Science (NCCS), Pune. Culturing of cells have been done as per the procedure of Pal et al. (2017). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), sodium bicarbonate, and antibiotics. Cultures were grown in a humidified incubator at 37 °C with 5% CO_2 and subcultured twice weekly upon reaching 10–90% confluency.

Cytotoxicity Assay: Cytotoxicity was evaluated using the Crystal Violet assay as per protocol of Saotome et al. (1989). ACHN cells were seeded in 96-well plates and treated with 20 µM and 30 µM sodium meta-arsenate for 24 hours. Following treatment, cells were fixed with methanol and stained with 0.2% crystal violet in 20% methanol. Excess dye was washed off with water, and bound dye was solubilized using Sorenson's buffer. Absorbance was measured at 540 nm using a spectrophotometer to determine cell viability.

RNA Isolation: Total RNA was extracted using TRIzol reagent as per the protocol of Rio et al. (2010). Briefly, cells were homogenized and lysed in TRIzol, followed by chloroform extraction and isopropanol precipitation. The RNA pellet was washed with 75% ethanol, air-dried, and resuspended in DEPC-treated water. RNA quality and concentration were determined spectrophotometrically at A260/280.

cDNA Synthesis and Real-Time RT-PCR: Reverse transcription of RNA into cDNA was performed as per the procedure of Carlini et al. (2010). The reaction included random primers and MultiScribe™ Reverse Transcriptase in a total volume of 20 µL. The thermal profile included incubation at 25 °C for 10 min, 37 °C for 120 min, and 85 °C for 5 min, followed by a hold at 4 °C. Gene expression was quantified by real-time PCR using TaqMan primer probes and master mix on a StepOnePlus™ Real-Time PCR System. GAPDH was used as the endogenous control. The thermal cycling conditions were 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 sec and 60 °C for 1 min. All samples were run in triplicate, and relative expression levels were calculated using the 2^–ΔCT method.

RESULTS

Cytopathic Effect: ACHN cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (FBS). After 24 hours of incubation at 37 °C in a 5% CO2 atmosphere, the cells reached 80% confluence in the culture flask. Following this, the cells were treated with sodium meta-arsenate (NaAsO3) at concentrations of 20 µM and 30 µM, respectively. After another 24 hours, the cells were observed for cytopathic effects under an inverted microscope. In the control, healthy cells were visible. In the cells treated with 20 µM and 30 µM, cytopathic effects were observed. In the 30 µM, most cells were lysed, and the surviving cells appeared rounded and were mostly detached from the surface of the flask. In contrast, the 20 µM treatment showed some cytopathic effects, but to a lesser extent than the 30 µM treatment. Even at 20 µM, the morphology of the cells had altered compared to the control group. The number of living cells was also significantly lower in the 30 µM and 20 µM treated wells compared to the control. Figure 1 illustrates the cell morphology in the control and those treated with 20 µM and 30 µM sodium meta-arsenate, respectively. The arrows indicate the rounded dead cells.

Figure Photomicrographs of ACHN cell lines exposed to Sodium meta-arsenate (NaAsO3): (A) Control; (B) 20µM NaAsO3; (C) 30 µM NaAsO3

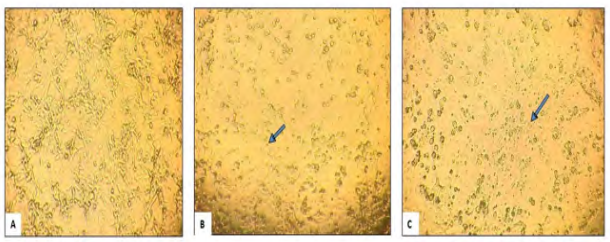
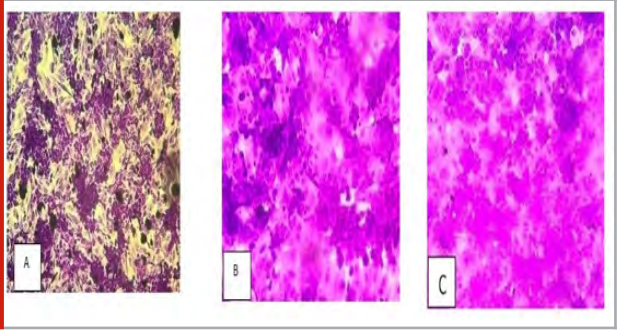


Table 1. Optical density of ACHN cells treated with sodium meta-arsenate ($NaAsO_3$) measured at 540nm: Data are presented as mean ± SEM.*p < 0.001 compared to control.**

S.No.	Concentration of $NaAsO_3$	O.D. at 540 nm Mean± SEM
1.	Control	2.44925±0.437124
2.	20µM	2.2605±0.013211***
3.	30µM	1.832±0.027057***

Crystal Violet Assay for Cell Viability: To determine the cytotoxic effects of sodium meta-arsenate on ACHN cells, a crystal violet staining assay was performed. Following microscopic observation, treated and control cells were stained, and optical density (O.D.) was measured at 540 nm to quantify the number of viable, adherent cells.As shown in Table 1, arsenic treatment significantly reduced cell viability in a dose-dependent manner. The mean O.D. value for the untreated control group was 2.449 ± 0.437, whereas cells treated with 20 µM and 30 µM sodium meta-arsenate showed significantly reduced O.D. values of 2.261 ± 0.013 and 1.832 ± 0.027, respectively ($p < 0.001$ for both compared to control).

Figure2. *Crystal violet-stained* ACHN cells: (A) Control, (B) 20 µM $NaAsO_3$, and (C) 30 µM $NaAsO_3$.Reduced staining indicates decreased cell viability with increasing dose.



These reductions in optical density reflect a significant loss of adherent, viable cells following arsenic exposure. This trend is visually supported by Figure 2, which displays the stained wells: (1) control cells with dense crystal violet staining, (2) cells treated with 30 µM, and (3) cells treated with 20 µM sodium meta-arsenate (NaAsO3), showing noticeably reduced cell density and dye retention. The corresponding Figure 3 illustrates the percentage of cell viability relative to the untreated control group, further confirming the dose-dependent cytotoxic effect of sodium meta-arsenate in treated groups.

Gene expression studies: Quantitative real-time PCR (qRT-PCR) was performed to assess the expression of KIM-1 in ACHN cells following treatment with sodium meta-arsenate (NaAsO3) at concentrations of 20 µM and 30 µM. Expression levels were normalized to GAPDH, and relative fold changes were calculated using the 2^(-ΔΔCt) method with the control group as reference.

As shown in Table 2 and Figure 4, KIM-1 expression increased in a dose-dependent manner in response to NaAsO3 treatment. At 20 µM, the ΔCt value decreased to 10.599, corresponding to a ΔΔCt of –0.8052 and a 1.75-fold increase in KIM-1 expression compared to control. A further decrease in ΔCt was observed at 30 µM (ΔCt = 10.5518), resulting in a ΔΔCt of –0.8524 and a 1.80-fold increase. These results indicate that $NaAsO_2$ induces upregulation of KIM-1 expression in ACHN cells, suggesting a potential role in arsenic-induced cellular response.

Table 2: Relative Expression of KIM-1 Normalized to GAPDH in Response to Sodium Arsenate ($NaAsO_2$) Treatment. ΔCt, ΔΔCt, and fold change were calculated using the 2^(-ΔΔCt) method relative to the control.

Concentrations	GAPDH (Ct)	KIM-1 (Ct)	ΔCt	ΔΔCt	Fold Change
Control	16.6149	28.0191	11.4042	0 (reference)	1
20 µM NaAsO3	14.5119	25.1109	10.599	-0.8052	1.75
30 µM NaAsO3	14.3836	24.9354	10.5518	-0.8524	1.8

Figure 3. Cell viability of ACHN cells after sodium meta-arsenate treatment. ACHN cells were treated with 20 µM and 30 µM $NaAsO_3$ for 24 hours. Cell viability was measured by crystal violet assay and expressed as a percentage of control. Data represent mean ± SEM (*$p < 0.001$ vs. control).**

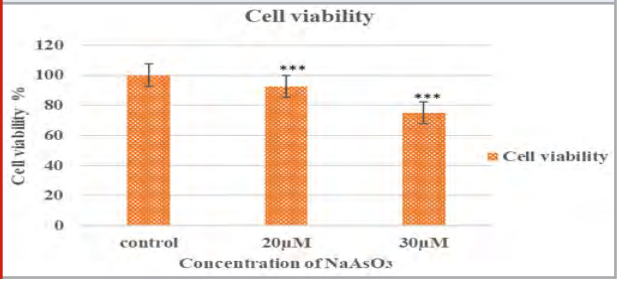
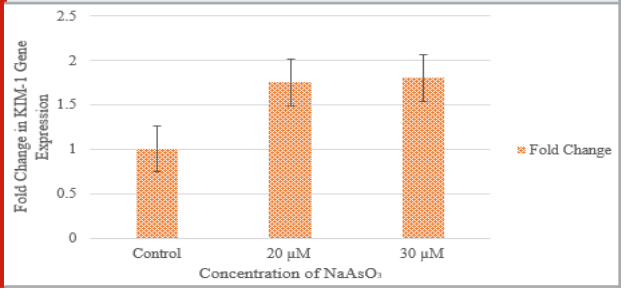


Figure 4: *Effect of sodium meta-arsenate (NaAsO3) on KIM-1 gene expression in ACHN cells.* Concentration v/s Relative fold change in KIM-1 gene expression. Error bars represent standard deviation (n=3).



DISCUSSION

Arsenic, a metalloid, is a naturally occurring environmental contaminant to which humans are frequently exposed through food, water, air, and soil. Approximately 200 million people worldwide have been exposed to high levels of arsenic in groundwater, particularly in regions of Mexico, the United States, and China (Podgorski and Berg, 2020). In some areas, such as parts of Taiwan and Bangladesh, arsenic concentrations have reached as high as 3,000 ppb (IARC, 2012). In India, significant examples of arsenic contamination can be found in Bihar, West Bengal, and various coastal regions, where it is estimated that nearly 230 million people have been drinking water contaminated with arsenic (Mondal et al., 2021; Banerjee et al., 2023; Biswas et al., 2023). The World Health Organization (WHO) recommends a limit of 10 µg/L for arsenic in drinking water (WHO, 2001), yet concentrations ranging from 50 to 3,200 µg/L have been reported (Sadee et al., 2025).

In our research, we investigated the cytotoxicity and molecular effects of sodium meta-arsenate (NaAsO3) on ACHN cells, a human renal carcinoma cell line. Our findings indicate that acute arsenic exposure (over 24 hours) leads to significant cytopathic changes, reduces cell viability in a dose-dependent manner, and upregulates KIM-1, a biomarker associated with early kidney injury and stress response. These results are consistent with those of Wei et al. (2022), who demonstrated that kidney-derived CAK cells from Cromileptes altivelis exhibit dose-dependent cytotoxicity when exposed to heavy metals like mercury, cadmium, and copper. Similarly, our study found that acute arsenic exposure induces notable cytopathic effects and decreases cell viability in a dose-dependent manner.

Additionally, we observed morphological changes in ACHN cells after treatment with NaAsO3, revealing progressive alterations. Cells treated with 20 µM exhibited early signs of cytopathic effects, including partial detachment and rounding. At 30 µM, these effects intensified, leading to widespread cell lysis and loss of adherence, indicating that NaAsO3 induces severe structural damage to renal epithelial cells. These qualitative findings are supported by de Almeida et al. (2023), who reported that alkylphenols caused dose-dependent cytotoxicity and structural damage in RTG-2 cells, marked by compromised membranes and oxidative stress. Notably, the optical density values decreased significantly at both 20 µM and 30 µM concentrations, suggesting substantial cytotoxicity. This aligns with prior studies indicating that arsenic exposure leads to cellular stress, mitochondrial dysfunction, and apoptosis in various renal and epithelial cell types (Cantoni et al., 2022; Liu et al., 2023).

The structural alterations we observed were accompanied by decreased cell viability and upregulation of KIM-1, a marker of early kidney injury. Molecular analysis revealed that KIM-1 expression significantly increased in response to arsenic exposure. The 2^(-ΔΔCt) analysis showed a 1.75-fold increase at 20 µM and a 1.80-fold increase at 30 µM, indicating a dose-responsive activation of this kidney injury marker. Similar mechanisms were noted in the study by Hu et al. (2025), where chromium-induced nephrotoxicity in rats was mediated through oxidative stress, inflammation, and apoptosis, with significant activation of the TLR4/MyD88, HMGB1/RAGE, and NF-κB pathways.

KIM-1 is a well-established biomarker for renal epithelial cell damage, commonly overexpressed during toxic or ischemic renal injury. Its upregulation in our study supports the hypothesis that arsenic induces nephrotoxic stress in ACHN cells and highlights the utility of KIM-1 as an early marker for arsenic-induced renal damage. Interestingly, while the increase in KIM-1 expression between 20 µM and 30 µM was relatively modest, the morphological damage and loss of cell viability were significantly more pronounced at the higher concentration. This suggests that KIM-1 induction may occur early in the injury process, even before extensive cell death, potentially serving as a predictive marker of cytotoxic progression.

It is also possible that beyond a certain threshold, additional molecular pathways, such as oxidative stress, DNA damage, or apoptosis, are activated, contributing to more severe cytopathic outcomes. Overall, our data provide mechanistic insights into the nephrotoxic effects of sodium meta-arsenate, reinforcing its potential to induce renal epithelial injury at sublethal concentrations. These findings are particularly relevant considering the environmental and occupational exposure risks associated with arsenic compounds, especially in regions with contaminated drinking water.

CONCLUSION

Sodium meta-arsenate exposure leads to significant cytotoxic and morphological alterations in ACHN cells, accompanied by dose-dependent upregulation of KIM-These results suggest that KIM-1 may serve as a sensitive biomarker for early detection of arsenic-induced renal injury. Further studies investigating oxidative stress markers, apoptotic pathways, and long-term gene expression responses will enhance our understanding of arsenic toxicity mechanisms in renal tissues.

Data availability: All data generated and analysed during this study are included in this published article.

Conflict of interest: None.

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The Diversity of Ants of Order Hymenoptera, Family Formicidae in Jhabua District of Madhya Pradesh, India

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ABSTRACT

The present communication deals with the diversity of ants within the Jhabua district of Madhya Pradesh, India, as there are no good enough statistics pertaining to the ant diversity of this place. The ants have been sampled by means of hand hand-picking method. The sampled specimens representing three species belonged to 2 genera and 1 subfamily. All through the present take a look at genera Monornorium and Crematogaster had been recorded. The species diversity of the genus Monomorium is better than genus Crematogaster. Ants, belonging to the family Formicidae, are the most diverse group within the order Hymenoptera. They serve as vital ecological indicators and ecosystem engineers, playing key roles in maintaining environmental balance. Ants benefit humans in various ways, including natural pest control by regulating insect populations. This study aims to document the ant species present in the region, offering important insights into their diversity and ecological significance. Overall, the research contributes valuable knowledge to the field of ecology, enhancing our understanding of ant communities and their impact on ecosystems.

KEY WORDS: ANTS, MONOMORIUM, ENVIRONMENTAL EFFECTS, SOCIAL BUGS, JHABUA TRIBAL AREA.

INTRODUCTION

Ants are one of the maximum numerous and ubiquitous groups of social bugs. Ants (Formicidae) are the largest family within the order Hymenoptera. They act as ecological indicators and atmospheric engineers. Ants are critical components of ecosystems not only due to the fact that they represent a high-quality part of the animal biomass but additionally because they act as atmosphere engineers. All the acknowledged species of ants are asocial (Gadagkar et.al. 1993). Ant species can be utilized in monitoring environmental effects, atmospheric investment, and equipment in ecological studies (Andersen 1988). Ant species are used as remarkable signs of land management practices (Bharti and Sharma 2009) and recuperation efforts (Sabu et.al. 2008, Hayarnnisa et al 2024).

Ants are important components of ecosystems not only because they constitute a great part of the animal biomass but also because they act as ecosystem engineers. Every species of ant has an enormous effect on its surroundings. It at once or circuitously impacts the development and destruction of flora and fauna of its surrounding environment. All the known species of ants are eusocial. Ant species can be used in monitoring environmental impacts, ecosystem funding, and tools in ecological studies (Ramesh & Jahir 2010 Hayarnnisa et al 2024).

Ant species are used as brilliant indicators of land management practices and recovery efforts. The food of ants consists of bugs, terrestrial arthropods, excretion from flora, honey dew excreted through aphids and mealy insects, secretion of the caterpillars of their own family Lycaenidae, seeds of plant life, and many other various and ubiquitous businesses of the social insects.

Ants belong to a single massive family, Formicidae, the largest of the order Hymenoptera. It is represented by way of 26 extant subfamilies with 14,711 valid species and 428 valid genera (Bolton 2011). Out of those, 152 species are indexed with the aid of IUCN and from India, 10 subfamilies are represented through 100 genera with 828 species. In India, the Himalaya and the Western Ghats harbor a huge variety of ant species; 656 species from 88 genera have been recorded from the Himalaya, and 455 species from 75 genera have been recorded from the Western Ghats, specifically in Tamil Nadu, 184 species from 51 genera have been recorded.

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They could function as model organisms for exploring the nature and dynamics of ecological groups because of the benefits with which they can be sampled and the potential for experimental manipulation (Bharti 2011).

METHODOLOGY

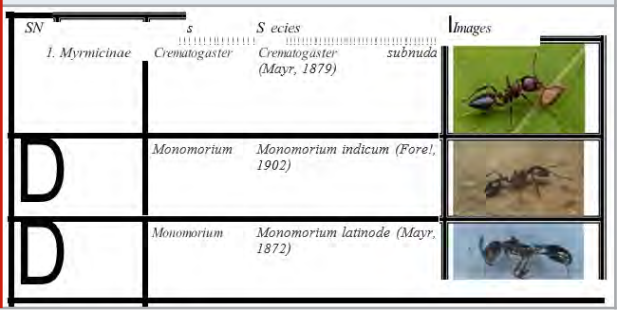
Study area: Jhabua (22°14-23°49-N Latitude, 73°30-75°42 Longitude) has a total area of 6782 square km. Having aboiut 1780 sq. kms of forest area where whereas there are 4 Tehsils, namely Jhabua, Petlawad, Ranapur, Thandala and 1025,048. Tribal population as per the census of 2011 is 87.0%. The Dense forest areas are Madrani, Kalyanpura. Narmada, Chambal are important rivers in this area. An in-depth survey was carried out to study the ant diversity in the region.

Sampling: Ant species were amassed at some point of the morning and night time the use of distinct techniques as described by Alonso (2000). Standard methods have been employed for the collection of ant samples within the university campus from October 2023 to Dec. 2024. Sampling methods were of two types (1) Bait Lure and (2) All-Out seek method.

Bait lure (BT): Several kinds of baits such as egg yolk, fried coconut, honey, unboiled rice, millet and useless bugs were used and placed within the quarter and sector inside the (%) area. The baits were left undisturbed for 4 hours, and later ant species were collected for twenty minutes from all six baits.

All-Out seek method (AOSM): an in-depth All-Out seek method was executed to acquire ant species seen in the different regions & sectors in the study area (Alonso 2000). Ants were hand amassed the usage of a broom and forceps and preserved in 70 % alcohol, and taken to the laboratory for similarly evaluation. Identification of ant species was made with the help of a stereozoom trinocular microscope. The accrued ant species have been identified up to genus and for a few species-level identifications, were finished with the help of keys (Bolton 1994). The amassed ant species have been identified and confirmed with the Department of Entomology (Rastogi et.al. 1997).

Figure Table 1: Ant (Hymenoptera: Formicidae) diversity in Jhabua



RESULTS AND DISCUSSION

Ants remain one of the least studied insect groups in India, particularly in terms of their taxonomy and ecology. Identifying ant species is challenging due to the scarcity of reference collections and outdated or region-specific identification keys that are often difficult to access. Despite this, ants play a crucial ecological role, making up nearly 30% of terrestrial biomass. They interact directly with soil, plants, and animals across various trophic levels, influencing ecosystem dynamics.

Ant communities are shaped by both biotic and abiotic factors, with species distribution changing along latitudinal gradients due to variations in climate and vegetation. Globally, there are approximately 12,571 known ant species, all belonging to the family Formicidae. This family is part of the superfamily Vespoidea within the order Hymenoptera, which falls under the class Insecta. Despite their ecological importance, much remains to be discovered about ant diversity and their role in different habitats.

During the present study, three species belonging to two genera and the subfamily Myrmicinae were found, as shown in Table 1. The members of genus *Crematogaster* were represented by one species and the genus *Monomorium* was recorded with two species.

Taxonomy:

Keys to the subfamily: Members of the subfamily myrmicinae are characterized by two-segmented pedicel, transversely rounded and unnamed pygidium, presence of eyes and frontal lobes and well-separated antenna. sockets. Most of the genera are polymorphic.

Posterior margin of clypes projection in between the antenna!sockets promesonotal

suture absent hind tibiae with only simple spur Myrmicinae Keys to the Myrmicinae genus:

Postpetiole attached to dorsal side of gaster; heart-shaped Crematogaster Crematogaster subnuda (Mayr, 1879)

Family: Formicidae, Subfamily: Myrmicinae, Genus: Monomorium, Species: subnuda

Characters: Head, thorax pedicel, antennae & legs brownish red pilosity sparsely spread on thorax and apex of gaster, pubescence white apprised widely, regularly arranged all over. Head smooth with a small straight surrounding antenna, hollow mandibles, straight clypeus, broad & anterior portion almost transverse eyes lateral on the middle,posterior part scape reach of head. Flagellum apical 3joints. Pronotum flat, rugolose antero-lateral suture weakly & mesonotal suture clear. Propodeal spines straight, acute, propodeum smooth petiole semi-circular, front angular post petiole shallow longitudinally grooved gaster broad, cordate,4.5 - 5 mm.

Distribution: India, Sri Lanka,Burma.

2.Propodium unnamed, evenly rounded
Mandible not as above
Monomorium
Monomorium indicum* (Forel, 1902), *Family: Formicidae, Subfamily: Myrmicinae Genus: Monomorium, Species: indium

Characters: Head,thorax and pedicel red, the legs and head brown; abdomen brownish black; head, thorax, abdomen rugolose and opaque, head and thorax anterior in certain light, dense, and fine, striate; abdomen minute reticulate; smooth, polish & shine; pilosity entire wanting. Head are broad, broader anterior than posterior, the hinder concave; mandibles narrow, obscure, striate, the latter obtuse bicarinate, antennae moderate long, eyes large,flat, placed about the middle of the head. Thorax anterior, round, moderate broad, the metanotum narrow, strong, compress, the meso-metanotal suture, the thorax emarginate suture, the basal portion of the metanotum broade posterior. Pedicel: the nodes, above, near equal, the rounded 1st node high than the 2°d , petiolate anterir; and abdomen oval.

Distribution: India, Malaya, Burma

***Monomorium Latinode* (Mayr, 1872)**
Family: Formicidae, Subfamily: Myrmicinae, Genus: Monomorium, Species: latinode

Character: Head, thorax, pedicel yellow-brown, mandibles antennae, legs yellow, abdomen dark brown body smooth polishing, shine. Metanotum and side of mesonotum transversestriate pilosity modrate brown, suberect, pubescence absent. Head long than broad. scape of antennae reach top of head. Club of flagellum thick, formed of apical 3 segments, eyes lateral situated in the front of middle promesonotum. Metanotum narrow, strait mesometanotal suture distinct propodeal spiracles distinct indicated; propodeum with upper margine apex smooth, petiole conical postpetiole distinct broad than petiole, gaster broad oval, 3 to 4 mm

Distribution: India, Bhutan, Malaya, Burma.

CONCLUSION

Ants, belonging to the family Formicidae, are the most diverse group within the order Hymenoptera. They serve as vital ecological indicators and ecosystem engineers, playing key roles in maintaining environmental balance. Ants benefit humans in various ways, including natural pest control by regulating insect populations. This study aims to document the ant species present in the region, offering important insights into their diversity and ecological significance. Overall, the research contributes valuable knowledge to the field of ecology, enhancing our understanding of ant communities and their impact on ecosystems.

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Data Availability: Data are available with the corresponding author

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In silico Evaluation of Plant-Derived Compounds as Potential α-Amylase Inhibitors for Treatment of Type 2 Diabetes

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ABSTRACT
This study investigates the potential of phytochemical compounds as natural inhibitors of human α-amylase (PDB ID: 1B2Y) for the management of type 2 diabetes mellitus (T2DM). Molecular docking was performed on a library of 3,320 phytochemicals derived from 20 medicinal plants and synthetic anti-diabetic compounds. The top-ranked compounds exhibited superior binding affinities (-11.7 to -18.6 kcal/mol) compared to the control drug Acarbose (-8.7 kcal/mol). Goyaglycoside-g, Goyaglycoside-e, and Momordicoside R emerged as the most potent inhibitors, with Momordicoside R specifically targeting the catalytic residue TYR151. Compounds from *Momordica charantia* (Kuguacin M, β-Amyrin, α-Amyrin) and *Azadirachta indica* (Melianoninol, Odoratone) also showed remarkable inhibitory effects. Interaction analysis revealed diverse binding mechanisms, including van der Waals forces, hydrogen bonding, and hydrophobic interactions with key residues such as TYR151 and TRP58. ADME analysis highlighted the challenges of limited bioavailability for some glycosides, while toxicity predictions identified Melianoninol as a promising candidate with a balanced efficacy-safety profile. This study validates the ethnopharmacological use of *Momordica charantia* and *Azadirachta indica* in T2DM management and emphasizes the potential of phytochemicals as safer alternatives to synthetic drugs. Future research should focus on structural optimization and advanced formulations to enhance the bioavailability of these compounds. The present analysis demonstrates the superior efficacy of phytochemicals as natural α-amylase inhibitors over synthetic drugs, with identified compounds from *Momordica charantia* and *Azadirachta indica* showing 2.14-fold better binding affinity than Acarbose, establishing a foundation for developing safer, bioavailable anti-diabetic therapeutics through computational-guided structural optimization and advanced formulation strategies for clinical translation in type 2 diabetes management.

KEY WORDS: *PHYTOCHEMICALS, TYPE 2 DIABETES, MOLECULAR DOCKING, MOMORDICA CHARANTIA, AZADIRACHTA INDICA.*

INTRODUCTION

Diabetes mellitus, particularly type 2 diabetes mellitus (T2DM), poses a significant global health challenge due to its high prevalence and associated complications. The prevalence of diabetes mellitus among adults has notably increased, from 4.7% in 1980 to 8.5% in 2014, affecting over 422 million people globally by 2014 (Kanter and Bornfeldt, 2016). In 2017 alone, approximately 462 million individuals were affected by type 2 diabetes, accounting for about 6.28% of the world's population (Khan et al., 2019). This condition is now the ninth leading cause of mortality, with over 1 million deaths yearly attributed to diabetes causes alone. One Recent study found that gamma-mangostin interacts with the active site of the alpha-amylase enzyme, showing a binding affinity of -9.1 kcal/mol, which is comparable to the control, acarbose (-16.4 kcal/mol). The interactions involve hydrogen, alkyl, and van der Waals bonds. In vitro testing further supported its potential, with gamma-mangostin reducing blood sugar levels by 43.33% compared to acarbose's 56.25% at the same concentration (Kurniawan, Marfu'ah and Fazriah 2025).

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Type 2 diabetes is primarily characterized by insulin resistance and pancreatic beta-cell dysfunction, leading to chronic hyperglycemia (DeFronzo et al., 2015). Factors contributing to insulin resistance include impaired insulin signaling, increased hepatic glucose production, and low-grade systemic inflammation. Furthermore, mitochondrial dysfunction in pancreatic cells exacerbates insulin and glucagon secretion issues (Grubelnik et al., 2020). Insulin resistance is closely linked to lipid metabolism alterations, which manifest as low HDL cholesterol levels, contributing to cardiovascular risks associated with T2DM (Vollenweider, Von Eckardstein and Widmann, 2015).

Recent research has shown promise in the development of novel therapeutic compounds. For example, a study on certain pyridazine derivatives (Molecules 45, 46, and 29) found they had good docking interaction scores with alpha-amylase receptors. While ADMET analysis revealed some limitations, such as lower solubility and poor distribution, these molecules generally maintained drug-like properties with good oral bioavailability. Notably, Molecule 46 was identified as non-toxic. In vitro tests further confirmed their potent alpha-amylase inhibition, with significantly lower IC50 values (a measure of how much of a substance is needed to inhibit a given biological process by half) compared to the standard drug acarbose (Varshney et al., 2024).

Current therapeutic approaches for managing T2DM focus on lifestyle modifications and pharmacological interventions. First-line treatments typically involve lifestyle changes complemented with metformin, which helps improve glycemic control. Other medications include sulfonylureas, thiazolidinediones, alpha-glucosidase inhibitors, glucagon-like peptide-1 agonists, and insulin therapy. However, these treatments often have limitations such as side effects and limited effectiveness over time (El-Kaissi and Sherbeeni, 2011a). The compounding issue of side effects with oral hypoglycemic agents severely affects patient compliance. The development of novel therapeutics, such as nucleic acid-based therapies, aims to overcome these limitations by targeting the expression of genes that cause insulin resistance and hyperglycemia (Kokil et al., 2015). Mesenchymal stem cells also present a promising therapeutic avenue due to their potential to facilitate beta-cell regeneration and immune system regulation, although the associated risks require further investigation. Efforts are continually being made to discover effective interventions that can better manage T2DM and its complications, and these efforts emphasize the complex nature of the disease and the need for multidisciplinary approaches to treatment (El-Kaissi and Sherbeeni, 2011a Mikłosz and Chabowski, 2023).

α-Amylase is a critical enzyme in carbohydrate digestion and glucose absorption, being instrumental in breaking down dietary starch into simpler sugars like maltose and maltotriose. The enzyme's activity leads to the liberation of glucose, which subsequently influences blood glucose levels and can aggravate postprandial hyperglycemia, a significant concern in diabetes management (Kashtoh and Baek, 2023). Given its role, α-amylase presents a promising therapeutic target for managing diabetes. The inhibition of α-amylase slows down carbohydrate digestion and delays glucose absorption, thus helping regulate blood glucose levels (Sales et al., 2012a). Numerous studies have focused on natural plant-based compounds to inhibit α-amylase effectively, offering an alternative to synthetic inhibitors like acarbose and miglitol, which are known for their side effects such as gastrointestinal discomfort. Recent Study found that chlorogenic acid exhibited the highest docking scores against both AMPD1 (-8.41 kcal/mole) and PKA (-12.56 kcal/mole), suggesting its potential as a potent antidiabetic compound (Devakrishna, Taj and Upadhyay, 2024).

Phytochemicals, bioactive compounds derived from plants, offer significant potential as natural therapeutics, especially in the context of diabetes management. These compounds, including flavonoids, terpenes, phenolic acids, and alkaloids, have been investigated for their health-promoting properties and potential to act as anti-diabetic agents (Silva et al., 2017a; Vinayagam, Xiao and Xu, 2017a). The primary advantages of plant-derived compounds include their natural origin, potential lower toxicity, and fewer side effects compared to synthetic drugs . These attributes make phytochemicals an appealing alternative in therapeutic interventions, particularly where synthetic medications have limited effectiveness or cause adverse side effects over long-term use (A. Omara et al., 2010). Additionally, phytochemicals can be incorporated into dietary regimes, thus providing a cost-effective strategy for disease prevention and management (Silva et al., 2017b).

Classes of Phytochemicals with Anti-Diabetic Properties: Different classes of phytochemicals possess anti-diabetic properties through varied mechanisms. For instance:

- Flavonoids and Terpenes: Known for their antioxidant and anti-inflammatory actions, these compounds can inhibit key enzymes like α-amylase and α-glucosidase that are involved in carbohydrate digestion, thereby reducing blood glucose levels (Vinayagam, Xiao and Xu, 2017b).
- Phenolic Compounds: These are abundant in edible plants and have been associated with the regulation of glucose metabolism and improvement of insulin sensitivity (Silva et al., 2017b).
- Alkaloids and Lignans: These compounds enhance insulin secretion and sensitivity, contribute to glycogen synthesis, and modulate oxidative stress, which are beneficial in diabetes management (Ardalani et al., 2021).

Molecular docking plays a pivotal role in drug development by simulating and predicting interactions between small molecules and target proteins, thereby streamlining the process of drug discovery (Mursal et al., 2024a). As a structure-based method, it aids in the identification of novel therapeutic compounds and the prediction of ligand-target interactions (Pinzi and Rastelli, 2019). Molecular docking is integral to virtual screening and lead optimization, where it expedites the identification of potential drug candidates by allowing high-throughput evaluation of molecular interactions. The software used in molecular docking, such

as AutoDock and GOLD, utilizes algorithms to predict binding modes and assess binding affinities, significantly contributing to the drug development process (Mursal et al., 2024b).

Structure-based drug design (SBDD) relies heavily on the three-dimensional structure of target biomolecules to guide drug development (Schneuing et al., 2024). It involves the fitting of drug-like molecules into a protein's binding site, enhancing the search for drug candidates through rational drug design strategies (Bentham Science Publisher, 2006). SBDD benefits from advanced computational techniques such as virtual screening and ensemble docking, which improve the precision of docking predictions and accelerate drug discovery (Mathur et al., 2024). Despite its advantages, SBDD faces challenges such as the need for accurate binding site identification and predicting the effects of protein-ligand interactions accurately.

While molecular docking and structure-based drug design offer significant advances in drug development, careful application and understanding of their limitations are necessary for their effective use in pharmaceutical research (Patel et al., 2022). In silico docking and ADMET studies on clinical targets for type 2 diabetes correlated to in vitro inhibition of pancreatic alpha-amylase and alpha-glucosidase by rutin, caffeic acid, p-coumaric acid, and vanillin," published in 2025, investigate the potential of several natural compounds as inhibitors for Type 2 Diabetes. The study combines in silico (computational) docking and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analyses with in vitro (laboratory) inhibition studies of pancreatic alpha-amylase and alpha-glucosidase (McMillan, Bester and Apostolides, 2025).

α-Amylase plays a crucial role in carbohydrate digestion by catalyzing the hydrolysis of starch into sugars. The inhibition of α-amylase is considered a significant strategy for managing conditions like diabetes and obesity due to its role in reducing postprandial blood glucose levels (Sales et al., 2012a). The enzyme functions as a calcium metalloenzyme, facilitating the breakdown of polysaccharides, which subsequently leads to increased blood glucose levels (Kaur et al., 2021).

The α-amylase enzyme is a well-characterized protein with a detailed structural analysis underscoring its catalytic mechanisms. The crystal structure of α-amylase has been studied extensively using techniques like X-ray diffraction, providing insights into its active sites and functionality. The active site of α-amylase involves key amino acid residues that are essential for its catalytic action of hydrolyzing starch. The process of starch breakdown is initiated through the enzyme's active site, where the polysaccharide chains are cleaved into monosaccharides and disaccharides. This enzyme's action is central to carbohydrate metabolism, particularly in the digestion process (Jayaraj, Suresh and Kadeppagari, 2013a).

Multiple studies have explored the development of α-amylase inhibitors as therapeutic agents, particularly for diabetes management. These inhibitors function by binding to key sites on the enzyme, thereby preventing its interaction with substrates. Acarbose, a well-known α-amylase inhibitor, interacts with the enzyme's active site, effectively reducing glucose production from carbohydrate digestion (Li et al., 2021). Additionally, novel inhibitors like those based on the pyrazole motif have shown promising potential due to their strong binding affinities and effective inhibitory actions on α-amylase.

Research into plant-based inhibitors has identified several compounds, particularly flavonoids, that exhibit significant α-amylase inhibitory activities. The efficacy of these inhibitors is often linked to their molecular structures, such as the number of hydroxyl groups, which enhance their binding affinity to the enzyme (Sales et al., 2012a; Ayorinde et al., 2025). Flavonoids, in particular, have been highlighted for their dual inhibition capabilities against α-amylase and α-glucosidase, making them promising candidates for natural antidiabetic therapies(Ayorinde et al., 2025).

Phytochemical inhibitors, particularly those targeting the enzyme α-amylase, hold promise as natural treatments for diabetes and related metabolic disorders. Recent studies have focused on identifying and validating such inhibitors derived from plant sources, exploring their structure-activity relationships (SAR), and experimentally validating their efficacy. The primary objective of this study is to screen phytochemicals against the 1B2Y protein target, which is likely an α-amylase enzyme. The secondary objectives involve analyzing the binding modes of these phytochemicals with 1B2Y and predicting their ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties. The hypothesis suggests that natural compounds have the potential to effectively inhibit α-amylase activity, which could have implications for managing conditions related to carbohydrate metabolism.

METHODOLOGY

The Phytochemical Library creation: An extensive literature review was conducted to investigate the anti-diabetic properties of plants using an in-silico method, leading to the selection of bioactive plants. The selected plants were analyzed using the KNApSAcK: A Comprehensive Species-Metabolite Relationship (Afendi et al., 2012) and IMPPAT: Indian Medicinal Plants, Phytochemistry, and Therapeutics databases (Mohanraj et al., 2018), from which phytochemicals linked to different plants were collected. The assembled list of phytochemicals was subsequently organized, and their 3D structural representations were acquired in SMILE format from the PubChem database (Kim et al., 2025).

The target alpha-amylase main protease preparation: The crystal structures of the alpha-amylase (PDB ID: 1B2Y, 3.20 Å) were obtained from the protein data bank (https://www.rcsb.org). The selected protein structures were downloaded along with its co-crystallized Acarbose inhibitors. The protein crystal structures were protonated, where hydrogen atoms were introduced with their 3D geometry. Furthermore, Swiss-PdbViewer was used to add

missing amino acids and correct any faults discovered in the connection or type of distinct atoms.

Ligand Library preparation for molecular docking: Chemical structures of phytochemicals were obtained in the structure-data file (.sdf) format from the PubChem database (https://pub-chem.ncbi.nlm.nih.gov/). Using Open Babel, chemical structures of ligands in .sdf format were converted to .pdb format (O'Boyle et al., 2011). Ligand structures were generated by incorporating nonpolar hydrogens, Gasteiger modifications, and rotatable bonds, and then exported in .pdbqt format with the integration of AutoDock Vina 1.2.0 (Eberhardt et al., 2021).

Molecular Docking: Initially, the phytochemical structures were loaded with Alpha amylase and Acarbose target proteins using AutoDock Wizard. A three-dimensional grid box for target protein was generated using the MGL tools, and docking was accomplished with the PyRx-Python script V.0.8.(Oleg trott, 2012). Nine binding poses with both target proteins were produced for each ligand. The binding affinity was estimated as negative Gibbs free energy scores (kcal/mol), which used to rank the results.

The ADME (Absorption, Distribution, Metabolism, and Excretion) properties of the top-ranked phytochemical compounds were evaluated using the SwissADME web server (Daina, Michielin and Zoete, 2017) to assess drug-likeness parameters, including Lipinski's rule of five, bioavailability score, and pharmacokinetic properties, while toxicity profiles were predicted using ProTox-II server (Banerjee et al., 2018) to determine LD50 values, toxicity classes, and potential adverse effects.

RESULTS AND DISCUSSION

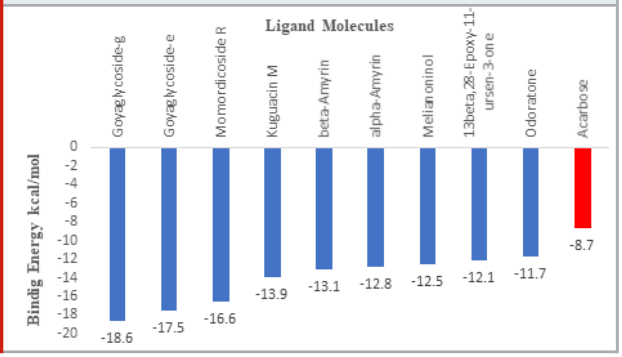
A comprehensive phytochemical library comprising 3,320 compounds was successfully assembled from different sources, including 20 medicinal plants (*Allium sativum, Aloe vera, Andrographis paniculata, Azadirachta indica, Cinnamomum verum, Cheilocostus speciosus, Costus speciosus, Eclipta prostrata, Ficus racemosa, Gymnema sylvestre, Mangifera indica, Momordica charantia, Ocimum sanctum, Ocimum tenuiflorum, Pterocarpus marsupium, Solanum americanum, Syzygium cumini, Trigonella foenum-graecum*, and *Zingiber officinale*) and 24 synthetic anti-diabetic compounds (including Acetobexamide, Bezafibrate, Chlorpropanide, Dexamethasone, Duloxetine, Gilbenclamide, Gliciazide, Glimepiride, Glipizide, Glisoxepide, Glymidine, Hydrochlorothiazide, Irbesartan, Mazindol, Metformine, Mifepristone, Pentamidine, Phenformin, Pioglitazone, Prednisolone, Quinapril, Streptozocin, Tolazamide, Tolbutamide, and Troglitazone). The SMILES structures were retrieved from IMPPAT (1,412 compounds) and KNAPSACK (1,883 compounds) databases, with an additional 25 compounds obtained from PubChem standards. Acarbose was employed as a positive control for molecular docking validation against the α-amylase enzyme (PDB ID: 1B2Y).

Docking Study

Figure 1: Figure 1 Three-dimensional structure of protein 1b2y (center) surrounded by the chemical structures of ten ligand molecules: (1) Goyaglycoside-g, (2) Goyaglycoside-e, (3) Momordicoside R, (4) Kuguacin M, (5) beta-Amyrin, (6) alpha-Amyrin, (7) Melianoninol, (8) 13beta,28-Epoxy-11-ursen-3-one, (9) Odoratone, and (10) Acarbose. The figure illustrates the structural diversity of these ligands, which are investigated for their potential interactions with protein 1b2y in this study.

The molecular docking study revealed significant binding interactions between phytochemical compounds and the α-amylase enzyme 1B2Y (Figure 1). The comparative binding energy analysis (Figure 2) demonstrated that all ten top-ranked compounds exhibited superior binding affinities compared to the positive control Acarbose (-8.7 kcal/mol), with binding energies ranging from -11.7 to -18.6 kcal/mol.

Figure 2: Binding energies (kcal/mol) of the top ten molecules docked against alpha-amylase (protein 1b2y), shown alongside the positive control Acarbose.



Goyaglycoside-g emerged as the most potent inhibitor with the highest binding affinity of -18.6 kcal/mol, representing a 2.14-fold improvement over Acarbose. Goyaglycoside-e ranked second with -17.5 kcal/mol (2.01-fold improvement), while Momordicoside R demonstrated strong binding at -16.6 kcal/mol (1.91-fold improvement). The results demonstrate that natural compounds can significantly outperform the established inhibitor Acarbose, offering promising alternatives for diabetes management. The top-ranked compounds exhibited remarkable binding affinities ranging from -11.7 to -18.6 kcal/mol, substantially superior to Acarbose (-8.7 kcal/mol). Goyaglycoside-g showed the highest potency with a 2.14-fold improvement,

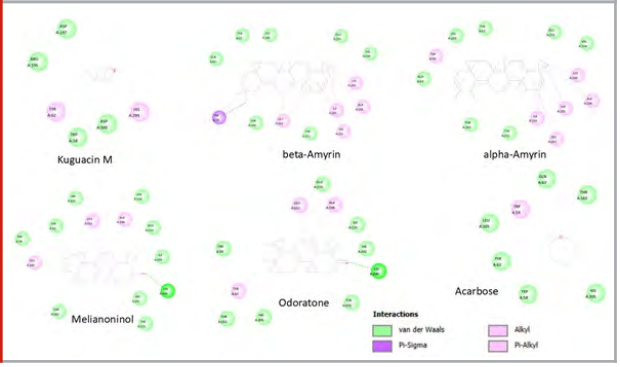
while Momordicoside R demonstrated specific targeting of the catalytic residue TYR151, crucial for starch hydrolysis (Jayaraj, Suresh and Kadeppagari, 2013b). These findings align with previous studies emphasizing the effectiveness of plant-derived compounds in enzyme inhibition (Sales et al., 2012b; Kashtoh and Baek, 2022).

Table 1. Top ranked compounds from molecular docking analysis showing binding affinities, key molecular interactions, and significant residues involved in protein-ligand binding.

Rank	Compound Name	Binding Affinity (kcal/mol)	Key Interactions	Significant Residues
1	Goyaglycoside-g	-18.6	van der Waals	TRP58, TYR62
2	Goyaglycoside-e	-17.5	vdW + H-bond (LEU162)	LEU162
3	Momordicoside R	-16.6	vdW + TYR151 interaction	TYR151
4	Kuguacin M	-13.9	PI-Alkyl + vdW	TYR62, TRP58, ASP197, ASP300
5	β-Amyrin	-13.1	Pi-Sigma + Pi-Alkyl	Hydrophobic pocket
6	α-Amyrin	-12.8	Alkyl	Hydrophobic pocket
7	Melianoninol	-12.5	vdW + H-bond	A1.01, A2.24
8	13β,28-Epoxy-11-ursen-3-one	-12.1	vdW	Hydrophobic pocket
9	Odoratone	-11.7	vdW + H-bond + Pi-Alkyl	Hydrophobic/aromatic residues
10	Acarbose	-8.7	vdW + PI-Alkyl	TRP58, TYR62, HIS305, LEU165

The detailed interaction analysis (Table 1) revealed distinct binding patterns for the moderately active compounds. Kuguacin M (-13.9 kcal/mol) exhibited excellent inhibitory potential through multiple PI-Alkyl interactions with TYR62, TRP58, ASP197, and ASP300, along with extensive van der Waals contacts. The triterpenoid β-Amyrin (-13.1 kcal/mol) demonstrated strong binding through Pi-Sigma and Pi-Alkyl interactions within the enzyme's hydrophobic pocket, while α-Amyrin (-12.8 kcal/mol) showed similar alkyl-based interactions in the same hydrophobic region.

Figure 3: Binding poses and key residue contacts, highlighting van der Waals, Pi-Sigma, alkyl, and Pi-alkyl interactions for ligand Kuguacin M, beta-Amyrin, alpha-Amyrin, Melianoninol, Odoratone including the positive control Acarbose



Melianoninol (-12.5 kcal/mol) displayed a balanced interaction profile combining van der Waals forces with hydrogen bonding capabilities, indicating stable enzyme-ligand complex formation. Odoratone (-11.7 kcal/mol) showed good inhibitory activity through a combination of van der Waals interactions, hydrogen bonds, and Pi-Alkyl contacts with both hydrophobic and aromatic residues.

Remarkably, these five compounds (Kuguacin M, β-Amyrin, α-Amyrin, Melianoninol, and Odoratone) demonstrated binding affinities 1.34 to 1.60-fold superior to Acarbose, indicating their excellent potential as natural α-amylase inhibitors (figure 3). The interaction profiles suggest that these compounds effectively occupy the enzyme's active site through complementary molecular interactions, positioning them as promising anti-diabetic candidates with potentially fewer side effects than synthetic inhibitors.

The diverse interaction mechanisms observed, including van der Waals forces, hydrogen bonding, and Pi-Alkyl interactions, suggest stable enzyme-ligand complexes. The triterpenoids (β-Amyrin, α-Amyrin, and Kuguacin M) showed strong hydrophobic interactions, consistent with effective enzyme-ligand stability reported in previous studies (Salehi et al., 2019).

ADME Properties: The ADME (Absorption, Distribution, Metabolism, and Excretion) analysis of the phytochemical compounds revealed critical insights into their potential as anti-diabetic agents. Among the top candidates, Momordicoside R from *Momordica charantia* emerged as the most promising compound due to its strong binding affinity (-16.6 kcal/mol) targeting the catalytic residue TYR151 of α-amylase, which is crucial for inhibiting carbohydrate digestion. Despite its low gastrointestinal absorption and moderate solubility, Momordicoside R exhibits a favorable low logP (0.11), indicating better solubility compared to other glycosides like Goyaglycoside-g and -e. However, its

high molecular weight and excessive hydrogen bond donors limit its bioavailability (score: 0.17), a common challenge for natural glycosides.

In contrast, Kuguacin M, another compound from *Momordica charantia*, shows high GI absorption and no Lipinski violations, making it more drug-like, but its hydrophobicity (logP: 3.63) and weaker binding affinity (-13.9 kcal/mol) reduce its therapeutic potential. Meanwhile, β-Amyrin and α-Amyrin, despite their strong hydrophobic interactions, suffer from poor solubility (logP >7) and low bioavailability, limiting their clinical applicability.

The control drug Acarbose, while highly soluble, has poor permeability and requires high doses, leading to gastrointestinal side effects. Momordicoside R's targeted inhibition of TYR151 and natural origin make it a safer and more specific alternative. To overcome its bioavailability limitations, future research should focus on structural optimization (e.g., reducing molecular weight) and advanced formulations (e.g., nanoemulsions). In conclusion, Momordicoside R represents the best candidate for anti-diabetic development, provided its absorption challenges are addressed through further preclinical studies.

ADME analysis revealed that while Momordicoside R showed excellent binding affinity, its limited bioavailability (0.17) presents typical challenges for natural glycosides (Wang et al., 2015). However, its favorable logP value (0.11) indicates good solubility characteristics. Kuguacin M demonstrated better drug-like properties with high GI absorption and Lipinski compliance, despite moderate binding affinity.

Toxicity Prediction: The toxicity study of phytochemical compounds using ProTox-3.0 revealed important insights into their safety profiles as potential anti-diabetic agents. Among the evaluated compounds, Melianoninol emerged as a particularly promising candidate despite its moderate acute toxicity (LD50 = 2500 mg/kg, Class 5). While β-Amyrin and α-Amyrin showed the highest LD50 values (70,000 mg/kg, Class 6), indicating very low acute toxicity, their potential effects on GABA receptors and blood-brain barrier permeability raise concerns for therapeutic use. In contrast, Melianoninol demonstrated a more favorable overall toxicity profile, showing no hepatotoxicity or neurotoxicity, though it did present mild nephrotoxicity risk (0.61 probability) and significant immunotoxicity (0.98 probability). Importantly, Melianoninol's toxicity profile compares favorably to the control drug Acarbose, which, despite having a higher LD50 (24,000 mg/kg), showed active hepatotoxicity, nephrotoxicity, and cardiotoxicity.

Melianoninol's advantages include its natural origin, strong binding affinity to α-amylase (as shown in previous docking studies), and absence of severe organ toxicity risks. The main concerns for Melianoninol are its immunotoxicity potential and CYP450 inhibition (affecting CYP1A2, 2C19, 2C9, and 3A4), which could lead to drug-drug interactions in clinical use. However, these issues may be addressable through structural optimization and careful dosing strategies. When compared to other lead compounds, Melianoninol offers a balanced combination of efficacy and safety, making it a viable candidate for further development as a natural anti-diabetic agent.Toxicity predictions identified Melianoninol as particularly promising, showing no hepatotoxicity or neurotoxicity compared to Acarbose, which exhibited multiple organ toxicity risks. This supports the general observation that natural compounds often display better safety profiles than synthetic drugs (Ardalani, Avan and Ghayour-Mobarhan, 2017; Omara et al., 2020).The identified phytochemicals address key limitations of current α-amylase inhibitors, including side effects and patient compliance issues (Kokil et al., 2010; El-Kaissi and Sherbeeni, 2011b). Compounds from *Momordica charantia* and *Azadirachta indica* validate traditional medicinal uses and support ethnopharmacological approaches to drug discovery.

CONCLUSION

The molecular docking studies presented in this research highlight the significant potential of phytochemical compounds as natural inhibitors of human α-amylase 1B2Y for managing type 2 diabetes mellitus. The findings demonstrate that plant-derived ligands exhibit superior binding affinities compared to the synthetic inhibitor Acarbose, with Goyaglycoside-g, Goyaglycoside-e, and Momordicoside R emerging as the most potent candidates. Notably, compounds derived from *Momordica charantia* (Kuguacin M, β-Amyrin, α-Amyrin) and *Azadirachta indica* (Melianoninol, Odoratone) showed remarkable inhibitory effects, underscoring the therapeutic promise of these plants. The interaction analysis revealed that these phytochemicals bind effectively to key residues of α-amylase, such as TYR151 and TRP58, through diverse mechanisms including van der Waals forces, hydrogen bonding, and hydrophobic interactions.

Despite challenges in bioavailability for some glycosides, their natural origin and favorable toxicity profiles position them as safer alternatives to synthetic drugs. This study validates the ethnopharmacological use of *Momordica charantia* and *Azadirachta indica* in traditional medicine and emphasizes their role as rich sources of anti-diabetic agents. Future research should focus on structural optimization and advanced formulations to enhance the bioavailability of these compounds. Overall, the findings advocate for the continued exploration of plant-based therapeutics in diabetes management, combining computational and experimental approaches to develop novel, effective, and safer treatments.

Conflict of Interest Statement: The authors declare that there are no conflicts of interest.

Author Contributions: Dhyey Kothari designed the study and wrote the manuscript. Manoj Godhaniya performed docking, data interpretation, and assisted with literature review. Kunjan Kikani supervised the work and revised the manuscript critically.

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author.

Ethics Statement: This study does not involve human or animal participants; hence, ethical approval was not required.

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