PHYTOCHEMICAL INVESTIGATION OF SOME UNDERUTILIZED CEREALS IN INDIA

Thesis Submitted
in Partial Fulfilment of the Requirements for the
Degree of

DOCTOR OF PHILOSOPHY

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OCTOBER, 2025

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ACKNOWLEDGEMENTS

As I reach the culmination of my doctoral journey, I express my profound gratitude to those who have been instrumental in my academic success and personal growth.

First and foremost, I consider myself incredibly privileged to have worked with the invaluable guidance of **Prof. Devendra Kumar** and **Prof. Rajinder K. Gupta.** I express my deepest gratitude to Prof. Devendra Kumar, whose collective wisdom, unwavering support, and dedication have left a lasting imprint on my scholarly path and Prof. Rajinder K. Gupta**,** who not only guided me in research but also taught me lessons that go beyond the laboratory. His humility, wisdom, and passion for teaching have truly made him my role model. Your mentorship has shaped my research and taught me the true meaning of integrity, ethics, and selfless dedication.

I am deeply grateful to **Prof. Prateek Sharma**, Vice Chancellor of Delhi Technological University, for allowing me to pursue my research at this esteemed institution. I also thank **Prof. Anil Kumar,** Head of the Department of Applied Chemistry, for providing me with all the required facilities and support to carry out this research work. I sincerely appreciate the distinguished faculty members of the Department of Applied Chemistry at Delhi Technological University. Their guidance and support have been invaluable to my research endeavours. I gratefully acknowledge the technical and administrative staff of the Department of Applied Chemistry, DTU. for their day-to-day support. I would like to acknowledge the cleaning staff (specially Surender uncle and Manoj Rana bhaiya) for ensuring a clean and organized workspace during my research period.

My heartfelt appreciation goes to my lab colleagues whose mentorship has significantly enhanced my research capabilities. Specially my two juniors Nishtha & Tanvi. I extend my sincere gratitude to my wonderful chai-break friends - **Mr. Ashwani K. Tiwari, Ms. Versha Joshi, Ms. Priyanka, and Ms. Jigyasa,** for their unwavering emotional support and encouragement during my most stressful times.

Words cannot adequately express my gratitude to my beloved parents for their unwavering support, unconditional love, and constant encouragement. I express my

heartfelt gratitude to my mother, **Smt. Sunita Sharma,** and my father, **Shri Inder Pal Sharma,** whose life lessons and encouragement have been a candle at night. Their endless love and firm belief in me motivate me throughout this journey. Special thanks to my **sister (Neha) and brother (Sahil)** for their constant enthusiasm and positivity. My family has been the cornerstone of my academic journey, and this achievement would not have been possible without their blessings.

Lastly, I sincerely thank **Lord Krishna** for providing me with strength, guidance, and every opportunity needed to embark on this journey. I am humbled by the blessings and provisions bestowed upon me throughout this endeavour.

I extend my deepest gratitude to all who have played a part in this journey, whether through encouragement, assistance, or companionship. This thesis is a testament to the collective efforts and contributions of all who have touched my life along this transformative path.

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CANDIDATE'S DECLARATION

I, **Ritu Sharma,** hereby certify that the work which is being presented in the thesis entitled "**Phytochemical Investigation of Some Underutilized Cereals in India**" in partial fulfilment of the requirements for the degree of Doctor of Philosophy, submitted in the Department of **Applied Chemistry**, Delhi Technological University is an authentic record of my own work carried out during the period from 13/01/2021 to 24/07/2025 under joint supervision of **Prof. Devendra Kumar & Prof. Rajinder K. Gupta**. The work presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

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Certified that **Ritu Sharma (2K20/PHDAC/503)** has carried out her research work presented in this thesis entitled **"Phytochemical Investigation of Some Underutilized Cereals in India"** for the award of the degree of **Doctor of Philosophy** from Department of Applied Chemistry, Delhi Technological University, Delhi under our joint supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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ABSTRACT

The thesis titled “Phytochemical investigation of some underutilized cereals in India” focuses on the comprehensive exploration of the bioactive compounds, nutritional analysis, and biological profiling of the selected underutilized cereals of India. The present thesis aimed to investigate the phytochemical and biological profiling of selected underutilized cereal samples of India - adzuki beans (*Vigna angularis*), mung beans (*Vigna radiata*), horse gram/kulthi beans (*Macrotyloma uniflorum*), little millet (*Panicum sumatrense*), and rice beans (*Vigna umbellata*), followed by proper identification, authentication, and sequential Soxhlet extraction using different polarity solvents and to further evaluate their potential for functional food applications.

All five selected cereal samples were subjected to a detailed proximate analysis, fatty acid methyl ester analysis, elemental analysis, amino acid profiling, and phytochemical analyses using GC-MS and UHPLC-QTOF-MS to establish a comprehensive profile of their nutritional and metabolite content. The phytochemical screening focused on both qualitative and quantitative estimation of major as well as minor phytoconstituents such as phenolics, flavonoids, tannins, saponins, alkaloids, and phytosterols, using GC-MS and UHPLC-QTOF-MS. Based on the distinct phytochemical profile and literature evidence, adzuki bean and mung bean were selected for the evaluation of anti-obesity activities through in-vitro pancreatic lipase and HMG-CoA reductase inhibition assays.

The nutritional assessment highlights that these underutilized cereals are a valuable source of essential macronutrients - namely carbohydrates, protein, and dietary fiber - in addition to a wide array of amino acids (essential, non-essential, and non-proteinogenic amino acids). The phytochemical profiling of these underutilized cereals revealed that the different solvent extracts contained flavonoids, terpenoids, and phenols. Various secondary metabolites were identified using a non-targeted approach, which belong to several natural product classes, including flavonoids, sugars, amino acids, fatty acids, fatty acid derivatives, and other organic acids. Through a targeted approach,

metabolites including catechin-7-O-glucoside, catechin, epicatechin, quercetin, gallo-catechin, gallic acid, caffeic acid, para-coumaric acid, and glycitein were identified using UHPLC-QTOF-MS. In-vitro anti-obesity activity revealed the effectiveness of adzuki and mung beans as potent HMG-CoA reductase and pancreatic lipase inhibitors. The findings of anti-obesity activities indicate that adzuki beans and mung beans can be utilized to prevent obesity and related disorders.

This research not only emphasizes the nutritional and biological value of these lesser-known cereals but also explores the formulation and evaluation of novel breakfast cereals/flakes and nutritional cookies developed from underutilized legumes - *Vigna radiata* (mung bean) and *Vigna angularis* (adzuki bean)- as sustainable, plant-based interventions to combat obesity and related disorders. Incorporating these legumes enhances the formulations' protein, fiber, and micronutrient content and introduces bioactive compounds with significant health benefits like weight loss and cholesterol lowering benefits. An important milestone of this work was the commercial interest, leading to collaboration with *Kalsubai Purest Company,* a local food enterprise based in Maharashtra. Based on my research formulations, their R&D team created a trial batch and is currently working toward market launch as a nutritious and functional food. The significance of this research was further recognized through an appreciation letter from *Kalsubai Purest Company*, acknowledging its practical value and real-world applicability.

In addition to product development application of these underutilized cereals, we also investigated the potential of underutilized cereals' mucilage and further explored their application by synthesizing mucilage-based hydrogels. For this purpose, we have explored four new sources of mucilage, namely adzuki beans, amaranth, proso millet, and little millet. The underutilized cereals' mucilage application has been examined by developing hydrogels through the free radical copolymerization technique. This study demonstrated that the mucilage isolated from underutilized cereals might be a good feedstock for a hydrogel-forming agent, which can be explored in the food, cosmetics, and pharmaceutical industries.

Overall, this work provides a comprehensive evaluation of the selected underutilized Indian cereals, identifying key bioactive metabolites and demonstrating their potential in the development of anti-obesity and cholesterol-lowering novel functional foods. The study not only contributes to scientific understanding of these neglected cereals but also supports their integration into sustainable food systems, promoting both nutrition security and agricultural biodiversity.

Dedicated

to

Lord Krishna

For giving me endurance, positivity, and healthy life to achieve my goals

To the unwavering support of ***Shrimati Sunita Sharma and Shri Inder Pal Sharma****, my cherished parents;*

To my beloved ***sister*** *(Neha) &* ***brother*** *(Sahil)*

and to my two life mentors (my dearest friends)

You all are my constants in a world of variables and the driving force behind this achievement.

LIST OF THESIS PUBLICATIONS

1. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2024) Bioactive profiling of two varieties of Indian legumes: Adzuki and mung beans, *International Journal of Food Science and Technology*, *59*(9), 6218-6230. doi:10.1111/ijfs.17358. (IF-2.6)

2. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2024) Phytochemical profiling of little millet (*Panicum sumatrense* Roth.), *Indian Journal of Natural Products and Resources*, *15*(4), 555-564. https://doi.org/10.56042/ijnpr.v15i4.9518. (IF-0.8)

3. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2025) Profiling of *Naurangi* and Kulthi dal used in traditional Indian cuisines and medicines, *Indian Journal of Traditional Knowledge, 24*(7), 698-707. (IF-0.7)

4. **Ritu Sharma**, Rajinder K. Gupta & Archna Rani (2023) Hydrogels based on mucilage of underutilized cereals: Synthesis and characterization, *Indian Journal of Chemical Technology*, *30*(4), 524-533. https://doi.org/10.56042/ijct.v30i4.70238%20. (IF-1.0)

5. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2025) Novel cereals and cookies to combat obesity using adzuki and mung beans, *ChemistrySelect* (Under review).

6. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2025) Underutilized food crops of India as potential nutraceuticals: A review on nutritional profile, health benefits, and their applications in food, pharmaceutical, and cosmetics sectors (To be submitted).

CONFERENCE PROCEEDINGS

1. **Ritu Sharma**, Rajinder. K. Gupta and Archna Rani, "Isolation and Characterization of Mucilage from *Paspalum scrobiculatum* (Kodo millet)", at International conference and exhibition," Evolving Technology and New Paradigms in Analytical Sciences" organized by Graphic Era (Deemed to be University) held from 26-28th May,2022.

2. **Ritu Sharma**, Rajinder K. Gupta & Archna Rani, "*Panicum sumatrense*: A new source of mucilage for food additives" at International Conference on green and sustainable chemistry, organized by Manav Rachna University held from 17-19th November, 2022.

3. **Ritu Sharma**, Tamanna Beniwal, Nishtha & Rajinder K. Gupta, International Conference on "Chemical and Allied Science and their Applications", Organized by Department of Applied Chemistry, Delhi Technological University held on 20th January,2023.

4. **Ritu Sharma**, D. Kumar & Rajinder K. Gupta, Isolation and characterization of natural polysaccharide from kodo millet: A sustainable approach, International Conference on "Emerging techno-economic development for sustainable environment, organized by IPS academy institute of engineering and science, Indore held from 12 -13th January, 2024.

5. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta, Application of *Paspalum scrobiculatum* (Kodo millet) mucilage as a bio-flocculant for wastewater treatment, National Conference on "Desalination, Brine Management and Water Recycling", organized by IIT Bombay, held from 12 - 13th August, 2024.

6. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta, Unveiling the Bioactive Potential of Indian Adzuki Bean (*Vigna angularis*) for Nutritional and Health Benefits, 7th International Conference on Frontiers in Chemical Sciences Indian Institute of Technology Guwahati, organized by IIT Guwahati, held from 4th -6th December, 2024.

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A_b	Adzuki beans
^{13}C-NMR	Carbon Nuclear Magnetic Resonance Spectroscopy
^{1}H-NMR	Proton Nuclear Magnetic Resonance Spectroscopy
AAc	Acrylic acid
ABF	Adzuki bean flour
AFLPL/SOP/CH/INH	Avon Food Lab Private Limited/Standard Operating Procedure/Chemical/Instrumentation
AI	Acceptability Index
A_m	Amaranth
AOAC	Association of Official Analytical Chemists
BLQ	Below Limit of Quantification
BMI	Body Mass Index
BP	Before Present
CSIR-NIScPR-RMHMD	Council of Scientific & Industrial Research - National Institute of Science Communication and Policy Research-Raw Materials Herbarium and Museum Delhi
CV	Cardiovascular Disease
DCM	Dichloromethane
DM	Diabetes Mellitus
DMAB	4-(Dimethylamino)benzaldehyde
DMEM	Dulbecco's Modified Eagle Medium
DPPH	Diphenylpicrylhydrazyl Radical
E. coli	*Escherichia coli*
EAA	Essential Amino Acids
EI	Electron Ionization
ESI	Electron Spray Ionization
EUCF	Extrusion Modified Underutilized Cereal Flour

FAME	Fatty Acid Methyl Ester
FAO	Food and Agriculture Organization
$FeCl_3$	Ferric Chloride
FSSAI	Food Safety and Standards Authority of India
FTIR-ATR	Fourier Transform Infrared Spectroscopy - Attenuated Total Reflectance
GC-MS	Gas chromatography-Mass Spectrometry
GI	Glycemic Index
HCl	Hydrochloric acid
HDL	High Density Lipoprotein
HMG-Co-A	3-Hydroxy-3-methylglutaryl coenzyme A
IC_{50}	Inhibitory Concentration for 50% Inhibition
ICMR	Indian Council of Medical Research
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICUC	International Centre for Underutilized Crops
IL-1β	Interleukin
KOH	Potassium hydroxide
KPS	Potassium Persulphate
Ku	Kulthi beans
LC-MS	Liquid Chromatography Mass Spectrometry
LDL	Low Density Lipoprotein
Lm	Little millet
MALDI	Matrix Assisted Laser Desorption Ionization
MBA	N,N'-Methylenebisacrylamide
M-co-AAc	Mucilage-co-Acrylic acid
MF	Molecular formula
Mg	Mung beans
MGF	Mung bean flour
MUFA	Monounsaturated Fatty Acids

MW	Molecular Weight
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NaOH	Sodium hydroxide
NAS	National Academy of Sciences
NASA	National Aeronautics and Space Administration
NCCS	National Centre for Cell Science
NF-κB	Nuclear Factor Kappa B
NIFTEM	National Institute of Food Technology, Entrepreneurship and Management
NIST	National Institute of Standards and Technology
NRU	Neutral Red Uptake
PBS	Phosphate Buffer Saline
PUFA	Polyunsaturated Fatty Acids
PXRD	Powder X-Ray Diffraction
Rb	Rice beans
RT	Retention Time
S. aureus	*Staphylococcus aureus*
S.D.	Standard Deviation
SEM	Scanning Electron Microscopy
SI	Swelling Index
T2D	Type 2 Diabetes
TGA	Thermogravimetric Analysis
TNF-α	Tumor Necrosis Factor-Alpha
UHPLC-QTOF-MS	Ultra-High Performance Quadrupole Time of Flight Mass Spectrometry
UV-Vis	Ultraviolet- Visible Spectroscopy
WAI	Water Absorption Index
WHO	World Health Organization
WSI	Water Solubility Index

Symbols

°C	Degree Celsius
β	Beta
α	Alpha
γ	Gamma
ω	Omega
δ	Delta
h	Hour
g	Gram
mg	Milligram
min	Minute
mL	Millilitre
mm	Millimetre
Kg	Kilogram
ppm	Parts Per Million
µg	Microgram
µg	Micrometre
µL	Microlitre
%	Percentage
mM	Millimolar
w/v	Weight by Volume
rpm	Revolutions per minute

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

With the growing population of India, which is projected to exceed 1.5 billion by 2030, the demand for food is expected to rise significantly. This demographic trend puts immense pressure on the agricultural sector to produce a more significant quantity of food, feed, and biofuel. India is known for its rich agricultural heritage, encompassing various crops cultivated for centuries. Mankind depends on a few crops like rice, maize, and wheat to meet the needs of staple diets, while a treasure trove of many crops remains unexplored and underutilized (Figure 1.1) [1]. The disparity between the human population and food production poses a global threat, and therefore, researchers are trying to explore underutilized crops for multifarious uses.

Underutilized crops have been used for centuries for food but have lost their importance and cultivation with time. They are plant species grown for food, oil, fiber, fodder, etc., but have yet to be addressed due to their unrecognized nutritional value. These underutilized crops, often called "neglected and underexplored species", are crucial in ensuring food and nutritional security, especially in marginal and ecologically fragile areas [2]. A daily serving of these cereals increases the intake of protein, zinc, dietary fiber, magnesium, and folate, while simultaneously lowering the consumption of saturated and total fat [3]. Several global organizations, such as the International Centre for Underutilized Crops (ICUC), the U.S. National Academy of Sciences (NAS), and

Biodiversity International have identified 200 underutilized crop species. These span diverse eco-geographical regions and comprise 29 types of millets, 27 pulses, 25 root and tuber crops, 10 oilseeds, 52 fruits and nuts, 24 lesser-known fruits, 39 vegetables, and 5 species used for fiber and pulp production [4]. The deficiency of micronutrients causes different illnesses, particularly iron deficiency, which can cause low birth weight in children. According to the Food and Agriculture Organization (FAO) statistics, approximately 800 million children, women, and men are suffering from protein-calorie deficiencies, and over 2 billion suffer from malnutrition [5].

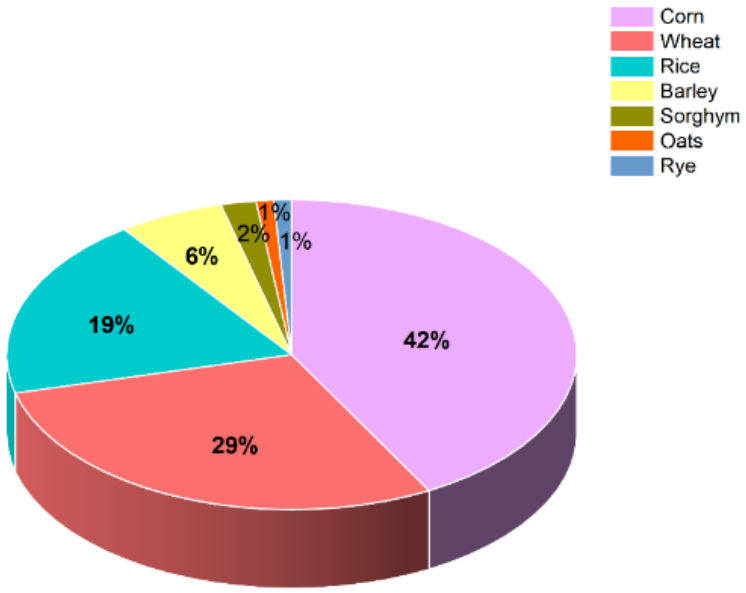

Figure 1.1: Contribution of different cereals and grains consumed in India

Cereals, legumes, and millets are staple food crops that offer vital nutrients and bioactive compounds beneficial to human health. Among them, millets (Family: Poaceae) stand out for their superior nutritional profile, often surpassing common staples like rice and wheat in fiber, minerals, and antioxidant content. Their high protein content, dietary fiber, micronutrients, amino acids, and phytochemicals, combined with exceptional health

benefits, provide ample energy. In addition to their nutritive value, several potential health benefits, such as anticancer, antidiabetic, anti-obesity, anti-tumorigenic, antimicrobial, and antioxidant properties, have been reported from millets [7], [8]. Realizing the nutritional values of these underutilized millets, they are considered nutri-cereals. Legumes/grains are excellent sources of nutrients, high-quality proteins, amino acids, and fatty acids, which can help improve overall health. India, the world's largest millet producer, faces declining output due to low awareness and rising reliance on processed foods [9]. The main legumes of India include chickpea, kidney beans, lentils, pigeon pea, and black gram, which are consumed in various ways. Other lesser-known legumes include adzuki bean, rice bean, mung bean, horse gram/kulthi bean, and moth bean. Legumes are considered more nutritious than vegetables and contain a balanced ratio of protein to carbohydrates, making them viable protein sources for the global vegetarian population [10]. Legumes are rich in high-quality proteins and are commonly known as “poor man’s meat” due to their low cost and accessibility. Similarly, pseudo-cereals are underutilized cereal crops valued for their high protein content, essential nutrients, vitamins, and various bioactives, which have many pharmacological and nutraceutical applications [11]. They include crops like amaranth, quinoa, and buckwheat, which resemble the function and composition of true cereals, which is why they are called pseudo-cereals. They also contain saponins that have hemolytic and antilipemic activity and can lower cholesterol levels in blood serum [12]. They are known for being gluten-free, making them ideal for people with gluten intolerance or celiac disease [13]. They are increasingly gaining attention due to their health benefits, culinary flexibility, and ability to thrive in diverse growing conditions.

In India, cereals and legumes might be consumed based on regional preferences. Every cereal crop has distinct and significant nutraceutical relevance due to the existence of specific bioactive compounds. These cereals are rich in beneficial phytochemicals, including polyphenols (like flavonoids, phenolic acids, and lignans), carotenoids, phytic acids, phytosterols, and β-glucans. These compounds exhibit antioxidant, anti-inflammatory, and anti-carcinogenic effects, contributing to a reduced risk of chronic health conditions. The presence of these unique bioactive compounds has led to growing research interest in developing cereal-based functional foods that support immunity and help reduce or prevent chronic diseases.

These cereal crops produce a variety of compounds; some are healthy, whereas others are harmful, inedible, and can prevent the absorption of some nutrients or delay the digestion process. Such compounds are considered as anti-nutritional compounds. The anti-nutritional compounds primarily present in the legumes include tannins, oligosaccharides, saponins, phytic acids, lectins, toxic amino acids, and enzyme inhibitors like trypsin or chymotrypsin inhibitors [14]. According to the reports, the effect of these anti-nutrients on human health can be reduced by applying different processing methods such as soaking, roasting, dehulling, germination, and fermentation [15].

To meet the increasing global food demand and ensure nutritional security, it is essential to explore and promote the cultivation of crops that are currently neglected and underutilized but possess high nutritional and functional potential. In the present study, an effort has been made to investigate selected underutilized cereals of India with a focus on their nutritional composition, phytochemical profiling, biological activities, and their potential applications.

1.2 Overview

1.2.1 Definition and classification

India is one of the most diverse and prosperous agricultural countries in the world, with thousands of legumes, cereals, and pseudocereals varieties that are staples in the diet of the local population [16],[17]. In addition to offering nutritional benefits and environmental sustainability, these crops are crucial to the country's agriculture, economy, and food security. Legumes are tiny seeds of the Leguminous family that encompass a variety of peas, beans, lentils, and similar crops. Legumes can be categorized into major and minor species according to their utility and economic value. Major legumes such as soybean, chickpea, pigeon pea, and kidney bean are widely grown, well-known, and have a long history of domestication and cultivation. On the other hand, minor legumes are obscure and are considered underutilized/unexplored. Examples include adzuki bean, rice bean, winged bean, horse gram, mung bean, lablab bean, and many more. These minor legumes could be exploited for various purposes, such as food, medicine, and agriculture. It is a common misconception that legumes and pulses are interchangeable terms; however, according to the Codex Alimentarius Commission of the WHO Food Standard, the fat content serves as the key criteria for discriminating between the two terms [18]. Pulses are dry seeds (cowpea, chickpea, pigeon pea) while legumes are oil seeds (soybean, peanuts) [19].

Cereals belong to the Poaceae family and are considered rich in carbohydrates, with a significant source of proteins, vitamins, and minerals. They are those food categories that have a good health impact on our human body. They contain a necessary amount of

nutrients as well as some other important compounds that have a positive impact on the consumer [20]. Millets are classified under cereals as small seeded grasses that have a significant nutritional content (high fiber and mineral content) when compared to other major grains like rice, wheat, and maize. They are considered as "Nutri-cereals" due to their high nutritional profile [21]. Owing to their ability to withstand drought, resistance to pests, and short cultivation period, they are gaining attention for applications in food, biofilm manufacturing, and bioethanol production [7]. Cereals are classified into two types: major and minor cereals. Major cereals are prominent, widely cultivated, and meet more than 50% of the world's caloric requirement [22]. Although these significant cereals comprise a censorious portion of many diets, they lack consequential micronutrients and phytonutrients. Minor cereals are obscure, neglected, underutilized, and seen as underexploited.

Pseudocereals, such as amaranth, quinoa, and buckwheat, are plants that produce seeds similar to grains but do not belong to the Poaceae family, which includes true cereals like wheat, rice, and corn. They resemble that of true cereals in functional aspects; however, they differ in nutritional and phytochemical aspects. Unlike true cereals, they are composed of less starch and more lipids and proteins, including essential amino acids like lysine, cysteine, and methionine. In cereals, proteins are stored in the form of prolamins, whereas in pseudocereals, they are present in the form of albumins and globulins. High concentration of prolamins in cereals is responsible for celiac disease. Pseudocereals are naturally gluten-free, making them suitable for individuals with celiac disease. They are also high in dietary fiber, antioxidants, vitamins, and minerals, contributing to their growing popularity as health-promoting functional foods. They are

dicotyledonous plants that differ from true cereals in both structure and function. Unlike cereals, which are derived from grasses, pseudocereals originate from broadleaf plants belonging to various botanical families. Additionally, pseudocereals are rich in bioactive compounds that promote human health, contributing to a reduced risk of conditions such as cancer, diabetes, hypertension, and heart disease, as well as mitigating oxidative stress due to their high phenolic content. Notably, the northeastern hills of India cultivate a variety of pseudocereals, including amaranth, buckwheat, and quinoa.

1.2.2 Literature review on underutilized cereals and legumes (2020 - 2024)

Over the past four to five years, underutilized cereals and legumes have seen a surge in comprehensive literature reviews, with researchers critically emphasizing on their growing importance as sustainable food sources. These reviews have covered a wide range of topics highlighting the nutritional richness of various millets and cereals, which are often overlooked in mainstream agriculture. Research during this period focused on their high protein content, dietary fiber, and an array of bioactive compounds such as polyphenols, flavonoids, and phytochemicals that offer significant antioxidant, anti-inflammatory, and anti-diabetic properties. Additionally, these reviews emphasize the potential of underutilized crops in addressing food security and climate resilience due to their adaptability to marginal environments and low-input agriculture. However, few gaps remain recurring challenges highlighted across studies. An analysis of a few review papers conducted over the past five years has revealed significant insights and highlighted critical research gaps that still need to be addressed. Table 1.1 below summarizes these reviews' major findings and research gaps.

Table 1.1: Main points and research gaps from some review publications on underutilized cereal crops of India from 2020-2024

Ref.	About	Cereals/legumes/millet used	Major findings	Research gaps
[23]	Underutilized vegetables and fruit crops	Pearl, finger, foxtail, proso, barnyard, kodo, wheat, rice, maize	❖ Resource conservation, production potential, and marketing strategies of underutilized crops of India.	❖ In-depth nutritional profile of underutilized crops (cereals, legumes, millets, pseudo-cereals) was less studied.
[24]	Extrusion modified underutilized cereal flour	Finger millet, proso millet, sorghum, quinoa, amaranth, oats, adlay	❖ Chemical composition of extrusion-modified underutilized cereal flour (EUCF). ❖ Extensive study of pasting property, water absorption capacity, in-vitro enzyme digestion, and physiological functions of EUCF.	❖ Limited study on nano structural effect. ❖ Limited study on how extrusion impacts the bioavailability of nutrients. ❖ Extrusion effect on many other underutilized cereals and legumes is underexplored.
[25]	Underutilized pulses in improving global food security and building climate-resilient food systems	African yam bean, adzuki beans, moth beans, horse gram, lablab, rice bean, winged bean, bambara bean, lima bean, yard long bean, tepary bean.	❖ Genetic diversity and breeding potential. ❖ Detailed study of germplasm. ❖ Abiotic stress adaptation. ❖ Nutritional and health benefits of thirteen pulses. ❖ Genetic resources conservation. ❖ Genome sequencing. ❖ Potential for transgenic manipulation and breeding.	❖ Nutritional bioavailability studies are missing. ❖ Little focus on the environmental sustainability. ❖ In-depth nutritional quality of pulses is missing.
[25]	Underutilized millets	Finger millet, pearl millet, foxtail millet, proso millet, little millet, kodo millet, barnyard millet	❖ Nutritional benefits. ❖ Climate resilience. ❖ Solutions and policy recommendations for the promotion of millets.	❖ Limited information on millet-based food products. ❖ Little information on consumer preferences. ❖ Phytochemicals and their health benefits were not covered.

Ref.	About	Cereals/legumes/millet used	Major findings	Research gaps
[26]	Pseudocereals	Amaranth, quinoa, buckwheat, chia, wattle seeds	❖ Different methods of protein extraction and isolation from pseudocereals. ❖ Effect of processing method on protein quality. ❖ Health benefits. ❖ Anti-nutritional substances.	❖ Limited research on underutilized pseudo-cereals. ❖ Lack of knowledge on digestibility of pseudocereal's protein.
[27]	Underutilized grains	Amaranth, chia, lupin, quinoa, buckwheat, barley	❖ Nutritional value of underutilized grains incorporated into functional foods. ❖ Production of bioactive peptides. ❖ Extraction methods for bioactive peptides and their application in food, pharmaceuticals, and nutraceuticals.	❖ Limited knowledge of specific grains. ❖ Lack of in-vivo studies. ❖ Studies on environmental impact are missing.
[28]	Underutilized legumes	Mung beans, horse gram, Bambara groundnut	❖ Significance and nutritional profile of underutilized legumes. ❖ Potential benefits in agriculture, nutrition, and environmental sustainability.	❖ Lack of comparative study. ❖ Impact of climate change. ❖ Limited research data on specific legumes.
[29]	Underutilized legumes	Bambara groundnut, jack bean, lima bean, sword bean, velvet bean, winged bean, fenugreek, marama bean, African yam bean	❖ Nutritional profiling ❖ Health benefits ❖ Intercropping and nitrogen fixation ❖ Food security	❖ Limited knowledge of processing and anti-nutritional factors. ❖ Scalability of nitrogen fixation. ❖ Intercropping benefits.
[30]	Underutilized legume	Moth bean	❖ Nutritional composition ❖ Agricultural significance ❖ Health benefits ❖ Processing	❖ Comparative studies ❖ Novel processing techniques were not discussed. ❖ Breeding and yield improvement.

Ref.	About	Cereals/legumes/millet used	Major findings	Research gaps
[31]	Underutilized legumes	Bambara, lima bean, jack bean, sword bean	❖ Nutritional benefits and physicochemical properties. ❖ Bioactive compounds ❖ Advanced breeding techniques	❖ Lack of comprehensive nutritional profiling. ❖ Limited data on genetic diversity. ❖ Commercialization of legumes challenges.
[32], [33]	Underutilized dessert legumes	*Prosopis cineraria*, *Acacia senegal*	❖ Nutritional profile ❖ Bioactive compounds ❖ Bioenergy potential ❖	❖ In-depth studies on bioactives for health applications were missing. ❖ Limited data on the commercial viability of these legumes.
[34]	Underutilized cereals	Wheat, rice, maize, millets, pseudocereals	❖ Nutraceutical potential ❖ Positive influence of cereals on gut microflora ❖ Cereal-based products and their health benefits	❖ Lack of knowledge on antinutritional factors ❖ Application of cereals in food and nutraceuticals was not explored.

1.3 Neglected and Underutilized Cereals in India

Underutilized cereals refer to plant species that are traditionally grown and consumed within specific regions but have not gained widespread recognition or use on a larger scale [35]. These cereal crops are often overshadowed by staple crops like wheat, rice, and maize, despite their potential to contribute significantly to food security, nutrition, and sustainability. They are notable crops in their countries of origin but are generally deserted by policy makers, plant breeders, agricultural researchers, consumers, and technology providers [1]. These crops are an essential component of the local diet and are locally well-suited to marginal areas. They include a diverse range of species such as millets, pseudocereals, and legumes. These species are known by many appellations

like neglected, underutilized, underexploited, under-researched, miscellaneous crops. Despite their nutritional benefits and adaptability to different climates, these grains are often under-represented in global agriculture and food markets. Table 1.2 represents the classification of these cereals based on their botanical families, regional names, and regions of origin. There are several reasons why these cereals are being neglected or underutilized and are less prominent than staple crops of India, which are listed below:

(i) Limited Awareness and Demand: Many consumers lack familiarity with underutilized crops, resulting in lower demand. The global food system is largely dominated by staple crops, which are more prominently marketed and consumed.

(ii) Economic Factors: Staple grains are often supported by large-scale industrial agriculture, making them more cost-effective to produce, store, and distribute. Underutilized grains, on the other hand, are often grown on a smaller scale, which can make them more expensive and less accessible.

(iii) Agricultural Research and Development: Research, breeding programs, and technological advancements have focused heavily on staple grains, improving their yields and resilience. Underutilized grains receive far less investment in terms of agricultural development, resulting in lower yields or more limited availability.

(iv) Cultural Preferences: Many regions have long-established traditions centred around staple grains, which have shaped culinary habits and dietary patterns. Underutilized grains, though nutritionally beneficial, may not be as integrated into the local food culture.

(v) Processing and Infrastructure: The infrastructure for processing and distributing staple grains is highly developed, while underutilized grains may require different processing techniques and technologies, which can limit their market presence.

Table 1.2: Classification of some underutilized cereal crops in India

Botanical name	**Family**	**English name**	**Regional name**	**Cultivation regions**
Millets				
Panicum miliaceum	Poaceae	Proso	Cheena, Barri	Uttar Pradesh, Punjab, Haryana, Madhya Pradesh.
Panicum sumatrense		Little	Kutki	Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra
Paspalum scrobiculatum		Kodo	Kodra	Odisha, West Bengal, Jharkhand, Chhattisgarh.
Urochloa ramose L.		Browntop	Koral	Karnataka, Tamil Nadu, Maharashtra, Andhra Pradesh
Echinochloa frumentacea		Barnyard	Sanwa	Uttar Pradesh, Haryana, Gujarat.
Setaria italica		Foxtail	Kangni	Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra
Pseudo cereals				
Chenopodium quinoa	Amaranthaceae	Quinoa	Khaire	Rajasthan, Andhra Pradesh, Maharashtra, Gujrat, Tamil Nadu, Uttar Pradesh
Amaranthus spp.	Amaranthaceae	Amaranth	Chaulai, Rajgira, Ramdana	Gujrat
Avena sativa	Poaceae	Oats	Common oat	Punjab, Haryana, Uttar Pradesh, Odissa
Salvia hispanica	Lamiaceae	Chia	Chia seeds	Karnataka, Tamil Nadu, Andhra Pradesh
Fagopyrum esculentum	Polygonaceae	Buckwheat	Kuttu	Uttarakhand
Chenopodium	Amaranthaceae	Pigweed	Bathua	Uttar Pradesh, Punjab, Haryana

Botanical name	Family	English name	Regional name	Cultivation regions
Legumes				
Vigna angularis	Fabaceae	Adzuki bean	Aduki, azuki	Uttarakhand
Vigna umbellata		Rice bean	Mountain bean, *Naurangi* dal	Manipur, Mizoram, Nagaland
Macrotyloma uniflorum		Horse gram	Kulthi beans, Garhat dal	Karnataka
Vicia faba		Faba bean	Broad bean	Himachal Pradesh, Manipur, Nagaland
Vigna aconitifolia		Moth bean	Matki	Rajasthan, Gujrat, Maharashtra
Vigna radiata		Green gram	Mung/moong	Uttar Pradesh, Rajasthan
Psophocarpus tetragonolobus		Winged bean	Goa bean, Four-angled bean	Kerela, West Bengal, Assam, Tamil Nadu
Vigna unguiculata		Cowpea	Lobhia, black-eyed pea	Uttar Pradesh, Madhya Pradesh, Bihar, Maharashtra
Mucuna pruiens		Velvet bean	Kevanch, kaunch	Kerela, Tamil Nadu, Karnataka, Andhra Pradesh
Lablab purpureus		Hyacinth bean	Lablab bean, Indian bean	Uttar Pradesh, Bihar, West Bengal, Odisha
Canavalia ensiformis		Jack bean	Magic bean, Mole bean	Kerela, Karnataka, Tamil Nadu
Canavalia gladiate		Sword bean	Khadsampal, Badi sem	Kerela, Karnataka, Andhra Pradesh, Tamil Nadu

1.4 Historical & cultural importance in human nutrition

India is home to a rich diversity of underutilized millets and legumes, which have played a significant role in the country's agricultural, cultural, and culinary heritage. Historically, these cereals have been cultivated for thousands of years, adapting to India's diverse climates and regions. They were staples in traditional diets, especially in rural and semi-

arid areas, providing essential nutrition and resilience against climate extremes. Despite being overshadowed by modern staples like rice, wheat, and pulses like chickpeas and kidney beans, these underutilized cereals have a deep historical and cultural significance. Underutilized cereal crops like horse gram, adzuki beans, mung beans, moth beans, rice beans, and little millet have been central to traditional agriculture and local diets across different parts of India. Adzuki beans have been integrated into Indian cuisine and traditional practices, and it is often used to make dal dishes, soups, stews, sprouted salads, and sometimes in sweet dishes, similar to how they're used in Japanese cuisine [36]. These beans are considered light and easy to digest, making them ideal for people with kapha and vata imbalances. Due to their rich protein and fiber content, they are often recommended for people facing digestive issues, helping regulate bowel movements and improve gut health. Similarly, horse gram/kulthi beans are regarded as a "heating" food in Ayurveda, which means it increases internal heat and metabolism. It's often recommended for weight loss and reducing fat [37]. One of the most common traditional uses of horse gram is its ability to help dissolve kidney stones [38]. In both Ayurvedic and folk medicine, people with kidney stones are advised to consume horse gram water or soup regularly. In some parts of India, especially during harvest festivals like Pongal in Tamil Nadu and Makar Sankranti, horse gram is used in traditional dishes to celebrate abundance and prosperity.

1.5 Brief description of some selected underutilized cereals in India

1.5.1 Underutilized Pseudo-cereals

1.5.1.1 Amaranth (*Amaranthus spp.)*

Amaranth, commonly known as ramdana, chaulai or pigweed, belongs to the Amaranthaceae family. There are over 70 distinct species and 400 varieties of amaranth;

however, not all of them are seen on daily menus [39]. This crop is essential and widely grown in India, mainly cultivated in the Kulu, Kinnaur, and Sirmour districts of Himachal Pradesh [40]. It is a cosmopolitan herbaceous plant cultivated as leafy vegetables, ornamental plants, and pseudo-cereals [41]. It is an annual plant, having broad leaves with inflorescence and foliage. The seeds of amaranth are smooth, shiny, small, and circular in shape, having 1-1.5 mm diameter with varying colors and lustrous seed coats. Several species of amaranth, like *Amaranthus dubius, Amaranthus blitus, Amaranthus hypochondriacus, Amaranthus tricolor, Amaranthus edulis, and Amaranthus cruentus,* are grown for their leaves that are used in salads and soups. Some species, like *Amaranthus retroflexus, Amaranthus viridis,* and *Amaranthus spinosus,* are unfit for human or cattle food due to the presence of antinutritional factors like oxalates, phytates and saponins [42]. It is a crop of interest because of its extraordinarily nutritious protein, high micronutrient content, and contains a better balance of essential amino acids than staple crops.

1.5.1.2 Quinoa (*Chenopodium quinoa* Willd.)

Chenopodium quinoa Willd is a starchy dicotyledonous seed having more than 250 species. It is cultivated as leafy vegetables, ornamental plants, and pseudo-cereals [41]. It is an annual plant, having broad leaves with inflorescence and foliage. Its high nutritional value, well-balanced amino acid content, excellent source of micronutrients, and wide adaptability to climate change, make it a “super-food” or “golden grain”. NASA considered quinoa as the optimal food for a controlled ecological life support system, making it a key choice for equipping its rockets for long-duration space missions and addressing protein intake challenges. It is a great source of linoleic acid, which has numerous health benefits. Historically, quinoa was a staple for the Inca civilization, who referred to it as “chisaya mama”, meaning "mother of all grains”.

1.5.2 Underutilized millets

1.5.2.1 Kodo millet (*Paspalum scrobiculatum)*

Paspalum scrobiculatum, also known as cow grass or Indian crown grass. It is cultivated in India, the Philippines, Indonesia, Pakistan, Vietnam, and West Africa. [43] It is recognized by a variety of regional names like kodo (Bengali), kodon (Hindi), varagu (Telegu), kodra (Gujrati, Punjabi, and Marathi), and koduain (Odia). Madhya Pradesh and Tamil Nadu have the maximum kodo millet production and promotion proportion. Kodo millet has more than 400 species, some of which are perineal [44]. This drought-tolerant crop is often grown in semi-arid regions with no intercultural operations. The seeds are monocotyledonous and ellipsoidal, having approximately 2 mm height and 1.5 mm width.

1.5.2.2 Proso millet (*Panicum miliaceum* L.)

Panicum miliaceum L., a grain crop, belongs to the Poaceae family. This crop was domesticated in Northern China about 10,000 BP [45]. It is widely grown in China, India, Nepal, Ukraine, Russia, Africa, Turkey, Romania, and the United States. It is popularly known as common millet, hog millet, broomcorn millet, red millet, gijang, or kashfi millet. It is an annual crop with a 3 mm × 2 mm seed size. It is one of the crops most adapted to rainfed agricultural systems. This crop can be grown in various soil types and under challenging growth circumstances since it is salt-, cold-, alkali-, and drought-tolerant.

1.5.2.3 Little millet (*Panicum sumatrense*)

Panicum sumatrense, commonly known as little millet or kutki, is a millet species in the Poaceae family. It resembles proso millet except that it is smaller. It is majorly

cultivated in Central India. It is an annual herb with round and smooth grains, having a grain size of approximately 2.5 mm × 1.5 mm [46]. This crop can withstand both drought and waterlogging.

1.5.3 Underutilized legumes

1.5.3.1 Adzuki bean (*Vigna angularis*)

Vigna angularis is a significant legume crop cultivated in over 30 countries worldwide. It is also known as adzuki bean, azuki bean, aduki bean, or small red bean. It is mainly grown in China, Japan, South Korea, and Taiwan. After soybean, adzuki bean is Japan's second most important legume. It has been used as an herbal medicine since the Tang dynasty in China to combat obesity [47]. Many varieties are available worldwide depending on grain size, color, climate, harvest time, and cultivation region. There are over 60 varieties available with grain sizes ranging from 3 to 5 mm in width and 5 to 8 mm in length. In India, it is cultivated in the Himalayan region specifically in Almorah. Nowadays, adzuki bean is also gaining attention due to their anti-obesity and antidiabetic properties. Recent studies, including our own investigation on the Indian variety of adzuki bean, have demonstrated its strong potential as an excellent source of bioactive compounds with anti-obesity potential [48].

1.5.3.2 Rice bean (*Vigna umbellata*)

The Fabaceae family member rice bean (*Vigna umbellata*) is a legume cultivated in different parts of the world. Himachal Pradesh, Manipur, Nagaland, Madhya Pradesh, Chhattisgarh, and Tripura are the primary producers of rice bean in India. The rice bean plant, a small vine with a distinctive hairy appearance, produces vibrant yellow flowers and yields small edible beans. These beans exhibit a range of colors, but red and yellow

hues are particularly prevalent. Rice bean is enriched with proteins (majorly albumins: 6.13-7.47% and globulins: 13.11-15.56%), amino acids (all essential amino acids), vitamins (higher content of ascorbic acid and niacin), minerals (sodium, potassium, calcium, phosphorus, magnesium) and fatty acids (higher content of linoleic and linolenic acid) [49],[50]. Their lower fat content and a significant proportion of beneficial unsaturated fatty acids make them a healthy dietary choice. It boasts higher nutritional quality when compared to numerous other legumes/grains within the *Vigna* family. The content of anti-nutritional factors such as phytic acids, trypsin, saponins, and tannins was less than that of other *Vigna* family pulses [51].

1.5.3.3 Mung bean (*Vigna radiata*)

Mung bean or green gram (Family: Fabaceae) has been cultivated in India since ancient times and is considered a native crop of the region. This crop is adapted to all soil types and can be grown for 75–90 days. It is a warm seasonal, annual legume that can flourish in adverse arid and semi-arid conditions [52]. The seed husks, green plants, and harvested pods serve as feed and fodder for animals. The beans are renowned for their high nutritional value and have the highest protein content, ranging from 20-31%. It is a significant source of dietary proteins due to the abundance of essential amino acids, including leucine, phenylalanine, valine, isoleucine, arginine, lysine, and tryptophan. It is a good source of carbohydrates (56%, starch as a principal carbohydrate), dietary fiber (4.1%), and minerals (calcium, iron, phosphorus) [53]. It is also rich in bioactives such as *p*-coumaric acid, protocatechuic acid, ferulic acid, gallic acid, quercetin, catechin, epicatechin, *p*-hydroxybenzoic acid, vitexin, sinapic acid, isovitexin, syringic acid, which offers various potential health benefits and is used in complementary and alternative medicine for its antibacterial, antioxidant, antifungal, hepatoprotective,

antiviral, anti-inflammatory, cardioprotective, antidiabetic, anti-obesity, hypolipidemic, anticancer, and potent chemo-preventive properties [54].

1.5.3.4 Horse gram (*Macrotyloma uniflorum*)

Macrotyloma uniflorum, commonly known as Kulthi beans, Horse gram, or Garhat Dal, is a staple food predominantly consumed in southern India. The seeds are rich in protein (18.5-28.5%), dietary fiber (23%), nutrients like thiamine, vitamin A, and riboflavin, and contain negligible fat [55]. The bioactives present in the seeds are responsible for various physiological benefits, such as relieving intestinal diseases and heart diseases and treating kidney stones, piles, constipation, ulcers, and irregular periods [37],[56].

1.6 Nutritional profile of underutilized cereals

Underutilized cereals in India have significant nutritional value, often surpassing more commonly consumed grains in terms of nutrient density. Using highly nutritious cereals like adzuki bean, amaranth, kodo millet, proso millet, and quinoa to either supplement or completely replace common grains such as corn, rice, and wheat is a beneficial strategy that caters to the modern consumer's preference for healthy, value-added products. These underutilized cereals may be the most excellent solution to suit the needs of the target population because of their good nutritional profile. This section will explore the macronutrient and micronutrient profiles of these crops, focusing on their potential as nutraceuticals.

1.6.1 Macronutrients

1.6.1.1 Carbohydrates

Most of the cereal’s dry weight (50% to 70%) comprises carbohydrates, generally categorized as digestible and non-digestible [57]. Besides their high vitamin, protein, and

mineral content, cereals contain elevated starch levels as a main biopolymeric constituent. Starch is the primary source of physiological energy in the human diet and is present in the seeds' perisperm. The amylase content and size of the starch granules differ between 12-88% and 5-104 μm, respectively. The dietary fiber in plants comes from the cell wall, which comprises a complex mixture of polysaccharides (cellulose, gums, & pectin), waxes, and lignin. [58] The outer layer of these cereals consists of mucilage, a physiological component produced under natural and damage-free conditions. Our previous study reported that the mucilage isolated from underutilized cereals like amaranth, adzuki bean, rice bean, and proso millet is rich in different monosaccharide units like arabinose, xylose, and mannose [59]. We also observed that the swelling index of the isolated mucilage from these cereals showed significant swelling which can be desirable for thickening agents, stabilizing agents, emulsifying agents, and binding agents. Dhillon P.K. & Tanwar B. (2018) reported that rice beans have carbohydrates ranging from 58.15% to 71.99% [60]. According to Saharan et al. (2002) and Katoch (2013), compared to faba bean, rice bean has more number of total sugars (5.0 – 5.6 g/100 g) and non-reducing sugars (4.7-5.3 g/100 g) and less amount of starch (50-55 g/100 g) [49],[61]. Soybean has more raffinose, verbascose, and stachyose than rice beans. Quinoa has more carbohydrate (67% to 74%) content than rice beans, and it also has a small amount of other carbohydrates like monosaccharides, disaccharides, pentosans, and crude fiber [11]. In comparison with buckwheat, faba bean, and quinoa, adzuki bean and mung bean contain proportionally more carbohydrates. Thamarsha et al. (2024), explained in their recent article about the legume-based edible coatings and films. The starch isolated from legumes can be a better source for developing edible films with high nutritional values [62].

1.6.1.2 Proteins

To meet the future protein needs of the expanding global population, alternatives to animal-based protein are required. To address these complex dietary problems, new and unconventional approaches should be used. In India, legumes are widely consumed as an important dietary protein due to a significant vegetarian population. Legume protein is present in the cotyledon, the embryonic axis of seeds, and in small amounts in the seed coat. The presence of essential amino acids, which the human body cannot synthesize, determines the nutritional quality of proteins. The essential amino acids (EAA), which the human body cannot synthesize because of the existence of the carbon skeleton, are the most significant component of protein from a nutritional point of view. Therefore, they must be included in the diet. Cereals are considered a substantial source of dietary protein. Cereals and pseudocereals like amaranth, kodo millet, and quinoa have higher protein quantities than grains like wheat, rice, and maize. The protein content varies between the cereals: amaranth contains 16.0 g (per 100 g) protein, while barley contains 11.0 g (per 100 g) protein [63]. The high arginine and histidine in amaranth and quinoa make them attractive for adults' nutrition.

1.6.1.3 Fat

Studies have shown that pseudo-cereals have a higher amount of fat (unsaturated fatty acids) than cereals. Fatty acids are generally classified as saturated, monounsaturated (MUFA), polyunsaturated (PUFA), and trans-fat. Among these fatty acids, MUFA and PUFA (also known as essential fats) have several beneficial impacts on cardiovascular diseases and insulin sensitivity. Squalene, a triterpene PUFA found in whale and shark liver oil, is abundant in palm and olive oil. Among pseudo-cereals, amaranth oil is the richest source of squalene. Squalene derived from pseudo-cereals shows a wide range

of applications in the cosmetic and pharmaceutical industries. Alvarez-Jubete et al. reported that quinoa and amaranth seeds are a rich source of ω-6 fatty acids [64]. They found that the greater the degree of unsaturation in lipids, the greater the nutritional value of the cereal. Adzuki beans, horse gram, and rice beans are rich sources of linoleic acid. In a study conducted by Zhu et al., it was found that α-linolenic acid can help regulate blood lipids, reduce blood pressure, protect against cardiovascular diseases, support weight loss, and cerebrovascular diseases, inhibit cancer, reduce inflammation, function as an antioxidant, and contribute to anti-aging effects [65].

1.6.2 Micronutrients

Cereals are enriched with several phytochemicals that not only contribute to their flavor, color, and odor, but also have potential effects on metabolic functions in humans (Figure 1.2). A brief discussion of phytochemicals present in underutilized cereal crops is given as follows:

1.6.2.1 Phenolic compounds

Phenolic compounds or polyphenols are naturally occurring organic molecules that contain at least one hydroxyl group directly attached to an aromatic ring. There are over 8000 phenolic compounds currently known that possess significant health-promoting properties [66]. Based on different molecular weights and basic chemical structures, there are different classes of phenolic compounds viz; tannins, lignans, flavonoids, coumarins, phenolic acids, xanthones, stilbenoids, and quinines. In underutilized cereals and pseudo-cereals such as amaranth, buckwheat, and millets, the most abundant polyphenols are flavonoids, tannins, and phenolic acids. Similarly, in underutilized legumes like adzuki bean, moth bean, horse gram, and mung bean, polyphenols like

phenolic acids, lignans, and flavonoids are ubiquitous [67]. Polyphenol content varies based on growing conditions, cereal variety, and processing techniques. Quinoa contains flavonoids, hydroxycinnamic acids, and betalains as the primary polyphenols, contributing to its total antioxidant potential. On the other hand, mung bean contains phenols, organic acids, esters, and aldehyde compounds as the major polyphenols. Yeon Ju An et al. (2020) identified 12 phenolic compounds in mung bean flour, including gallic acid, gentisic acid, p-coumaric acid, protocatechuic acid, p-hydroxybenzoic acid, salicylic acid, caffeic acid, rutin, luteolin, orientin, vitexin, and isovitexin, with vitexin and isovitexin being the most plentiful [68].

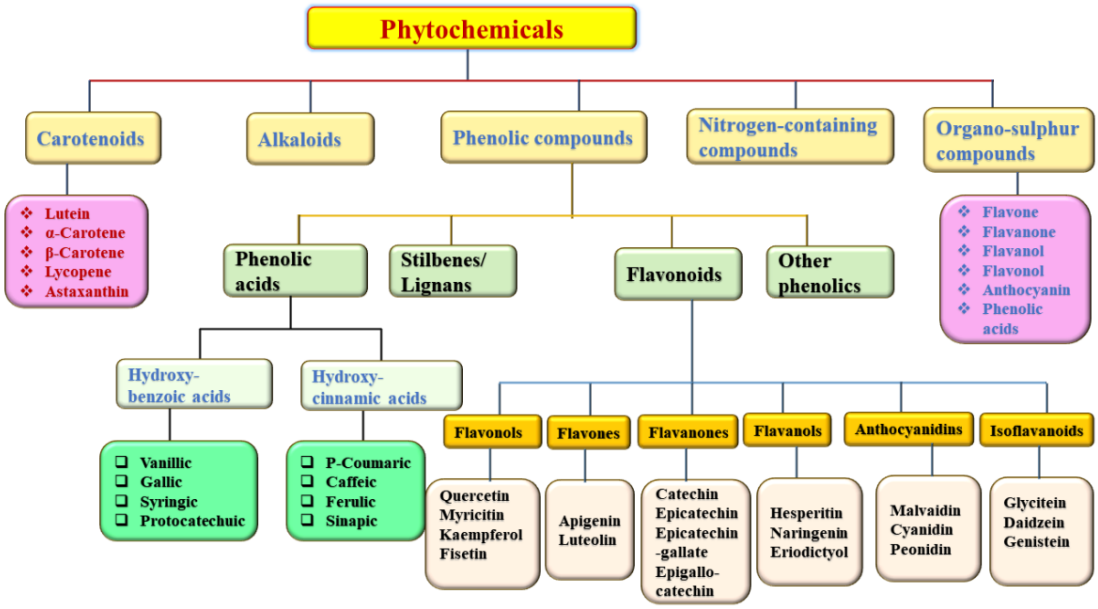

Figure 1.2: Classification of phytochemicals

1.6.2.2 Tannins

Tannins are water-soluble polyphenols with well-known astringent flavors and have been examined for their potential health benefits. It is found largely in the outer layer of cereals/grains, such as hull and bran. It acts as a plant defense system, protecting plants

from pathogens, pests, and environmental damage. Based on structure, tannins can be classified into two categories: a) Condensed tannins and b) Hydrolysable tannins. Condensed tannins or proanthocyanidins are widely present in the seed coat or outer layer of cereals and grains [69], [70]. They are formed by the polymerization of flavan-3-ols, like catechins and epicatechins. They are more stable than hydrolysable tannins due to their resistance to hydrolysis. They offer superior cell-reinforcing properties compared to monomeric phenolic compounds. Sorghum (broomcorn) is the richest source of condensed tannins, oligomers, or polymers composed of flavan-3-ol nuclei [71]. They are also abundant in legumes such as adzuki beans and kidney beans, which contribute to various health benefits such as lowering cholesterol levels, reducing inflammation, promoting the growth of beneficial gut bacteria, and reducing the risk of cardiovascular disease. Hydrolysable tannins are a type of polyphenol made up of a sugar molecule bonded with gallic or ellagic acid. These compounds break down easily when exposed to mildly acidic or basic environments. Their presence is linked to beneficial effects, including antioxidant, antimicrobial, and anti-inflammatory activity [72].

1.6.2.3 Flavonoids

Flavonoids are particularly found in the grain's outermost layers, and their concentration, profile, and types are related to the pericarp thickness and color. It is a secondary metabolite that incorporates flavonols, anthocyanins, and flavanones. Amaranth includes a variety of flavonoids, the most abundant of which are rutin and quercetin [41], [42]. Researchers are particularly interested in anthocyanin flavonoids, which have strong antioxidant properties, and are abundantly present in pigmented sorghum [73].

1.6.2.4 Tocols

Tocols are lipophilic phenolic antioxidants that incorporate tocopherols and tocotrienols. They can be characterised into eight types: α-tocotrienols, β-tocotrienol, γ-tocotrienol, δ-tocotrienol, α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol [74]. They are naturally occurring antioxidants found in many cereal grains like rice, oats, rye, barley, and many more, and are well-known for their bioactivity. The most prevalent tocol in amaranth (*Amaranthus cruentus*) is β-tocotrienol, and the least is δ-tocotrienol.

1.6.2.5 Other bioactive compounds

In addition to vitamins, proteins, and phenolic compounds, cereals are a great source of bioactive compounds such as β-glucans, alkyl-resorcinols, carotenoids, phytic acids, and phytosterols. These bioactive compounds play a significant role in the metabolism of humans and animals [75]. Due to the good nutritional profile of cereals, increasing the intake of whole grain food items in our diet might help us avoid diet-related disorders, including cardiovascular and cancer diseases.

1.7 Comparative analysis with staple crops

Underutilized legumes and millets, compared to staple crops like maize, rice and wheat, present a compelling alternative in India's agricultural and nutritional landscape. While mainstream crops have dominated global agriculture due to their yield and ease of cultivation, underutilized crops such as millets and legumes stand out for their nutrient density, resilience, and health-promoting properties.

(i) Nutritional impact: One key area of comparison is protein content. Underutilized legumes such as horse gram, adzuki bean, rice bean, and mung bean are an excellent source of plant-based protein, vital for addressing protein deficiency,

which is common in many parts of India. They contain a significantly higher concentration of protein compared to wheat and rice. For example, adzuki bean, mung bean, horse gram, and rice bean contain up to 19 g/100 g protein, 24 g/100 g, 21 g/100 g, and 21 g/100 g, respectively, compared to wheat, which has around 12-14% protein [48], [50], [76]. These legumes not only contain a higher content of protein but also offer a good profile of amino acids. Similarly, millets are an excellent source of micronutrients and dietary fiber that aid digestion. Finger millet (ragi) is known for its high calcium content, containing approximately 344 mg/100 g, which is far superior to that of rice or wheat.

(ii) Agricultural impact: The other key area of distinctions between underutilized legumes and millets and staple crops is their ability to thrive in marginal environments. These crops are hardy, drought-resistant, and can grow in poor soil conditions with minimal external inputs. Unlike rice and wheat, which require substantial irrigation, fertilizers, and pesticides to produce high yields, millets and legumes can withstand arid conditions and grow with limited water. This makes them particularly valuable in regions of India that face water scarcity and unpredictable monsoon patterns. For instance, crops like little millet, and kodo millet can grow in semi-arid regions where rice cultivation would be impossible due to water constraints. As climate change intensifies, the need for crops that can adapt to environmental stresses is increasingly crucial, and underutilized legumes and millets meet this requirement more effectively than many staple crops.

(iii) Environmental impact: The environmental footprint of growing rice and wheat is significantly larger compared to that of millets and legumes. Rice is notorious for its high-water demand, with vast amounts needed for paddy fields, contributing to

groundwater depletion. Additionally, rice cultivation is a major source of methane emissions, a potent greenhouse gas [77]. Wheat also requires considerable irrigation and the use of synthetic fertilizers, which can degrade soil health over time. Millets and legumes, on the other hand, enrich soil health through nitrogen fixation (in the case of legumes), reducing the need for chemical fertilizers, and can be grown using organic methods with minimal environmental degradation.

1.8 Potential health benefits

Cereal grains are considered one of the most important sources of dietary nutrition for the global population. They are considered the powerhouse of nutrients, and the abundance of numerous bioactive compounds, vitamins, dietary fiber, and protein offers many potential benefits to human health, such as the alleviation of various chronic diseases. In epidemiological research, whole-grain and dietary fiber consumption has been linked to decreased mortality and risks of obesity, cardiovascular diseases, and type 2 diabetes (T2D), and intervention trials have shown benefits in glucose and lipid metabolism, particularly for soluble fiber. Some of the health benefits are presented in Figure 1.3.

1.8.1 Antidiabetic activity

Diabetes mellitus (DM) is a widespread metabolic disorder prevalent worldwide. It is mainly associated with inadequate insulin levels. According to the recent study, secondary metabolites from plants possess excellent anti-diabetic properties [78]. The bioactive compound coumarin Cajanus lactone, present in pigeon peas (*Cajanus cajan L.*), has been shown to possess anti-diabetic properties [79]. Similarly, kidney beans (*Phaseolus vulgaris*) have various bioactive compounds like peonidin, pelargonidin, cyanidin, gallic

acid, and vanillic acid, which show antidiabetic, anti-inflammatory, and antimicrobial properties [80]. These cereals have a significant role in weight management because these cereal-based foods usually contain a low glycaemic index (GI) and thus augment the low-density lipoprotein (LDL) cholesterol and blood glucose regulation. Narasimhan et al. examined the effectiveness of ferulic acid, a bioactive compound in sorghum, barley, rice, and oats, in controlling T2D [81]. One primary therapeutic strategy for DM is to reduce postprandial hyperglycemia by inhibiting the enzyme activity necessary to hydrolyse complex sugars. Shen et al. reported that β-glucan in oats can significantly slow down the absorption of glucose and lower postprandial blood sugar levels [82]. Also, it can lower the GI of foods, and it can also delay starch digestion and absorption by inhibiting amylase activity, resulting in a reduction of blood sugar levels. Legumes like adzuki bean, mung bean, moth bean, and horse gram are excellent sources of protein and contain lower concentrations of fat. When glucose availability is reduced, the amount of glucose absorbed into the bloodstream decreases and insulin usage decreases, leading to a decrease in the glycemic index and postprandial insulinemia response. Legumes, millets, and cereals possess antidiabetic activity through numerous mechanisms, primarily by slowing carbohydrate absorption, regulating blood glucose levels, improving insulin sensitivity, reducing oxidative stress, and improving lipid profiles. Luo et al. (2016) investigated the in-vitro antidiabetic effect of hull and cotyledon of mung and adzuki bean by protease and aldose reductase inhibitory assays [83]. The study revealed that the bean's hull has great antidiabetic potential.

1.8.2 Anticancer activity

Several studies have reported that secondary metabolites in cereals have great potential to alleviate risk factors associated with various types of cancers such as colon, breast,

prostate, and others. The phenolic compounds in coarse cereals show anti-carcinogenic activity, which involves the inhibition and progression of cancerous cells by curbing the growth of lump angiogenesis, metastasis, and transformation of normal human cells [84]. According to Irakli et al., cereals such as rye, oats, barley, and corn are rich sources of phenolic acids like gallic acid, ferulic acid, coumaric acid, hydroxybenzoic acid, and vanillic acid [85]. Gallic acid is an effective anti-cancer agent as it significantly decreases the growth of various carcinoma cells, including human leukemia and human prostate cancer cells. Lycopene, an eminent carotenoid found in chickpea, has been reported to reduce the incidence of prostate cancer. According to the reports, ferulic acid and p-coumaric acid found in foxtail millet, adzuki bean, and mung bean can induce breast cancer cell death [86]. Moreover, several flavonoids, such as troxerutin, apigenin, and myricetin, have shown significant anti-cancer activity. Besides bioactive compounds, these underutilized cereals are excellent sources of dietary fiber. Recent research has revealed that increasing the content of dietary fiber is the best and most feasible way to control the onset of breast cancer [87].

1.8.3 Antiobesity activity

Several studies have suggested that high-fiber diets are directly related to improved body weight management. Cereals high in dietary fiber substantially lower total plasma cholesterol concentration; thus, dietary fiber-rich diets help people maintain a lower body mass index (BMI). Underutilized cereals like oats, rye, amaranth, adzuki beans, and little millet are high in dietary fiber and hence give substantial satiety value, which reduces hunger and thus aids in weight management [8]. Consuming low-GI meals increases cholecystokinin production, suppressing appetite and increasing feelings of fullness. Most cereals, including oats, millet, amaranth, chickpea, adzuki bean, and

mung bean, have low GI (due to high dietary fiber) that may aid in weight loss and obesity reduction [88],[86],[87].

Obesity is mainly associated with increased triglyceride levels, increased low-density lipoprotein (LDL) cholesterol, and decreased high-density lipoprotein (HDL) cholesterol levels. Consuming meals high in saponins causes a 16-24% reduction in plasma cholesterol, as it binds to cholesterol or bile acids and increases the excretion of bound cholesterol via stool [91]. Rice beans (*Vigna umbellata)* and soya beans (*Glycine max)* are rich sources of saponins. Similarly, β-sitosterol, abundant in adzuki beans, little millet, chickpea, and kodo millet, assists in lowering blood cholesterol and the risk of cardiovascular disease. One of our recent articles investigated the anti-obesity potential of two Indian underutilized legumes - adzuki and mung beans by HMG-Co-A reductase enzyme inhibitory activity [48]. The study revealed that the methanol extract of adzuki bean can effectively inhibit the HMG-Co-A reductase enzyme activity as compared to the mung bean. The inhibition of the HMG-Co-A reductase enzyme may reduce cholesterol levels and possibly mitigate obesity. Including low-GI cereals and legumes (such as amaranth and millets) in our daily diet can lead to the production of cholecystokinin (a hormone that suppresses hunger) and increased satiety.

1.8.4 Antimicrobial activity

Bioactive compounds derived from cereals and legumes that function as natural antibacterial agents are known as biocides. These biocides inhibit or kill the growth of microbes without damaging the adjacent tissues or cells. Natural sources such as phytochemicals have been studied for their antimicrobial activity against several microbes. Natural secondary metabolites found in the outermost layer of plant organs,

such as quinine, tannins, lignans, flavonoids, and phenolic acids, function as an inhibitory factor for the physical invasion by microbes. Polyphenols deplete vital micronutrients, hinder microbial metabolism, disrupt the cytoplasmic membrane, and promote cell membrane permeabilization, resulting in microbe death. Cereals and legumes also contain proteinase inhibitors that serve as antimicrobial components. It has been reported that polyphenols from finger millet (*Eleusine coracana)* exhibit antimicrobial activity [92]. The seed coat extract of finger millet had a higher ability to fight bacteria such as *Bacillus cereus* and *Aspergillus flavus*. The hull extract of pigeon pea (*Cajanus cajan)* and chickpea (*Cicer arietinum)*, rich in flavonoids and phenolic acids, shows antimicrobial activity against Gram-positive and Gram-negative bacteria. According to Lopez-Amoros et al., polyphenolic compounds like trans-ferulic acid, hydroxybenzoic phenolic compounds, p-coumaric acid, and vanillic acid found in these underutilized cereals have been linked with antimicrobial activity [93]. Polyphenols isolated from mung bean (*Vigna radiata)* have shown effective inhibition against *Helicobacter pylori* [94]. Moreover, according to Taguri et al., approximately twenty-two polyphenolic compounds showed antimicrobial activity against twenty-six microbial strains [95].

1.8.5 Antioxidant activity

Cereals have long been regarded to be less essential antioxidant sources than fruits and vegetables, even though they contain many antioxidants such as vitamin E, minerals, folates, carotenoids, phytic acid, lignin, and other compounds such as betaine, choline, sulphur amino acids, lignans, and alkyl-resorcinols, that are essential dietary components globally [96], [97]. Phenolic compounds are believed to offer numerous health benefits, which enable them to neutralize free radicals and bind metal ions, thereby preventing

oxidative stress. In both animals and humans, the endogenous production of antioxidants is limited. Consequently, they rely on exogenous sources to combat oxidative stress. These external antioxidants are primarily obtained through the diet, notably from plant-derived foods rich in vitamins A, C, and E, minerals, and polyphenols.

1.8.6 Anti-inflammatory activity

Inflammation is an essential biotic reaction to tissue damage. The immune system releases pro-inflammatory cytokines in response to various stimuli, such as irritation, injury, or infection [8]. Excessive production of pro-inflammatory cytokines like interferon, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) has been linked to the development of major adult health conditions, including cardiovascular disease, joint disorders, allergies, and various forms of cancer. Therefore, reducing the overproduction of these pro-inflammatory cytokines is crucial for managing and avoiding chronic diseases. The anti-inflammatory activity of some underutilized cereals is primarily due to their phytochemical composition, which includes a variety of bioactive components such as flavonoids, polyphenols, and antioxidants. These substances have been shown to have anti-inflammatory properties by inhibiting the production of inflammatory cytokines and enzymes. Sur et al. studied the anti-inflammatory potential of avenanthramides, phenolic compounds present in oats (*Avena sativa*), by observing the significant inhibition in TNF- α induced nuclear factor kappa B luciferase activity and subsequent reduction of interleukin-8 (IL-8) release [98]. According to Noratto et al., the phenolic compounds isolated from quinoa (*Chenopodium quinoa Willd*) were effective in reducing IL-8, IL-1, and TNF cytokine levels in cultured colonic epithelial Caco-2 cells, while also alleviating obesity-induced inflammation and enhancing gastrointestinal health in mice [99].

1.8.7 Celiac disease safety

Celiac disease manifests as gluten-sensitive enteropathy in genetically sensitive people. It is characterized by constant lesions of the intestinal mucosa caused by the ingestion of gluten, and the mucosa can completely recover because of the total removal of gluten from the diet [42]. The withdrawal of gluten-containing dietary products is the only therapy known to patients diagnosed with celiac disease. The underutilized crops such as millets, adzuki bean, horse gram, mung bean, and amaranth are gluten-free, making them highly suitable for individuals with celiac disease. According to the reports, amaranth grain has long been utilized by celiac disease patients since it does not induce allergic responses in the intestinal mucosa [100],[101].

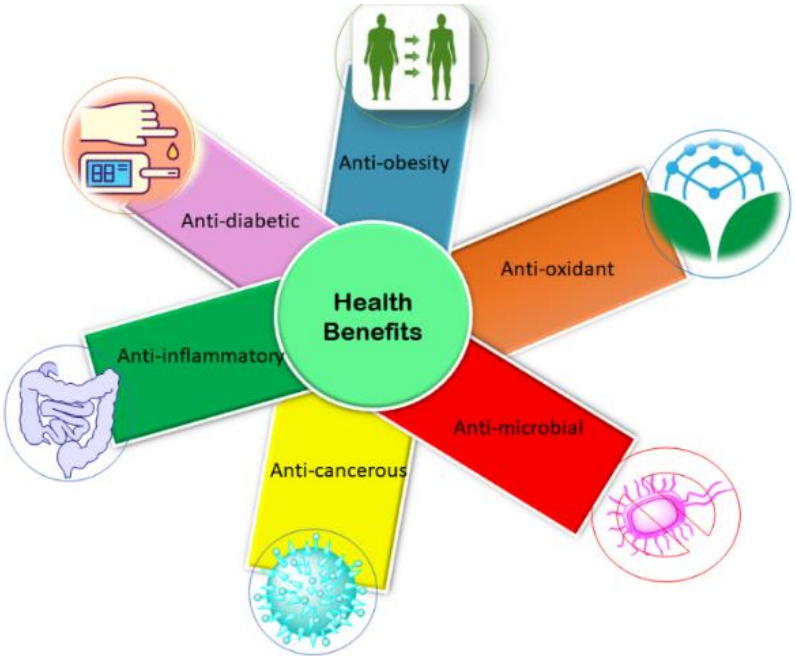

Figure 1.3: Health benefits associated with underutilized cereal crops

In summary, cereals are indispensable to global diets, yet overdependence on major staples has created nutritional gaps and health challenges. Underutilized cereals of India such as Kodo millet, Little millet, Foxtail millet, Proso millet, and Barnyard millet are

emerging as promising alternatives due to their rich nutrient composition, resilience to climate stress, and abundance of bioactive phytochemicals. A review of available literature shows that while some progress has been made in documenting their nutritional value, systematic phytochemical profiling, functional evaluation, and product development remain limited.

The gaps identified in existing studies underline the need for comprehensive investigation using advanced analytical techniques and biological assays, coupled with efforts to develop nutrient-rich, consumer-acceptable products. This research, therefore, seeks to fill these gaps by generating scientific evidence on the phytochemical composition, nutritional quality, and functional potential of selected underutilized cereals of India. Accordingly, the next chapter outlines the scope of the present work, detailing the specific research objectives, rationale, and the framework within which this study has been carried out.

CHAPTER 2
SCOPE OF WORK AND RESEARCH OBJECTIVES

2.1 Introduction

Over the past few decades, India has witnessed rapid economic growth and urban expansion, transforming the lives of millions. Yet, this progress has come with a hidden cost - the steep rise of chronic illnesses such as diabetes, hypertension, cardiovascular diseases, and obesity. The shift toward urban living, combined with sedentary lifestyles and unhealthy dietary habits, has fueled a growing preference for calorie-dense, refined foods while traditional, nutrient-rich options have been pushed aside. At the same time, malnutrition, lifestyle disorders, and unsustainable farming practices highlight the urgent need to rethink our food systems. This calls for a greater focus on crops that are not only nutritious and climate-resilient but also locally available. Among these, underutilized cereals stand out, having nourished indigenous communities for centuries, yet they remain largely neglected in both scientific research and commercial use.

India, with its rich agricultural heritage and biodiversity, is home to several such cereals - adzuki beans, horse gram, mung beans, little millet, kodo millet, proso millet, and many more - which grow well under low-input, rainfed conditions and have considerable potential for nutritional and functional food applications [102], [103]. These cereals are highly nutritious, rich in fiber, slow-digesting carbohydrates, essential amino acids, and bioactive phytochemicals. Their ability to grow under minimal input conditions makes them valuable for climate-resilient agriculture, particularly in marginal areas [26], [104]. However, they remain underexplored in terms of phytochemical composition, biological

activity, and food product development, representing a substantial gap in scientific knowledge.

The present research is driven by the need to bridge this gap by conducting a comprehensive investigation into the phytochemical composition, nutritional, and biological profiles of the selected underutilized cereals in India. Through advanced analytical techniques, biological assays, and food product development, this study aims to unlock the health benefits of these traditional cereals and support their reintroduction into modern diets as sustainable and health-promoting ingredients.

2.2 Importance of underutilized cereals

India is home to more than 50 species of small millets and minor cereals, many of which are locally adapted and climate resilient [1]. While major cereals such as rice and wheat dominate agricultural practices and consumption patterns, underutilized cereals contribute to dietary diversity and agro-biodiversity conservation.

- Kodo millet is known for its high dietary fiber and antioxidant activity [44].
- Proso millet is rich in protein and essential amino acids like leucine and isoleucine [105].
- Little millet is a good source of iron, magnesium, and zinc [106].

Despite being termed "Nutri-cereals," their production area and daily intake have declined, and public awareness of their value is minimal.

2.3 Nutritional and phytochemical potential

Several studies have indicated that underutilized cereals are not only sources of essential nutrients but also contain phytochemicals with therapeutic potential, such as:

- Phenolic acids (e.g., ferulic acid, p-coumaric acid)
- Flavonoids (e.g., quercetin, apigenin, luteolin)
- Tannins
- Phytosterols (e.g., β-sitosterol, stigmasterol)
- Saponins and alkaloids

These bioactive compounds contribute to various health benefits including antioxidant, anti-inflammatory, anti-diabetic, hypocholesterolemic, and anti-obesity effects [107], [108].

However, much of the existing research is limited to basic nutritional analysis, with little focus on comprehensive phytochemical profiling using high-end analytical platforms like GC-MS and UHPLC-QTOF-MS, or on linking these profiles with biological activities.

2.4 Statement of the problem

The growing dependence on a limited number of staple cereal crops such as rice, wheat, and maize has resulted in dietary monotony, micronutrient deficiencies, and increased vulnerability to climate change- driven production risks. In contrast, underutilized cereals of India, particularly small millets and minor grains, offer unique advantages due to their resilience, nutritional richness, and abundance of bioactive compounds. However, their potential remains largely untapped.

Although recent investigations have reported the presence of diverse phytochemicals such as flavonoids, phenolic acids, and phytosterols in cereals like little millet, kodo millet, adzuki beans, rice beans, and mung beans, the majority of research is limited to basic nutritional profiling and selective phytochemical screening [48],[109].

Comprehensive characterization using modern analytical tools (GC-MS, UHPLC-QTOF-MS) is still scarce, and systematic attempts to correlate phytochemical composition with biological activities such as antioxidant, anti-diabetic, and anti-obesity effects are minimal. Moreover, while studies highlight their therapeutic relevance, there has been limited progress in translating these findings into functional food products or validated nutraceutical applications.

Given the dual challenges of nutritional insecurity and lifestyle-related disorders, such neglect represents a critical research gap. There is a pressing need to establish scientific evidence that validates the nutritional and phytochemical potential of these cereals, links their bioactive compounds with health-promoting activities, and explores their incorporation into value-added food products. Furthermore, the promotion of underutilized cereals contributes not only to human health but also to sustainable agriculture, climate resilience, and biodiversity conservation.

2.5 Rationale and need for the study

This study is important because it addresses both nutritional and health challenges that India and the world are facing today. Modern diets rely heavily on a few staple cereals like rice and wheat, which provide energy but are often low in micronutrients and bioactive compounds. On the other hand, underutilized cereals such as kodo millet, little millet, foxtail millet, proso millet, and barnyard millet are naturally rich in dietary fiber, minerals, essential amino acids, and phytochemicals that can help in preventing diseases like diabetes, obesity, and heart problems.

Despite these benefits, these cereals have not been studied enough. Most available research only looks at their basic nutritional content, while detailed studies on their

phytochemical composition and biological activities are very limited. There is also very little work on how these cereals can be used to make modern, value-added food products that are both healthy and acceptable to consumers. This study becomes imperative for the following reasons:

- Lack of detailed phytochemical studies using modern techniques such as gas chromatography-mass spectrometry (GC-MS) and ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS).
- Limited understanding of their role in metabolic disorders, especially obesity, diabetes, and cardiovascular health.
- Scarce exploration of amino acid and mineral profiles, which are essential for understanding nutritional quality.
- Underdeveloped value chains and food products due to the absence of data on functional and sensory properties.

By using advanced analytical methods, this study will provide comprehensive information about the phytochemicals present in selected underutilized cereals and their health-promoting activities. It will also explore their use in developing nutrient-rich cookies and flakes, making them more relevant for present-day food habits.

Therefore, this research is justified because it will:

- Generate scientific evidence about the nutritional and phytochemical richness of underutilized cereals.
- Show their potential health benefits through biological activity studies.

- Promote their use in functional foods and nutraceuticals.
- Support sustainable agriculture and food diversity by encouraging the wider use of climate-resilient cereals.

In this way, the study will contribute not only to academic knowledge but also to public health, food industry innovation, and sustainable food security.

2.6 Research gap

The following gaps were considered for this research work based on the literature survey:

I. In-depth studies on the bioactive compounds of Indian-based underutilized cereals are not widely available.
II. These Indian-based underutilized cereals have not been studied for their nutraceutical application (weight loss).
III. There is a need to have cereal-based products to combat obesity.

2.7 Research objectives

This research work aimed to investigate the phytochemicals of underutilized cereals in India. To achieve this, the detailed objectives of the research work are as follows:

I. Isolation of active metabolites from the underutilized cereals
II. Characterization of active metabolites
III. Biochemical assay for anti-obesity
IV. Product formulation and other applications from the underutilized cereals

2.8 Overview of the thesis

To fulfil the above objectives, the work embodied in the thesis entitled **"Phytochemical Investigation of Some Underutilized Cereals in India"** has been divided into six chapters and is organized as follows:

Chapter 1 presents the introduction and literature review for the thesis investigating phytochemicals in selected underutilized cereals in India. It opens with a general overview of the research theme, followed by essential background on phytochemicals and various extraction methods. The chapter also highlights the importance of these lesser-known cereals in tackling contemporary food and nutritional security issues. Additionally, it reviews current scientific insights into their nutritional value and health-promoting properties, particularly concerning the prevention and management of non-communicable diseases.

Chapter 2 outlines the scope of the present work, which focuses on exploring the phytochemicals of underutilized cereals of India. It emphasizes the significance of exploring these crops for their health-promoting compounds and functional food applications. The chapter extensively examines and clarifies the methodology of the present work. The chapter defines the research problems and questions to be addressed, followed by a clear statement of the research objectives. It presents the organizational structure of the thesis, outlining how the subsequent chapters develop the research narrative. Finally, the chapter concludes by emphasizing the need for scientific validation of the therapeutic potential of these indigenous cereals through phytochemical investigations.

Chapter 3 provides detailed information on the materials and experimental procedures

used in the present study. It outlines the selection, collection, and authentication of underutilized cereals, followed by sample extraction steps. The chapter describes the procedures for proximate analysis, phytochemical extraction, and estimation of bioactive compounds. It also includes methodologies for evaluating antioxidant, antimicrobial, and anti-obesity activity, such as HMG-CoA reductase and pancreatic lipase inhibition assays.

Chapter 4 presents the experimental findings and a detailed discussion. It includes the results of proximate composition, revealing the nutritional richness of the selected underutilized cereals. The chapter also highlights the quantitative and qualitative estimation of key phytochemicals. The biological activity assays demonstrate significant lipase inhibitory potential, suggesting health-promoting properties. These findings are compared with existing literature to validate and interpret the results. The discussion emphasizes these cereals' nutritional and therapeutic relevance, supporting their potential use in functional foods and nutraceutical development.

Chapter 5 explores the practical applications of underutilized cereals through value-added product development and mucilage extraction. It describes the formulation of functional food products such as cookies and breakfast flakes using selected cereals, highlighting their nutritional and sensory attributes. Additionally, the chapter presents the extraction of cereal-derived mucilage and its potential application in the synthesis of mucilage-based hydrogels. The results demonstrate the versatility of these crops, promoting their utilization beyond traditional consumption and emphasizing their role in health-focused, sustainable product innovation. Also, this underutilized cereal-derived mucilage could be a suitable feedstock as a hydrogel-forming agent, which can

be explored in the food, cosmetic, and pharmaceutical industries.

Chapter 6 summarizes the entire research work reported in the thesis and future scope, highlighting the social relevance of the present research on phytochemical investigation of some underutilized cereals of India. It outlines the key findings, offers recommendations for practical applications, and proposes guidance for researchers aiming to explore this field further. The thesis employs a chapter-wise reference system to ensure clear organization and easy access to source materials.

CHAPTER 3

MATERIALS AND EXPERIMENTAL METHODS

3.1 Introduction

This chapter serves as the methodological backbone of the present research work, systematically detailing the materials, instruments, and experimental procedures adopted throughout the study. It begins with a description of the raw materials and chemicals used, followed by sample preparation steps. The primary characterization instruments employed in this work are outlined along with their working principles. These methodologies provide a comprehensive framework for achieving the research objectives and ensuring scientific rigor. It sets the foundation for understanding how data was acquired and interpreted, contributing directly to the reliability and validity of the research findings.

3.2 Materials

The following materials were selected and prepared specifically to ensure consistency and reliability across all experimental procedures described in this study:

3.2.1 Plant materials

Adzuki beans were purchased from Himjoli Products, Delhi, India. Mung beans were purchased from the Patanjali store, Delhi, India. Rice beans were purchased from Himjoli Products, Delhi, India. Horse gram was obtained from the ICAR-NBPGR, Delhi, India. Little millet and proso millet were procured from the Indian Council of Agricultural and Research, Hyderabad, India. Amaranth was purchased from the Patanjali store, Delhi, India.

3.2.2 Chemicals, reagents, & media

S. No.	Name of chemical	Brand name
1.	1,1-Diphenyl-2-picrylhydrazyl (DPPH)	Sigma, India
2.	Agar-agar	Merck, India
3.	Ascorbic acid	Merk, India
4.	Deuterated-water (D_2O)	Merck, India
5.	Ethanol	Merck, India
6.	Ferric chloride	Merck, India
7.	Dichloromethane	Sigma, India
8.	Methanol	Sigma, India
9.	n-Hexane	Sigma, India
10.	Acarbose	Sigma, India
11.	Nitric acid	Sigma, India
12.	Acetone	Sigma, India
13.	Lead acetate	Merck, India
14.	Dragendorff's reagent	Sigma, India
15.	Mayer’s reagent	Merck, India
16.	Hydrochloric acid	Merck, India
17.	Sulfuric acid	Merck, India
18.	Acetic acid	Merck, India
19.	Chloroform	Merck, India
20.	Fetal Bovine Serum	HIMEDIA, India

3.3 Instrumentation techniques

3.3.1 Mass spectrometry

Mass spectrometry serves as a powerful analytical technique for detecting, characterizing, and quantifying bioactive molecules. It is applied to a broad range of samples, including small organic molecules, metabolites, peptides, proteins, and complex plant extracts, making it highly valuable in fields such as chemistry, biology, food science, and pharmaceuticals. The basic operating principle of this technique is the generation of ions

from the sample, which are then separated according to their mass-to-charge (m/z) ratio. This separation yields valuable insights into the molecular weight and structural characteristics of the analyte.

The system is composed of three main components:

(i) Ionization Source

(ii) Mass Analyzer

(iii) Detector

Ionization of the sample takes place in the ion source through various methods, including matrix-assisted laser desorption/ionization, electrospray ionization, or electron ionization. These ions are then directed into the mass analyzer, where they are sorted according to their m/z values using devices like quadrupole, time-of-flight (TOF), or orbitrap analyzers. Finally, the detector captures the ions, quantifies their abundance, and produces a mass spectrum. This sequential process enables both qualitative and quantitative analysis, revealing the composition and diversity of compounds present in the sample. Unlike many conventional techniques, mass spectrometry can analyze complex mixtures with minimal sample requirements and provides both qualitative and quantitative information in a single experiment. It is capable of detecting compounds at trace levels, identifying unknown molecules, elucidating structural details, and studying molecular interactions. Due to its wide applicability, it is broadly used in pharmaceutical research, clinical diagnostics, food and environmental analysis, metabolomics, proteomics, and natural product investigations.

3.3.2 GC-MS

This is a sophisticated analytical technique that combines the separation capacity of gas chromatography with the detection and identification capabilities of mass spectrometry. It is commonly used to conduct qualitative and quantitative analyses of volatile and semi-volatile compounds found in complicated mixtures, making it useful in the field of food chemistry, medicines, forensics, and natural product research.

The principle of GC-MS involves two stages: (i) In the first stage, gas chromatography separates the components of a mixture based on their volatility and interaction with the stationary phase of the column. Each compound is eluted at a specific retention time. (ii) Secondly, the separated compounds enter the mass spectrometer, where they are ionized, fragmented, and analyzed according to their mass-to-charge ratio (m/z). This provides both the molecular weight and structural information of the analytes.

The instrumentation of GC-MS includes:

(i) Sample injection

(ii) Vaporization

(iii) Chromatographic separation

(iv) Ionization

(v) Mass analyzer

(vi) Detector

GC-MS is valuable because it offers high-resolution separation and accurate identification of compounds in complex mixtures. It is highly sensitive, able to detect very low concentrations of substances, and provides consistent results with both structural identification and quantification of analytes.

3.3.3 UHPLC-QTOF-MS

Ultra-High-Performance Liquid Chromatography coupled with Quadrupole Time-of-Flight Mass Spectrometry is an advantageous technology that combines the superior separation efficiency of UHPLC with the accurate mass detection capability of QTOF-MS. It is useful for the identification of non-volatile metabolites. It consists of two steps: First, UHPLC separates analytes based on their polarity and how they interact with the stationary phase under very high pressure. This allows for fast and high-resolution separation. Next, the compounds that are eluted are ionized, usually by electrospray ionization or atmospheric pressure chemical ionization, and sent to the QTOF mass spectrometer. There, the quadrupole filters precursor ions and the time-of-flight analyzer measures their exact mass-to-charge ratio with high accuracy and resolution. The working process of UHPLC-QTOF-MS is as follows:

(i) Sample injection

(ii) Chromatographic separation

(iii) Ionization

(iii) Quadrupole filtering

(iv) TOF analysis

(v) Detection

This technique is important because it provides highly accurate mass measurements with up to four decimals, detailed and accurate fragmentation patterns, and structural information, enabling reliable identification of both known and unknown compounds. This technique is a versatile tool for both identifying and measuring substances across fields like pharmaceutical research, metabolomics, and environmental monitoring. It delivers

sensitive, reproducible, and rapid results. Unlike GC-MS, it can analyze large or thermally fragile biomolecules that are not easily vaporized. However, its high cost and complexity can limit its use for routine applications. To identify maximum metabolites, we can analyse the samples using targeted and non-targeted approaches. In a targeted approach, the focus is on identifying specific known compounds and monitoring a predefined list of analytes with high sensitivity and precision. In contrast, the non-targeted approach detects and identifies a wide range of compounds in a sample without prior knowledge, providing a comprehensive chemical profile ideal for discovering new bioactive metabolites.

3.3.4 ICP-MS

This is a technique with a high level of sensitivity that can detect and quantify trace elements and isotopes at trace concentrations, often as low as parts-per-trillion levels. It is commonly utilized in environmental monitoring, food safety, clinical diagnostics, geology, metallurgy, and phytochemical studies that necessitate precise elemental analysis. The basic principle of ICP-MS involves the conversion of the sample into an aerosol, which is then introduced into an argon plasma operating at a very high temperature (about 6000–10,000 K). In the plasma, the sample atoms are ionized to form positively charged ions, which are then directed into the mass spectrometer. The instrument consists of a sample introduction system (usually a nebulizer), an ICP torch that generates the plasma, an interface region to transfer ions into the mass spectrometer, a mass analyzer, and a detector that records ion intensities. The stepwise process can be summarized as follows:

(i) Sample introduction

(ii) Atomization and ionization in plasma

(iii) Ion extraction

(iii) Mass analysis

(iv) Detection

(v) Data output

It plays a vital role in analytical science due to its capability for simultaneous multi-element detection, exceptional sensitivity, fast throughput, and precise isotopic ratio measurements. It is unique because it can handle a variety of sample matrices, such as liquid digests, biological specimens, and environmental extracts, which is what sets it apart from many other spectroscopic methods. Its ability to deliver accurate results at ultra-trace concentrations makes it an essential tool for elemental analysis and contamination assessment across various fields.

3.4 Experimental methods

3.4.1 Authentication of the plant material

The botanical specimens of all the selected plant material were authenticated by Dr. Sunita Garg, Ex-Chief Scientist, CSIR-NIScPR, Raw Materials Herbarium, and Museum, Delhi (RHMD), India.

3.4.2 Preparation of extracts

Different plant materials were extracted using a Soxhlet apparatus [110]. For this, 50 g of each sample (coarsely ground adzuki bean, mung bean, rice bean, little millet and horse gram) was placed in a thimble and sequentially extracted using different polarity solvents (n-hexane, DCM, and methanol). In order to ensure that the extraction process was complete, exhaustive extraction was used with each solvent for 10-12 h. The

obtained extracts were concentrated using a rotary evaporator maintained at 40 °C under reduced pressure. After labelling, the concentrated extracts were stored at 4 °C until further use.

3.4.3 Phytochemical analysis

The qualitative analysis of different extracts of all the selected legumes was done following the standard methods [110],[111]. Various chemical tests such as Dragendorff's test (for alkaloids), Shinoda's test (for flavonoids), Ferric chloride test (for tannins), Lead acetate test (for phenols), Killer killiani test (for glycosides), terpenoids test, and Liebermann-Burchard test (for steroids) were performed.

3.4.3.1 Alkaloids

Presence of alkaloid was estimated by Dragendorff's test. For this, 0.2 mL of prepared extract was treated with a few drops of Dragendorff's reagent. The absence of orange to reddish precipitate indicates the absence of alkaloids in the extracts.

3.4.3.2 Flavonoids

Presence of flavonoids was estimated by Shinoda's test. A small piece of magnesium ribbon was added to 2 mL of plant extract, then some concentrated HCl was added. The appearance of a red, orange, or pink coloration indicated the presence of flavonoids.

3.4.3.3 Tannins

Tannins were assessed by using the Ferric chloride method. 1 mL of extract was diluted with distilled water, and 2 drops of $FeCl_3$ were added. Instant appearance of black color indicates the presence of tannins.

3.4.3.4 Phenols

For the detection of phenols, a lead acetate test was performed. 2 mL of plant extract was treated with a few drops of lead acetate solution. The absence of white precipitate was taken as a negative indication of phenols in the sample.

3.4.3.5 Glycosides

For the detection of glycosides, the Keller–Killiani test was performed. 2 mL of plant extract, 1 mL of glacial acetic acid containing a trace of ferric chloride, was added, followed by 1 mL of sulfuric acid (concentrated) along the sides of the test tube. The appearance of a reddish-brown ring at the junction of the two layers, gradually turning bluish green, was considered a positive indication for the presence of glycosides.

3.4.3.6 Steroids

The presence of steroids was determined using the Liebermann-Burchard test. 2 mL of the prepared extract was mixed with 2 mL of acetic anhydride, followed by the slow addition of 1 mL of concentrated sulfuric acid along the wall of the test tube. The appearance of a green or bluish-green color confirmed the presence of steroids.

3.4.4 Proximate analysis

To quantify the nutritional composition of the selected cereals, a proximate analysis was conducted according to the Association of Official Analytical Chemists (AOAC) protocols to measure dietary fiber, protein, fat, and carbohydrate content [112].

3.4.5 Elemental analysis using ICP-MS

For elemental analysis, 0.25 g of each sample was diluted in 10 mL nitric acid and then analysed by ICP-MS. The same procedure was used to analyse NIST standards. The

blank sample solution was also prepared following the above procedure without adding the sample. The instrument calibration was assessed by analysing three certified samples from NIST [113].

3.4.6. Fatty acid profiling

Fatty acid content was assessed through fatty acid methyl ester (FAME) analysis, based on the protocol outlined by Ryan et al., with minor adjustments [114]. Precisely, 0.15 g portion of the extracted oil was placed in a 5 mL glass vial. To this, 200 µL of 2 N methanolic potassium hydroxide (KOH) was added, followed by vortexing for 2 min. The mixture was then incubated in a water bath shaker at 55 ºC for 10 min. After cooling, 1.0 mL of 5% hydrochloric acid (HCl) was introduced, and the sample was reheated at 70 ºC for another 10 min. Subsequently, 2 mL of petroleum ether was added, and the solution was vortexed again for 2 min. The upper organic layer was carefully separated and subjected to analysis using GC-MS (Shimadzu, GC-2010 instrument).

3.4.7 Gas-chromatography-mass spectrometry analysis

To determine the volatile compounds in the different crude extracts of selected samples, GC-MS analysis was done on GCMS-QP2010 (Shimadzu-Europe). Samples were injected into the GC-MS under the following conditions: Helium gas was used as the carrier gas. The samples were introduced in split mode at 260 ºC. The column flow rate was 1.23 mL/min in electron ionization (EI) mode. The ion source temperature was 220 ºC, and the interface temperature was 270 ºC. The oven temperature was planned for 60 ºC (2 min) to 280 ºC (26 min), and the solvent delay time was 3.50 min. The NIST (National Institute of Standards and Technology, version 1.10 beta, Shimadzu) mass spectral database was used to identify the separated peaks [115].

3.4.8 UHPLC-QTOF-MS analysis

To analyze secondary metabolites present in various crude extracts of the selected samples, UHPLC-QTOF-MS was employed, following a modified version of a standard protocol [116]. The mass spectrometry was carried out in electrospray ionization positive mode (ESI+), with the following source parameters: a source temperature of 120 °C, desolvation gas flow rate of 950 L/h, cone gas flow of 50 L/h, and a capillary voltage set at 3.22 keV. The instrumentation included a Waters SYNAPT-XS HDMS system (UK), equipped with a controller, autosampler, degasser, AD pump, and AD column, integrated with a QTOF-MS detector. For sample preparation, each extract was first combined with 10 mL of 1% formic acid in water and allowed to stand for 10 min. Subsequently, 10 mL of methanol and 10 mL of acetonitrile were added, followed by vortexing for one minute and centrifugation at 5000 rpm for five minutes. The resulting supernatant was diluted using acidified water before being introduced into the analytical system. Chromatographic separation was achieved using a 100 mm × 2.1 mm C18 column (Waters, Acquity BEH 2.1), with a 5 µL injection volume. Data acquisition and interpretation were carried out using ChemSpider software.

3.4.9 Amino acid analysis

The selected samples were examined for their amino acid composition using the L-8900 Automatic Amino Acid Analyzer (Hitachi Co. Ltd., Tokyo, Japan), in accordance with the methodology outlined by Chakrabarti *et al.* [117]. Each sample was dried, finely ground, and then hydrolyzed with 6 N HCl at 110 ºC for 22 h. Following hydrolysis, the samples were dried using a nitrogen evaporator (PCi Analytic Private Limited, Maharashtra, India) and then reconstituted in 0.02 N HCl prior to loading into the auto sampler. Each sample (20 µL) was injected for analysis.

Tryptophan, cysteine, and methionine required special treatment due to degradation during acid hydrolysis: cysteine and methionine were oxidized with performic acid and treated with hydrobromic acid; tryptophan was hydrolyzed using methane sulfonic acid and 3-(2-aminoethyl) indole. Other amino acids followed the standard protocol. Detection wavelengths were 440 nm for hydroxyproline and proline, and 570 nm for others. Quantification was based on peak area comparison with known standards, including Type B and AN-2 mixtures (Wako Pure Chemical Industries, Limited, Japan) and freshly prepared tryptophan and glutamine (Sigma-Aldrich, USA).

3.4.10 In-vitro biological assays

3.4.10.1 Antioxidant assay (DPPH method)

The antioxidant activity of different extracts of selected samples (horse gram, rice beans, & little millet) was evaluated using DPPH free radical scavenging assay, following the procedure explained by Banjara *et al*., with slight modifications [118]. Different concentrations of the methanol extract of both the samples were made, and 1 mL of each extract was mixed with 3 mL of DPPH solution. The prepared mixture was incubated for 30 min at room temperature, and the absorbance was measured at 517 nm using a UV spectrophotometer. Ascorbic acid was used as a positive control.

$$\% \text{ Scavenging activity} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of Control}}\right) \text{x } 100$$

3.4.10.2 Antimicrobial activity (Disc diffusion method)

The antimicrobial activity of the methanolic extract of selected samples (horse gram, rice bean, and little millet) was performed, following the method with slight

modifications [119]. After pouring 20 mL of the nutrient media into sterilized petri dishes, it was allowed to solidify. After that, 20 µL of each extract was tested against *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacteria. The bacteria were grown in an incubator at 37 °C and then left for 24 h. After the incubation period, the zone of inhibition was measured.

3.4.10.3 Cytotoxicity assay (Neutral red uptake assay)

Cytotoxicity of the methanol extract of adzuki and mung bean samples on 3T3-L1 cell line (Procured from NCCS Pune) was determined by NRU Assay. The 3T3-L1 cells (5000-8000 cells/well) were cultured in 96 well-plates for 24 h in Dulbecco's Modified Eagle Medium (DMEM- AT149-1L) supplemented with 10% Fetal Bovine Serum (FBS- HIMEDIA-RM 10432) and 1% antibiotic solution at 37 ºC with 5% CO_2. After 24 h, the medium was removed, and fresh culture medium was added to each well of the plate. 5 µL of treatment dilutions (1 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL, and 500 µg/mL) were added to the defined wells and incubated for 24 h. 100 µL of NRU (SRL Chem-36248) (40 µg/mL in PBS – phosphate-buffered saline) was added to the wells and incubated (Heal Force-Smartcell CO_2 Incubator-Hf-90) for 1 h. After that medium was removed, NRU was dissolved in 100 µL of NRU Destain solution. Plates were read at 550/660 nm using an Elisa Plate Reader (iMark BioRad-USA). IC_{50} values of both samples were calculated by using the software Graph Pad Prism -6 [120].

3.4.10.4 Anti-obesity activity (HMG-Co-A reductase enzyme activity)

The enzyme inhibitory activity of HMG-Co-A reductase in the methanol extract of selected samples (adzuki and mung beans) was evaluated using the HMG-Co-A reductase assay kit (Sigma Aldrich, St Louis, Missouri, USA). The enzyme experiments

were performed at 37 ºC as per the manufacturer's instructions. The reaction mixture (200 µL) for both samples was prepared by adding the reagents in the following order: reaction buffer; sample (10 µL); NADPH (10 µL); HMG-Co-A reductase enzyme substrate (20 µL). The samples were incubated for 1 h to complete the reaction. Finally, 100 µL of 4-(Dimethylamino) benzaldehyde reagent (DMAB) was added to each well and incubated for 5 min. The absorbance was then recorded at 490 nm with a microplate reader (iMark Biorad). Enzyme activity was calculated against the slope value obtained from the NADPH standard curve. All assays were performed in triplicate, and all the data were presented as mean ± standard deviation [121].

3.4.10.5 Anti-obesity activity (Pancreatic lipase inhibitory assay)

Following the method previously reported, selected samples (adzuki and mung beans) were evaluated for pancreatic lipase activity, with slight modifications [122]. A stock solution containing 1 mg/1 mL of pancreatic lipase enzyme was prepared using 0.1 mM potassium phosphate buffer at pH 6.0. Sample solutions with varying concentrations (20–100 µg/mL) were then pre-incubated in test tubes containing 200 µL of the pancreatic lipase enzyme solution (1 mg/mL) and 200 µL of phosphate buffer (pH 6.0) at 30 °C for one hour. After this, 200 µL of substrate (20 mM p-nitrophenyl butyrate in acetonitrile) was introduced to the mixture, which was then incubated for 5 min at 30 °C. The quantity of 2,4-dinitrophenol produced was determined by measuring absorbance at 405 nm using a spectrophotometer (Tecan, USA). Orlistat served as the reference standard at concentrations ranging from 20 to 100 µg/mL. The reproducibility of the biological analysis, the experiment was performed in triplicate, n=3.

$$\% \text{ Lipase Inhibition} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Extracts

The present study employed Soxhlet extraction for a more efficient method of isolating bioactive compounds from different cereal samples (adzuki bean, mung bean, rice bean, horse gram, & little millet), using a continuous hot solvent (hexane, DCM, & methanol) extraction process that maximizes extraction yield through repeated solvent cycling. This methodology not only ensured high extraction efficiency due to continuous use of fresh solvent, but also minimized manual intervention and potential variability, thereby producing reproducible results. Different colored extracts were obtained by the Soxhlet method, suggesting the presence of a diverse range of bioactive compounds in the selected samples (Figure 4.1).

The n-hexane extracts of samples generally exhibited a pale yellow color, which is characteristic of non-polar lipophilic substances such as fatty acids, waxes, and certain pigments. In contrast, DCM, being moderately polar, exhibited extracts of intermediate coloration, suggestive of semi-polar compounds including alkaloids, terpenoids, sterols, and certain phenolics. Methanol-based extracts appeared significantly darker, suggesting the presence of highly polar phytochemicals such as flavonoids, tannins, saponins, and glycosides. These findings demonstrate that the polarity-based extraction strategy employed was effective in extracting a wide spectrum of phytochemical compounds, thereby providing a comprehensive representation of the chemical diversity present in the cereal samples.

Figure 4.1: Different extracts of underutilized cereals

4.2 Phytochemical analysis

The qualitative analysis of different extracts (n-hexane, DCM, & methanol) of selected samples revealed the presence of several therapeutically significant classes of compounds as presented in Table 4.1. The qualitative analysis data revealed the presence of reducing sugars, flavonoids, and alkaloids, demonstrating the richness of the samples in essential metabolites with potential bioactive properties. Reducing sugars is vital for energy sources and plays a significant role in maintaining metabolic functions. The identification of flavonoids is of special significance due to their well-documented antioxidant, anti-inflammatory, and cardioprotective effects, demonstrating the potential of the investigated samples as functional food ingredients. In addition, alkaloids' presence suggests potential therapeutic relevance, as this group of compounds has been widely reported for various pharmacological functions, including antimicrobial, anti-hypertensive, and neuroprotective effects.

On the other hand, tannins, phenols, and saponins were not identified in any of the extracts. It is possible that the absence of tannins and phenolic compounds was due to their relatively low concentration in the selected samples. Similarly, the absence of saponins suggests that the selected plant sample may not be a significant source of this compound.

Overall, the qualitative assessment offers an initial yet valuable understanding of the phytochemical profiling of the selected samples. The detection of flavonoids and alkaloids points to promising therapeutic properties, whereas the lack of tannins, phenolic compounds, and saponins emphasizes the unique chemical profile characteristic of these underutilized cereals.

Table 4.1: Qualitative phytochemical analysis of selected underutilized cereals

S.No.	Sample	Phytochemicals					
		Alkaloid	**Reducing sugar**	**Flavonoid**	**Tannin**	**Phenol**	**Saponin**
1.	Ab-H	+	-	-	-	-	-
2.	Ab-D	+	-	-	-	-	-
3.	Ab-M	-	-	+	-	-	-
4.	Mg-H	+	-	-	-	-	-
5.	Mg-D	+	-	-	-	-	-
6.	Mg-M	-	+	+	-	-	-
7.	Ku-H	+	-	-	-	-	-
8.	Ku-D	+	-	-	-	-	-
9.	Ku-M	-	+	+	-	-	-
10.	Rb-H	+	-	-	-	-	-
11.	Rb-D	+	-	-	-	-	-
12.	Rb-M	-	+	+	-	-	-
13.	Lm-H	+	-	-	-	-	-
14.	Lm-D	+	-	-	-	-	-
15.	Lm-M	-	+	+	-	-	-

+ = Presence of phytochemicals
- = Absence of phytochemicals
H = n-Hexane
D = Dichloromethane
M = Methane

4.3 Proximate analysis

The proximate analysis of the selected samples was determined to assess their nutritional quality, and the results are presented in Table 4.2. Proximate analysis offers a

comprehensive assessment of the basic nutritional composition of food crops by systematically quantifying major components such as protein, fat, and carbohydrate, which are critical for understanding the relative proportions of macronutrients and their role in meeting dietary needs. This analysis included the estimation of protein, fat, dietary fiber, and carbohydrate content.

4.3.1 Protein content

Protein content in the selected samples varied between 8.7 ± 0.01 to 23.67 ± 0.02 g/100g, which suggests that the studied samples could serve as a significant dietary protein source. Protein is one of the most important macronutrients, vital for growth, repair, and maintenance of body tissues. Among all, the highest protein content was observed in mung bean (23.67 g/100g), followed by horse gram (21.26 ± 0.02 g/100g), rice bean (20.83 ± 0.01 g/100g) and adzuki bean (18.54 ± 0.01 g/100g). Little millet (8.7 ± 0.01 g/100g) showed the lowest protein content. The high protein content in mung beans indicates its potential as a high-protein cereal alternative. This also supports its application in the formulation of protein-rich functional foods.

4.3.2 Fat content

The fat content ranged between 0.56 ± 0.03 to 4.5 ± 0.02 g/100g. Among all, little millet had notably higher fat content as compared to other cereals suggesting its suitability for energy-rich diets. Other cereals having low-fat content are beneficial for low-fat formulations. The fat content in the selected cereals was relatively low compared to staple/traditional crops like cowpea and chickpea (4.8 g/100 g, 5.2 g/100 g, respectively), as reported by Azmah *et al.* [123]. Low fat content is advantageous for individuals seeking low-calorie diets and increases the shelf stability of the product by minimizing the risk of rancidity.

4.3.3 Carbohydrate content

The total carbohydrate content in the selected cereal samples ranged from 60.95 ± 0.01 to 72.2 ± 0.03 g/100g, which constitutes the major fraction of the proximate composition. Among all, little millet showed the highest carbohydrate content, making it an excellent source of energy. On the other hand, adzuki & mung bean had relatively lower carbohydrate content, making them ideal for diabetic or low-carbohydrate content diets. Carbohydrates provide the primary energy source for the body, and the observed levels confirm the suitability of the studied samples as staple food candidates. This highlights their potential role in combating malnutrition and energy deficiency.

4.3.4 Dietary fiber

The dietary fiber content of the selected samples ranged from 9.51 ± 0.01 to 29.44 ± 0.01 g/100g, reflecting notable variability among the studied cereal samples. From the data, it can be inferred that mung & adzuki bean showed the highest dietary fiber content, followed by rice bean, little millet, and horse gram. According to the reported study, the decrease in dietary fiber content is probably due to the increase in the carbohydrate content in the selected cereal samples [124]. Dietary fiber serves as a marker for non-digestible carbohydrate content in foods. Cereals with high fiber content support digestive processes and assist in weight regulation. Considering dietary fiber's well-documented benefits for gastrointestinal health, blood sugar modulation, and chronic disease prevention, the measured fiber levels underscore the nutritional significance of these samples. The substantial fiber content found in these cereals demonstrates their suitability for developing nutritionally enhanced, health-focused food products enriched with dietary fiber.

Table 4.2: Proximate analysis of selected underutilized cereals (on a dry basis)

Parameters	**Samples**				
	Adzuki bean	**Mung bean**	**Rice bean**	**Horse gram**	**Little millet**
Protein (g/100 g)	18.54 ± 0.01	23.67 ± 0.02	20.83 ± 0.01	21.26 ± 0.02	8.7 ± 0.01
Dietary Fiber (g/100 g)	16.41 ± 0.03	29.44 ± 0.01	12.46 ± 0.02	9.51 ± 0.01	10.3 ± 0.00
Carbohydrate (g/100 g)	61.91 ± 0.04	60.95 ± 0.01	62.73 ± 0.01	62.22 ± 0.05	72.2 ± 0.03
Fat (g/100 g)	2.56 ± 0.01	0.56 ± 0.03	0.60 ± 0.04	2.10 ± 0.01	4.5 ± 0.02
Saturated Fat (%)	0.91 ± 0.02	BLQ	0.20 ± 0.01	0.67 ± 0.0	1.0 ±0.01
MUFA (%)	BLQ	BLQ	BLQ	0.37 ± 0.03	1.6 ± 0.05
PUFA (%)	1.46 ± 0.01	0.24 ± 0.01	0.36 ± 0.03	1.06 ± 0.01	1.9 ±0.02
Trans Fat (%)	BLQ	BLQ	BLQ	BLQ	BLQ

Overall, from the data of nutritional composition, it can be inferred that the results were in agreement with the previous reports [125-127]. The previously reported data slightly differed from the present data, probably due to the difference in the cultivars of the cereals. Thus, the fiber-rich composition of these cereals underscores their potential as sustainable ingredients for the development of value-added functional foods.

4.4 Elemental analysis using ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) was employed to determine the elemental content of selected samples, focusing on nutritionally significant macro- and micro-elements, as well as potentially harmful heavy metals. In the elemental analysis of the selected samples, it was observed that a total of 21 macro-elements and micro-elements were present in these samples at different concentrations (as shown in Table 4.3).

Table 4.3: Mineral concentration of selected underutilized cereals

Minerals	Samples Mineral Concentration (ppm)				
	Adzuki bean	**Mung bean**	**Rice bean**	**Horse gram**	**Little millet**
Na	19.6204 ± 0.09	24.93173 ± 0.04	56.68 ± 0.01	31.26 ± 0.05	118.7 ± 0.02
Mg	3306.419 ± 0.02	2286.16 ± 0.09	3114.88 ± 0.01	1886.09 ± 0.01	1838.3 ± 0.01
Al	11.9918 ± 0.021	13.2238 ± 0.07	8.17 ± 0.02	18.21 ± 0.00	17.8 ± 0.00
K	15883.36 ± 0.03	16063.99 ± 0.12	17198.02 ± 0.03	13331.94 ± 0.01	2303.5 ± 0.03
^{43}Ca	448.0509 ± 0.04	190.9574 ± 0.09	599.51 ± 0.09	371.40 ± 0.02	49.7 ± 0.01
^{44}Ca	750.3771 ± 0.01	315.3264 ± 0.06	1011.09 ± 0.01	606.41 ± 0.03	84.6 ± 0.02
Cr	0.1021 ± 0.007	0.1211 ± 0.08	0.10 ± 0.01	0.041 ± 0.10	0.1 ± 0.05
Mn	28.5288 ± 0.1	15.5899 ± 0.07	24.09 ± 0.02	45.27 ± 0.03	22.3 ± 0.01
Fe	38.2998 ± 0.09	14.3856 ± 0.01	36.98 ± 0.01	56.79 ± 0.03	1.2 ± 0.02
Co	0.0807 ± 0.08	0.0623 ± 0.05	0.08 ± 0.00	0.37 ± 0.20	0.03 ± 0.04
Ni	1.2351 ± 0.01	2.6272 ± 0.07	0.88 ± 0.02	5.01 ± 0.01	1.7 ± 0.01
Cu	7.7803 ± 0.03	11.9612 ± 0.16	5.95 ± 0.01	10.04 ± 0.02	5.3 ± 0.01
Zn	33.0152 ± 0.08	25.4955 ± 0.21	32.33 ± 0.09	27.64 ± 0.05	20.3 ± 0.02
As	0.0053 ± 0.01	0.0138 ± 0.15	0.002 ± 0.05	0.01 ± 0.01	0.003 ± 0.01
Se	0.1797 ± 0.03	0.4138 ± 0.06	0.16 ± 0.04	0.09 ± 0.01	0.1 ± 0.03
Rb	26.1909 ± 0.06	29.1123 ± 0.05s	15.06 ± 0.01	37.50 ± 0.04	6.9 ± 0.01
Sr	3.8409 ± 0.03	6.0270 ± 0.03	5.21 ± 0.02	11.96 ± 0.09	2.1 ± 0.05
Mo	4832.165 ± 0.01	12240.99 ± 0.01	5772.41 ± 0.02	2012.17 ± 0.01	540.9 ± 0.04
Cd	0.0818 ± 0.02	0.1900 ± 0.09	0.09 ± 0.05	0.09 ± 0.00	0.01 ± 0.01
Pb	0.0169 ± 0.12	0.0180 ± 0.09	0.01 ± 0.00	0.02 ± 0.01	0.1 ± 0.02
U	0.0115 ± 0.21	0.0123 ± 0.17	0.02 ± 0.01	0.01 ± 0.03	0.05 ± 0.01

The data clearly showed that the samples were a good source of potassium, molybdenum, and magnesium, which are known to play vital roles in metabolic regulation, bone health, and electrolyte balance. Among all, rice beans emerged as a good source of potassium, adzuki bean rich in magnesium, and mung bean showed a

higher content of molybdenum. The highest concentration was found for potassium among all the elements that were analysed. Potassium is an essential mineral for the efficient functioning of tissues, the body's cells, and organs. Increased potassium intake in diets can help maintain healthy blood pressure levels. The concentration of heavy metals like cadmium, arsenic, and lead was below the standard limit values set by FSSAI. Trace elements such as iron, zinc, copper, and manganese were also detected in substantial concentrations, signifying the potential of these cereals to contribute towards alleviating micronutrient deficiencies. Overall, the ICP-MS results highlight the nutritional value and safety of the selected cereals, confirming their suitability for use in developing health-supporting functional foods. Higher mineral content supports the use of these cereals in functional foods and nutraceuticals, especially for populations prone to mineral deficiencies.

4.5 FAME analysis

The fatty acid composition of studied samples was characterized through methyl ester derivatization, enabling both qualitative identification and quantitative measurement of specific fatty acids. The percentage of fatty acid methyl esters varied between the selected legumes (as shown in Table 4.4). The analysis revealed the presence of twenty-four fatty acids, including both saturated and unsaturated fatty acids, with a predominance of essential PUFAs, such as linoleic acid and α-linolenic acid. MUFAs, particularly oleic acid, were also detected in appreciable amounts, while palmitic acid and stearic acid constituted the major saturated fatty acids.

In adzuki bean, the predominant constituents were linoleic acid (36.80%) and palmitic acid (27.20%), followed by oleic acid (7.01%), and linolenic acid (19.50%),

accompanied by a very small amount (0.08-7.01%) of others. In mung bean, the major fatty acids were palmitic acid (27.19%), linoleic acid (27%), oleic acid (17.88%), and ϒ- linolenic acid (12.73%). In little millet, the predominant constituents were linoleic acid (42%), and palmitic acid (15.74%), followed by oleic acid (34.10%), linolenic acid (1.1%), accompanied by a very small number of others (0.1- 5.21%). Among all, linoleic acid was the predominant component and was present in the highest quantity in little millet. In horse gram, the predominant fatty acid was linoleic acid (37.76%), followed by palmitic acid (20.64%), oleic acid (17.09%), and linolenic acid (11.40%), with smaller amounts (0.05–4.05%) of other fatty acids. Similarly, rice bean contained a high proportion of linoleic acid (37.73%) and palmitic acid (23.76%), followed by linolenic acid (20.72%) and minor quantities (0.05–7.25%) of other fatty acids. Among all detected components, linoleic acid was the most prevalent, appearing in the highest concentration in horse gram (37.76%), closely followed by rice beans (37.73%). Linoleic acid (ω-6 PUFA) is an essential fatty acid that has various biological effects on human body cells, such as flexibility, cell membrane, lower cholesterol levels, reduced cardiovascular disease, inflammation regulation, and immune system function. It is the most abundant PUFA in the legumes, accounting for about 90% of dietary ω-6 PUFA consumption, a higher intake of which is associated with a 15% lower risk of coronary heart disease incidence [123]. Oleic acid contributes to cholesterol regulation and supports heart health. The nutritional value of legumes can be significantly improved by the presence of a single essential fatty acid, which can be beneficial for human health [128].

Table 4.4: Fatty acid composition of selected underutilized cereals

Fatty acids composition	**% Area (g/100 g oil sample)**				
	Adzuki bean	**Mung bean**	**Rice bean**	**Horse gram**	**Little millet**
Lauric acid	0.08 ± 0.01	0.11 ± 0.02	0.00	0.07 ± 0.05	0.00
Caproic acid	0.00	0.02 ± 0.01	0.00	0.00	0.00
Myristic acid	0.33 ± 0.02	0.61 ± 0.03	0.34 ± 0.01	0.49 ± 0.03	0.10 ± 0.01
Pentadecanoic acid	0.19 ± 0.03	0.18 ± 0.01	0.22 ± 0.03	0.11 ± 0.05	0.00
Palmitic acid	27.20 ± 0.01	27.19 ± 0.05	23.76 ± 0.01	20.64 ± 0.05	15.74 ± 0.02
Palmitoleic acid	0.18± 0.01	0.13 ± 0.04	0.21 ± 0.08	0.32 ± 0.01	0.20 ± 0.04
Heptadecanoic acid	0.32 ± 0.02	0.27 ± 0.01	0.34 ± 0.02	0.15 ± 0.01	0.10 ± 0.01
Stearic acid	4.98 ± 0.01	6.20 ± 0.04	4.77 ± 0.04	4.05 ± 0.00	5.21 ± 0.03
Oleic acid	7.01 ± 0.04	17.88 ± 0.03	7.25 ± 0.01	17.09 ± 0.01	34.10 ± 0.06
Linoleic acid	36.80 ± 0.05	27.00 ± 0.02	37.73 ± 0.09	37.76 ± 0.04	42.00 ± 0.01
Arachidic acid	0.80 ± 0.01	0.00	0.96 ± 0.06	1.19 ± 0.00	0.80 ± 0.04
ϒ- Linolenic acid	0.10 ± 0.02	12.73 ± 0.01	0.09 ± 0.01	0.04 ± 0.00	0.00
Linolenic acid	19.50 ± 0.01	3.28 ± 0.02	20.72 ± 0.02	11.40 ± 0.01	1.10 ± 0.02
Cis-11-Eicosenoic acid	0.18 ± 0.03	1.60 ± 0.01	0.16 ± 0.04	0.41 ± 0.02	0.20 ± 0.01
Heniecosanoic acid	0.11 ± 0.03	0.00	0.15 ± 0.09	0.22 ± 0.06	0.00
Cis-11-14-Eicosadienoic	0.00	0.08 ± 0.01	0.00	0.00	0.00
Cis-8,11,14-Eicosatrienoic	0.06 ± 0.02	0.00	0.08 ± 0.01	0.10 ± 0.01	0.00
Cis-10-Pentadecanoic acid	0.09 ± 0.01	0.00	0.00	0.11 ± 0.01	0.00
Behenic acid	1.06 ± 0.01	0.00	1.30 ± 0.03	2.98 ± 0.07	0.20 ± 0.03
Erucic acid	0.00	1.42 ± 0.06	0.05 ± 0.02	0.00	0.00
Methylcis-5,8,11,14 Eicosatetraenoic acid	0.50 ± 0.02	0.00	0.49 ± 0.05	0.63 ± 0.01	0.10 ± 0.03
Cis-5,8,11,14,17-Eicosapentaenoic acid	0.17 ± 0.01	0.00	0.00 ± 0.01	0.05 ± 0.03	0.00
Lignoceric acid	0.34 ± 0.01	0.36 ± 0.05	0.95 ± 0.00	1.81 ± 0.01	0.20 ± 0.01
Nervonic acid	0.00	0.94 ± 0.01	0.00	0.00	0.00

Overall, cereals were rich sources of palmitic acid, linoleic acid, and linolenic acid. The observed fatty acid composition indicates a favorable balance between saturated and unsaturated fatty acids, which is nutritionally desirable for cardiovascular and metabolic health. The high PUFA and MUFA content enhance the functional value of the studied cereals/legumes by contributing to cholesterol regulation, anti-inflammatory activity, and overall cardiovascular protection. In general, the FAME analysis confirms that the lipid fraction of the samples is nutritionally advantageous, positioning them as promising raw materials for the development of health-oriented, lipid-enriched functional food products.

4.6 GC-MS analysis

The bioactive compounds in the extracts of selected samples were analyzed using GC-MS, allowing for the identification of volatile and semi-volatile phytoconstituents through their characteristic mass spectral fragmentation patterns and retention times. Compounds with high similarity indices were identified after matching each peak in the chromatogram with the NIST library. The GC-MS data revealed the presence of a diverse class of phytochemicals including hydrocarbons, terpenes, alkaloids, phenolic compounds, fatty acids, and their esters.

A total of 95 compounds were identified in the adzuki bean extract, as shown in Table 4.5. In adzuki bean extracts, the major bioactive constituents were glycerol (38.62%), 2-azidocholestan-3-ol (23.15%), z-7-tetradecenal (21.55%), palmitic acid (14.53%), and stigmasterol (10.09%). The previous study revealed that adzuki beans contained the highest concentration of aliphatic aldehydes and alcohols [129]. The previously

reported data differed from that described here, probably because Bi *et al*. used a Chinese variant of adzuki bean. According to them, only two hydrocarbons were detected in adzuki bean, but in the present study, more than two hydrocarbons were detected. For mung bean extracts, a total of 38 compounds were identified (Table 4.6). In the methanol extract of mung bean, ethyl benzoate (20.75%), palmitic acid (13.63%), and linoleic acid (17.67%) were identified as the major phytoconstituents. The data was comparable with the previous report [130]. The little millet data revealed that the extract encompassed a total of 84 compounds (as presented in Table 4.7). Two major compounds identified were palmitic acid (33.89%) and cis-9-hexadecenal (57.12%). The data obtained was comparable to the previous study, with some additional compounds [106]. According to Liu et al., aldehydes and benzene derivatives are the primary volatile compounds found in the Poaceae family [131]. Palmitic acid, dodecane, stearic acid, tetracontane, stigmasterol, δ-tocopherol, and γ-tocopherol were also present in little millet. On the other hand, 70 volatile compounds were identified in horse gram extracts (as shown in Table 4.8). The major phytochemicals were myo-inositol (52.87%), palmitic acid (15.27%), 9,12-linoleic acid (15.99%), and caprylic acid monoethanol amide (17.75%) were identified as the prominent phytochemicals. Similarly, 70 volatile compounds were identified in rice bean extracts (as shown in Table 4.9). The prominent phytochemicals were myo-inositol (14.89%), hexadecanoic acid (14.02%), 9,12-octadecadienoic acid (10.64%), (7z)-7-tetradecenal (12.64%), 9,10-dibromopentacosane (30.26%), stigmasterol (10.87%), and β-amyrin (10.22%). The findings aligned with earlier reports, which identified inositol as the primary component of horse gram [132,133].

Table 4.5: Bioactive compounds identified using GC-MS in adzuki bean

RT	Compound name	%Area	MW	MF	n-Hexane	DCM	Methanol
5.341	2,4-Dihydroxy-2,5-dimethyl-3(2h)-furan-3-one	1.46	144	$C_6H_8O_4$	-	-	+
5.400	Decane	0.19	142	$C_{10}H_{22}$	+	-	-
6.508	Butyl acetoxyacetate	0.69	174	$C_8H_{14}O_4$	-	-	+
6.625	Tricyclo(5.2.1.0(2,6))dec-4-ene	0.14	134	$C_{10}H_{14}$	+	-	-
7.058	Endo-tricyclo[5.2.1.0(2.6)]decane	0.10	136	$C_{10}H_{16}$	+	-	-
7.422	3-(2-Aminoethyl)-4-hydroxy-4-methyl-5- oxazolidin-2-one	2.26	228	$C_{11}H_{20}N_2O_3$	-	-	+
7.630	Maltol	0.20	126	$C_6H_6O_3$	-	+	-
7.859	2-Butyl-1-octanol	0.03	186	$C_{12}H_{26}O$	+	-	-
7.933	5-Butylnonane	0.02	184	$C_{13}H_{28}$	+	-	-
8.013	N-Hexadecane	0.02	226	$C_{16}H_{34}$	+	-	-
8.113	3-Methylundecane	0.04	170	$C_{12}H_{26}$	+	-	-
8.159	3-Hydroxy-2,3-dihydromaltol	1.28	144	$C_6H_8O_4$	-	-	+
8.570	Dodecane	0.43	170	$C_{12}H_{26}$	+	+	-
9.023	Glycerol	38.62	92	$C_3H_8O_3$	-	-	+
8.748	6-Methyltridecane	0.05	198	$C_{14}H_{30}$	+	-	-
9.348	1-Chloroctadecan	0.05	288	$C_{18}H_{37}Cl$	+	-	-
9.608	2,7,10-Trimethyldodecane	0.07	212	$C_{15}H_{32}$	+	-	-
9.674	5-Hydroxymethylfurfural	3.96	126	$C_6H_6O_3$	-	-	+
10.032	Tridecane	0.06	184	$C_{13}H_{28}$	+	-	-
11.001	2-Bromo dodecane	0.04	248	$C_{12}H_{25}Br$	+	-	-
11.053	Docosane	0.06	310	$C_{22}H_{46}$	+	-	-
11.406	Tetradecane	0.68	198	$C_{14}H_{30}$	+	-	-
12.698	Pentadecane	0.10	212	$C_{15}H_{32}$	+	+	-
13.916	N-Hexadecane	0.49	226	$C_{16}H_{34}$	+	-	-
15.068	Heptadecane	0.03	240	$C_{17}H_{36}$	+	-	-
16.164	Octadecane	0.35	254	$C_{18}H_{38}$	+	+	-
16.410	Isopropyl myristate	0.08	270	$C_{17}H_{34}O_2$	-	-	+
16.552	Neophytadiene	0.06	278	$C_{20}H_{38}$	-	+	-
16.641	6,10,14-Trimethylpentade can-2-one	0.16	268	$C_{18}H_{36}O$	+	-	-
17.490	Methyl hexadecanoate	3.65	270	$C_{17}H_{34}O_2$	+	+	+
18.187	Palmitic acid	14.53	256	$C_{16}H_{32}O_2$	+	+	+

RT	Compound name	%Area	MW	MF	n-Hexane	DCM	Methanol
18.946	Allyl stearate	0.17	324	$C_{21}H_{40}O_2$	-	-	+
19.135	Linoleic acid, methyl ester	1.68	294	$C_{19}H_{34}O_2$	+	+	+
19.180	Methyl linolenate	0.73	292	$C_{19}H_{32}O_2$	+	-	+
19.247	Methyl 9,10-dibromostearate	0.10	454	$C_{19}H_{36}Br_2O_2$	+	-	-
19.426	Methyl stearate	0.18	298	$C_{19}H_{38}O_2$	+	-	+
19.596	Palmidrol	1.25	299	$C_{18}H_{37}NO_2$	-	+	+
19.778	Z-7-tetradecenal	21.55	210	$C_{14}H_{26}O$	-	-	+
19.868	2- Azidocholestan-3-ol	23.15	429	$C_{27}H_{47}N_3O$	+	+	-
20.038	Stearic acid	1.79	284	$C_{18}H_{36}O_2$	+	+	+
20.520	Tributyl acetylcitrate	0.36	402	$C_{20}H_{34}O_8$	+	-	-
20.755	Allyl stearate	0.13	324	$C_{21}H_{40}O_2$	-	-	+
20.852	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.25	213	$C_{12}H_{23}NO_2$	-	-	+
20.954	Glycidyl palmitate	0.26	312	$C_{19}H_{36}O_3$	-	-	+
21.159	Caprylic acid monoethanol amide	0.17	187	$C_{10}H_{21}NO_2$	+	+	+
21.215	2-Dodecylcyclobutanone	0.10	238	$C_{16}H_{30}O$	+	-	-
21.220	1-(1-Adamantyl)-3-(1-piperidinyl)-1-propanone	0.87	275	$C_{18}H_{29}NO$	-	+	-
21.394	Lauric acid ethanolamide	0.24	243	$C_{14}H_{29}NO_2$	-	+	+
21.417	Palmidrol	0.06	299	$C_{18}H_{37}NO_2$	+	-	-
21.636	8-Methyl-6-nonenamide	0.23	169	$C_{10}H_{19}NO$	-	-	+
21.780	Heneicosane	0.07	296	$C_{21}H_{44}$	+	-	-
21.846	Octadecanamide	0.07	283	$C_{18}H_{37}NO$	+	-	+
21.967	Triphenyl phosphate	0.39	326	$C_{18}H_{15}O_4P$	+	+	+
22.262	2-Formylhexadecane	0.18	254	$C_{17}H_{34}O$	-	-	+
22.409	Methyl 2-cyclohexyl-2-methylpentanoate	0.73	212	$C_{13}H_{24}O_2$	-	-	+
22.415	Ethyl 3-oxooctadecanoate	0.44	326	$C_{20}H_{38}O_3$	-	+	-
22.587	2-Ethylbutyric acid, decyl ester	0.14	256	$C_{16}H_{32}O_2$	-	+	-
22.588	Pentacosane	0.07	352	$C_{25}H_{52}$	+	-	-
22.628	1,2-Dipalmitin	0.76	568	$C_{35}H_{68}O_5$	-	-	+
23.288	Hexadecanoic acid, phenylmethyl ester	0.69	346	$C_{23}H_{38}O_2$	+	-	-
23.361	Tetratetracontane	0.23	618	$C_{44}H_{90}$	+	-	-
23.369	1,22-Dibromodocosane	0.07	466	$C_{22}H_{44}Br_2$	-	+	-
23.407	Docosyl docosanoate	0.19	648	$C_{44}H_{88}O_2$	-	+	-

RT	Compound name	%Area	MW	MF	n-Hexane	DCM	Methanol
23.990	4-Cyanobenzoic acid, undec-10-enyl ester	0.37	299	$C_{19}H_{25}NO_2$	-	-	+
24.196	1-Glyceryl stearate	0.34	358	$C_{21}H_{42}O_4$	-	-	+
24.665	Benzyl linoleate	0.99	370	$C_{25}H_{38}O_2$	+	-	-
24.741	Benzyl linolenate	0.44	368	$C_{25}H_{36}O_2$	+	-	-
24.893	1-Bromotetracosane	0.15	416	$C_{24}H_{49}Br$	-	-	+
24.911	9-Octadecenamide	0.36	281	$C_{18}H_{35}NO$	+	-	-
24.933	Dioctyl sebacate	1.47	426	$C_{26}H_{50}O_4$	-	+	-
25.845	2-Phenyl-1,3-dioxan-5-yl icosanoate	0.20	474	$C_{30}H_{50}O_4$	+	-	-
26.045	3,5-Cholestadiene	0.88	368	$C_{27}H_{44}$	-	-	+
26.057	Stigmasteryl acetate	3.12	454	$C_{31}H_{50}O_2$	+	+	+
26.575	8-Methyltocol	5.27	402	$C_{27}H_{46}O_2$	+	+	+
26.680	(3-beta)-Stigmast-5-en-3-ol	0.35	414	$C_{29}H_{50}O$	+	+	-
26.805	3,5-Dedihydro-stigmastan-6,22-dien	0.08	394	$C_{29}H_{46}$	+	+	-
27.142	Stigmasteryl acetate	3.12	454	$C_{31}H_{50}O_2$	+	-	+
27.277	Campesterol, methyl ether	0.12	414	$C_{29}H_{50}O$	-	+	+
27.851	O-Xylotocopherol	4.87	416	$C_{28}H_{48}O_2$	+	+	+
28.310	Tetracontane	0.59	562	$C_{40}H_{82}$	+	-	-
28.445	Stigmast-5-en-3-ol, oleate	2.21	678	$C_{47}H_{82}O_2$	+	+	+
28.566	Cerotic acid	0.09	396	$C_{26}H_{52}O_2$	+	-	-
30.052	1-Nonyl-1,2,3,4-tetrahydronaphthalene	0.23	258	$C_{19}H_{30}$	+	-	-
30.848	Campestrol	0.61	400	$C_{28}H_{48}O$	+	+	-
31.430	Stigmasterol	10.09	412	$C_{29}H_{48}O$	+	+	+
32.779	Clionasterol	7.95	414	$C_{29}H_{50}O$	+	+	+
33.090	Fucosterol	0.55	412	$C_{29}H_{48}O$	+	-	-
33.584	Olean-12-en-3- one	4.82	424	$C_{30}H_{48}O$	-	-	+
33.656	Olean-12-en-3-ol	7.93	426	$C_{30}H_{50}O$	+	+	-
34.179	Stigmasta-4,22-dien-3-one	1.01	410	$C_{29}H_{46}O$	+	+	+
34.529	Handianol	0.15	426	$C_{30}H_{50}O$	+	-	-
34.741	Cycloartane-3beta.,25-diol	0.28	444	$C_{30}H_{52}O_2$	+	-	-
34.831	Stigmasta-3,5-dien-7-one	0.22	410	$C_{29}H_{46}O$	-	+	-
35.846	(24s)-Stigmast-4-en-3-one	0.73	412	$C_{29}H_{48}O$	+	+	+
35.876	Stigmast-4-en-3-one	0.20	412	$C_{29}H_{48}O$	-	+	+

Table 4.6: Bioactive compounds identified using GC-MS in mung bean

RT	Compound name	%Area	MW	MF
Methanol extract				
5.325	Ethyl benzoate	20.75	150	$C_9H_{10}O_2$
5.918	Benzaldehyde diethyl acetal	0.98	180	$C_{11}H_{16}O_2$
8.338	1-Chloro-2-(diethoxymethyl)benzene	0.12	214	$C_{11}H_{15}ClO_2$
8.850	Ethyl 2-ethoxybenzoate	0.09	194	$C_{11}H_{14}O_3$
9.619	2,4-Ditert-butylphenol	0.42	206	$C_{14}H_{22}O$
10.251	Guanosine	0.21	283	$C_{10}H_{13}N_5O_5$
10.616	1-Hexadecene	0.06	224	$C_{16}H_{32}$
11.529	α-Methyl-d-galactopyranoside	0.33	194	$C_7H_{14}O_6$
11.865	3-Hydroxypropyl benzoate	0.19	180	$C_{10}H_{12}O_3$
12.563	Tetradecanoic acid	0.17	228	$C_{14}H_{28}O_2$
12.883	1-Heptadecene	0.03	238	$C_{17}H_{34}$
14.274	Methyl hexadecanoate	1.34	270	$C_{17}H_{34}O_2$
14.358	Methyl 3-(3,5-ditert-butyl-4-hydroxyphenyl) propanoate	0.74	292	$C_{18}H_{28}O_3$
15.045	Palmitic acid	13.63	256	$C_{16}H_{32}O_2$
15.478	Methyl 2-hydroxyhexadecanoate	0.27	286	$C_{17}H_{34}O_3$
15.699	Heptadecanoic acid	0.17	270	$C_{17}H_{34}O_2$
15.941	Cis-Linoleic acid methyl ester	1.84	294	$C_{19}H_{34}O_2$
15.995	Methyl linolenate	0.83	292	$C_{19}H_{32}O_2$
16.215	Methyl stearate	0.69	298	$C_{19}H_{38}O_2$
16.757	Linolic acid	17.67	280	$C_{18}H_{32}O_2$
16.895	Stearic acid	2.33	284	$C_{18}H_{36}O_2$
17.713	Carbonic acid, 2-dimethylaminoethyl neopentyl ester	2.03	203	$C_{10}H_{21}NO_3$
18.214	Lauric acid ethanolamide	0.26	243	$C_{14}H_{29}NO_2$
18.391	Eicosanoic acid	0.33	312	$C_{20}H_{40}O_2$
19.175	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.51	213	$C_{12}H_{23}ONO_2$
19.313	Fumaric acid, 2-dimethylaminoethyl dodecyl ester	0.33	355	$C_{20}H_{37}NO_4$
19.355	Diglycol dibenzoate	0.57	314	$C_{18}H_{18}O_5$
19.645	2-Monopalmitin	7.18	330	$C_{19}H_{38}O_4$
19.957	Docosanoic acid	0.11	340	$C_{22}H_{44}O_2$
20.387	Methyl tricosanoate	0.10	368	$C_{24}H_{48}O_2$
21.055	Glycerol 1-monolinolate	6.05	354	$C_{21}H_{38}O_4$
21.182	Glycerin 1-stearate	0.83	358	$C_{21}H_{42}O_4$
22.764	δ-Tocopherol	0.32	402	$C_{27}H_{46}O_2$
23.694	γ-Tocopherol	2.55	416	$C_{28}H_{48}O_2$
25.683	Ergost-5-en-3-ol	1.29	400	$C_{28}H_{48}O$
26.066	Stigmasterol	3.05	412	$C_{29}H_{48}O$
26.971	γ-Sitosterol	5.43	414	$C_{29}H_{50}O$
27.969	Cycloartenol	0.13	426	$C_{30}H_{50}O$

Table 4.7: Bioactive compounds identified by GC-MS in the different solvent extracts of little millet

RT	Compound name	%Area	MW	MF	n-hexane	DCM	Methanol
5.398	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone	0.77	144	$C_6H_8O_4$	-	-	+
5.409	Decane	0.08	142	$C_{10}H_{22}$	+	-	-
5.841	Caproic acid	0.73	116	$C_6H_{12}O_2$	-	-	+
6.218	1-Methylpyrrolidone	0.24	99	C_5H_9NO	+	-	-
6.411	Benzeneacetaldehyde	0.15	120	C_8H_8O	-	-	+
6.573	Neo-hexanoic acid	0.49	116	$C_6H_{12}O_2$	-	-	+
6.633	Dihydrodicyclopentadiene	0.04	134	$C_{10}H_{14}$	+	-	-
7.068	Tetrahydrodicyclopentadiene	0.03	136	$C_{10}H_{16}$	+	-	-
8.183	Pyranone	5.12	144	$C_6H_8O_4$	-	-	+
8.579	Dodecane	0.21	170	$C_{12}H_{26}$	+	+	-
9.670	Dihydrobenzofuran	0.38	120	C_8H_8O	-	-	+
9.720	5-Hydroxymethylfurfural	4.40	126	$C_6H_6O_3$	-	-	+
10.517	4-Hydroxy-3-methoxystyrene	1.95	150	$C_9H_{10}O_2$	-	-	+
11.059	Docosane	0.02	310	$C_{22}H_{46}$	+	-	-
11.155	Isosorbide	0.47	146	$C_6H_{10}O_4$	-	-	+
11.392	2-Heptyl acetate	0.84	158	$C_9H_{18}O_2$	-	-	+
11.416	Tetradecane	0.31	198	$C_{14}H_{30}$	+	+	-
12.397	6-Demethoxyageratochromene	0.26	190	$C_{12}H_{14}O_2$	-	-	+
12.507	1-Dodecanol	0.04	186	$C_{12}H_{26}O$	+	-	+
12.823	Benzaldehyde methylimine	0.17	119	C_8H_9N	-	-	+
13.922	Hexadecane	0.19	226	$C_{16}H_{34}$	+	+	-
13.931	1,6-Anhydro-beta-D-glucopyranose	2.05	162	$C_6H_{10}O_5$	-	-	+
15.491	(2E)-3-(1-Methylcyclopropyl)-2-propenoic acid	4.99	126	$C_7H_{10}O_2$	-	-	+
15.767	Cyclohexanone, 2-methyl- semicarbazone	3.72	169	$C_8H_{15}N_3O$	-	-	+
16.168	Octadecane	0.09	254	$C_{18}H_{38}$	+	+	-
17.500	Palmitic acid, methyl ester	0.69	270	$C_{17}H_{34}O_2$	+	+	+
17.830	(+)-Dihydrorecifeiolide	0.33	198	$C_{12}H_{22}O_2$	-	-	+
18.187	Palmitic acid	33.89	256	$C_{16}H_{32}O_2$	+	+	+
18.954	Allyl stearate	0.29	324	$C_{21}H_{40}O_2$	-	-	+
19.124	Methyl linoleate	0.29	294	$C_{19}H_{34}O_2$	-	+	+
19.181	Methyl 7-octadecenoate	0.13	296	$C_{19}H_{36}O_2$	-	-	+
19.210	Methyl (9E)-9-octadecenoate	1.02	296	$C_{19}H_{36}O_2$	+	+	-
19.442	Methyl stearate	0.23	298	$C_{19}H_{38}O_2$	-	+	-
20.043	Cis-9-hexadecenal	57.12	238	$C_{16}H_{30}O$	-	+	+
20.136	Stearic acid	2.30	284	$C_{18}H_{36}O_2$	-	+	+
20.533	6-Nitro-cylohexadecane-1,3-dione	0.20	297	$C_{16}H_{27}NO_4$	-	-	+
20.847	3-Cyclopentylpropionic acid, 2-dimethyl aminoethyl ester	0.24	213	$C_{12}H_{23}NO_2$	-	-	+
20.958	Octadecanedioic acid	0.24	314	$C_{18}H_{34}O_4$	-	-	+
21.208	3,5,5-Trimethyl-5,6-dihydro-2H-pyran-2-one	0.13	140	$C_8H_{12}O_2$	-	+	-
21.463	Leinoleic acid	0.26	280	$C_{18}H_{32}O_2$	+	+	-
21.663	Hexadecanoic acid, hexyl ester	0.32	340	$C_{22}H_{44}O_2$	+	+	-
21.935	(Z)-11-Eicosenic acid	0.08	310	$C_{20}H_{38}O_2$	+	-	-

RT	Compound name	%Area	MW	MF	n-hexane	DCM	Methanol
22.259	Isometheptene	0.26	141	$C_9H_{19}N$	-	-	+
22.263	2-Mono-Linolein	0.26	354	$C_{12}H_{38}O_4$	-	+	-
22.296	Fumaric acid, 2-dimethylaminoethyl nonyl ester	0.17	313	$C_{17}H_{31}NO_4$	-	-	+
22.439	Methyl 2-cyclohexyl-2-methylpentanoate	0.47	212	$C_{13}H_{24}O_2$	-	+	-
22.503	Fumaric acid, 2-dimethylaminoethyl octadecyl ester	0.29	439	$C_{26}H_{49}NO_4$	-	-	+
22.559	Trans-9-Octadecenoic acid, pentyl ester	0.37	352	$C_{23}H_{44}O_2$	+	-	-
22.602	Palmitoyl chloride	2.13	274	$C_{16}H_{31}ClO$	-	-	+
22.801	Heneicosane	0.11	296	$C_{21}H_{44}$	+	-	-
23.025	Trans, trans-9,12-Octadecadienoic acid, propyl ester	0.14	322	$C_{21}H_{38}O_2$	-	+	-
23.355	Hexyl stearate	0.06	368	$C_{24}H_{48}O_2$	+	-	-
23.392	1,22-Dibromodocosane	0.33	466	$C_{22}H_{44}Br_2$	+	+	+
23.418	Hexadecanoic acid, phenylmethyl ester	0.05	346	$C_{23}H_{38}O_2$	+	-	-
23.786	3-Isopentylthiophene 1,1-dioxide	0.97	186	$C_9H_{14}O_2S$	-	+	-
23.995	Oleoyl chloride	3.47	300	$C_{18}H_{33}ClO$	+	-	+
24.174	Pentacosane	0.18	352	$C_{25}H_{52}$	+	-	-
24.185	Glycerin 1-monostearate	1.00	358	$C_{21}H_{42}O_4$	-	-	+
24.461	Widdrol	0.27	222	$C_{15}H_{26}O$	-	-	+
24.680	Lanost-7-en-3-one	0.11	426	$C_{30}H_{50}O$	+	-	+
24.720	Dodecahydro-3a,6,6,9a-tetramethyl naphtho[2,1-beta] furan	0.25	236	$C_{16}H_{28}O$	-	+	-
24.744	Linoleic acid, phenylmethyl ester	0.36	370	$C_{25}H_{38}O_2$	+	-	-
24.951	Dioctyl sebacate	1.96	426	$C_{26}H_{50}O_4$	-	+	-
24.992	Carbonic acid, eicosyl prop-1-en-2-yl ester	0.13	382	$C_{24}H_{46}O_3$	+	-	-
25.137	Squalene	0.85	410	$C_{30}H_{50}$	+	-	-
25.295	Betulin	0.07	442	$C_{30}H_{50}O_2$	-	-	+
25.450	(2e)-5-Hydroxy-3,4,4-trimethyl-2-hexenoic acid	0.04	172	$C_9H_{16}O_3$	-	-	+
25.558	3-Methyl-3-(palmitoylperoxy)butyl palmitate	0.09	596	$C_{37}H_{72}O_5$	-	-	+
25.692	3-Bromocholest-5-ene	0.11	448	$C_{27}H_{45}Br$	-	-	+
25.873	Tetratetracontane	0.24	618	$C_{44}H_{90}$	+	+	-
25.950	Tetratriacontane	0.87	478	$C_{34}H_{70}$	+	-	-
26.053	Cholestadiene	0.62	368	$C_{27}H_{44}$	-	+	+
26.613	δ-Tocopherol	0.13	402	$C_{27}H_{46}O_2$	+	+	-
27.016	Tetracontane	0.05	562	$C_{40}H_{82}$	+	+	-
27.355	(22E)-Stigmasta-4,7,22-trien-3-ol	0.20	410	$C_{29}H_{46}O$	-	+	-
27.921	γ-Tocopherol	0.08	416	$C_{28}H_{48}O_2$	+	-	-
28.159	Stigmasterol acetate	0.30	454	$C_{31}H_{50}O_2$	-	+	-
28.459	Stigmast-5-en-3-ol, oleate	0.20	678	$C_{47}H_{82}O_2$	-	+	-
31.214	Triacontanal	0.06	436	$C_{30}H_{60}O$	+	-	-
31.894	3β-methoxy-delta.14-serratene	7.20	440	$C_{31}H_{52}O$	+	+	-
32.422	Methyl commate b	0.73	470	$C_{31}H_{50}O_3$	+	+	-
32.800	γ-Sitosterol	1.06	414	$C_{29}H_{50}O$	+	+	-
34.829	3,5-Stigmastadien-7-one	0.22	410	$C_{29}H_{46}O$	-	+	-
35.898	Lupenyl acetate	1.44	468	$C_{32}H_{52}O_2$	-	+	-

Table 4.8: Quantitative identification of bioactive constituents in the different solvent extracts of Horse gram by GC-MS

RT	Compound name	%Area	MW	MF	n-hexane	DCM	Methanol
6.267	1-Methyl pyrrolidone	1.90	99	C_5H_9NO	+	-	-
6.338	N-α, n-omega-di-cbz-l-arginine	1.90	442	$C_{22}H_{26}N_4O_6$	+	-	-
6.631	Dihydrodicyclopentadiene	0.51	134	$C_{10}H_{14}$	+	-	-
7.021	Undecane	0.18	156	$C_{11}H_{24}$	+	-	-
7.066	2,3-Trimethylenenorbornane	0.39	136	$C_{10}H_{16}$	+	-	-
7.147	4,5-Dimethyl-1,3-oxazinane-2-thione	0.27	145	$C_6H_{11}NOS$	-	-	+
7.669	4,7-Methano-1h-indene, octahydro-2-methylene	0.54	148	$C_{11}H_{16}$	+	-	-
8.120	3-Methylundecane	0.14	170	$C_{12}H_{26}$	+	-	-
8.299	3-Hydroxy-2,3-dihydromaltol	1.44	144	$C_6H_8O_4$	-	-	+
8.577	Dodecane	1.43	170	$C_{12}H_{26}$	+	-	-
9.615	2,6,10-Trimethyldodecane	0.19	212	$C_{15}H_{32}$	+	-	-
9.964	5-Hydroxymethylfurfural	1.06	126	$C_6H_6O_3$	-	-	+
10.040	Tridecane	0.19	184	$C_{13}H_{28}$	+	-	-
10.595	2-Methoxy-4-vinylphenol	0.30	150	$C_9H_{10}O_2$	-	-	+
11.069	1,3-Dimethyl pyrogallate	0.28	154	$C_8H_{10}O_3$	-	-	+
11.413	Tetradecane	2.10	198	$C_{14}H_{30}$	+	-	-
11.430	Hydratropaldehyde	0.59	134	$C_9H_{10}O$	-	-	+
11.630	L- Glutamine	1.53	146	$C_5H_{10}N_2O_3$	-	-	+
13.106	1,3:2,5-Dimethylene-l-rhamnitol	1.11	190	$C_8H_{14}O_5$	-	-	+
13.774	3,5-Dimethoxyacetophenone	0.16	180	$C_{10}H_{12}O_3$	-	-	+
13.924	Pentadecane	1.43	212	$C_{15}H_{32}$	+	-	-
14.070	2-Ethylquinoline	1.35	157	$C_{11}H_{11}N$	-	-	+
14.289	N-Pentylcyclohexane	1.29	154	$C_{11}H_{22}$	-	-	+
16.176	Nonadecane	0.96	268	$C_{19}H_{40}$	+	-	-
16.653	6,10,14-Trimethylpentade can-2-one	0.26	268	$C_{18}H_{36}O$	+	-	-
17.197	Myo inositol	52.87	194	$C_7H_{14}O_6$	-	-	+
17.502	Methyl hexadecanoate	0.91	270	$C_{17}H_{34}O_2$	+	+	+
18.074	Palmitic acid	15.27	256	$C_{16}H_{32}O_2$	+	+	+
19.144	Methyl linoleate	0.95	294	$C_{19}H_{34}O_2$	+	+	+
19.202	Lineoleoyl chloride	0.45	298	$C_{18}H_{31}ClO$	+	-	+
19.611	Lauric ethylolamide	7.67	243	$C_{14}H_{29}NO_2$	+	+	+
19.720	9,12-Linoleic acid	15.99	280	$C_{18}H_{32}O_2$	+	+	+
19.759	Z-9-Hexadecenal	3.78	238	$C_{16}H_{30}O$	-	+	+
19.924	9,10-Octadecenoic acid	0.41	282	$C_{18}H_{34}O_2$	-	+	-
19.935	Stearic acid	0.68	284	$C_{18}H_{36}O_2$	+	-	+

RT	Compound name	%Area	MW	MF	n-hexane	DCM	Methanol
20.179	N-Octadecyl ethanoate	0.32	312	$C_{20}H_{40}O_2$	+	-	-
20.547	3-Acetoxy- 7,8-Epoxylanostan-11-ol	0.07	502	$C_{32}H_{54}O_4$	-	-	+
20.859	2-Dimethylaminoethyl ester Octanoic acid	1.65	215	$C_{12}H_{25}NO_2$	-	-	+
20.978	3-Fluoro-4-(octyloxy)benzoic acid	0.63	268	$C_{15}H_{21}FO_3$	-	-	+
21.145	Caprylic acid monoethanol amide	17.75	187	$C_{10}H_{21}NO_2$	+	+	+
21.410	Lauric acid monoethanolamine	0.85	243	$C_{14}H_{29}NO_2$	+	+	-
21.795	Henicosane	0.21	296	$C_{21}H_{44}$	+	-	-
21.979	Triphenyl phosphoric acid ester	0.38	326	$C_{18}H_{15}O_4P$	+	+	+
22.274	2-(dimethylamino) ethyl 3-Cyclopentyl propanoate	2.19	213	$C_{12}H_{23}NO_2$	-	-	+
22.311	Fumaric acid, 2-dimethylaminoethyl nonyl ester	2.00	313	$C_{17}H_{31}NO_4$	-	-	+
22.416	Ethyl 3-oxooctadecanoate	0.66	326	$C_{20}H_{38}O_3$	-	+	-
22.422	Methyl 2-cyclohexyl-2-methylpentanoate	0.80	212	$C_{13}H_{24}O_2$	+	-	-
22.601	Heneicosane	0.73	296	$C_{21}H_{44}$	+	-	-
22.627	2-monopalmitoylglycerol	0.29	330	$C_{19}H_{38}O_4$	-	-	+
23.377	2-Methyloctacosane	0.34	408	$C_{29}H_{60}$	+	-	-
23.763	1,1-Dichloro-2,2,3,3-tetramethylcyclopropane	0.90	166	$C_7H_{12}Cl_2$	+	+	-
23.780	3-Isopentylthiophene 1,1-dioxide	0.57	186	$C_9H_{14}O_2S$	-	-	+
23.900	Octocrilene	0.14	361	$C_{24}H_{27}NO_2$	+	-	-
24.372	Tetratetracontane	0.26	618	$C_{44}H_{90}$	+	-	-
24.637	Laurylethanolamide	0.28	243	$C_{14}H_{29}NO_2$	-	+	-
24.929	Carbonic acid, eicosyl prop-1-en-2-yl ester	0.79	382	$C_{24}H_{46}O_3$	+	-	-
24.942	Dioctyl sebacate	6.63	426	$C_{26}H_{50}O_4$	-	+	-
26.095	δ-3,5-cholestadiene	0.72	368	$C_{27}H_{44}$	+	+	+
26.566	8-Methyltocol	0.77	402	$C_{27}H_{46}O_2$	+	+	+
26.621	Stigmasterol acetate	0.64	454	$C_{31}H_{50}O_2$	+	+	+
27.859	O-Xylotocopherol	10.42	416	$C_{28}H_{48}O_2$	+	+	+
28.460	Stigmast-5-en-3-yl (9Z)-9-octadecenoate	2.59	678	$C_{47}H_{82}O_2$	-	+	+
28.333	Tetracontane	1.00	562	$C_{40}H_{82}$	+	-	-
28.458	Stigmasta-3,5-diene	1.15	396	$C_{29}H_{48}$	+	-	-
30.851	24-Epicampesterol	1.19	400	$C_{28}H_{48}O$	+	+	+
31.359	Stigmasterol	5.15	412	$C_{29}H_{48}O$	+	+	+
32.674	Stigmast-5-en-3-ol	6.19	414	$C_{29}H_{50}O$	+	+	+
33.602	Olean-12-en-3-ol	7.97	426	$C_{30}H_{50}O$	+	+	+
34.102	Stigmast-7-en-3-ol	2.03	414	$C_{29}H_{50}O$	+	+	-
34.776	Viminalol	2.34	426	$C_{30}H_{50}O$	+	+	-

Table 4.9: Quantitative identification of bioactive constituents in the different solvent extracts of rice bean by GC-MS

RT	Compound name	%Area	MW	MF	n-Hexane	DCM	Methanol
5.404	Decane	0.52	142	$C_{10}H_{22}$	+	+	-
5.449	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3.62	144	$C_6H_8O_4$	-	-	+
6.313	1-Methylpyrrolidin-2-one	0.26	99	C_5H_9NO	-	+	-
6.333	Benzyl alcohol	0.97	108	C_7H_8O	+	-	-
6.631	Tricyclo(5.2.1.0(2,6))dec-3-ene	0.33	134	$C_{10}H_{14}$	+	-	-
6.987	Glycerol	1.71	92	$C_3H_8O_3$	-	-	+
7.021	Undecane	0.12	156	$C_{11}H_{24}$	+	-	-
7.065	Trimethylenenorbornane	0.25	136	$C_{10}H_{16}$	+	-	-
7.507	Maltol	1.21	126	$C_6H_6O_3$	-	+	+
7.968	Ethyl 3-(2,6-dimehylmorpholino) propionate	0.06	215	$C_{11}H_{21}NO_3$	-	+	-
8.119	3-Methylundecane	0.09	170	$C_{12}H_{26}$	+	-	-
8.192	3-Hydroxy-2,3-dihydromaltol	6.49	144	$C_6H_8O_4$	-	-	+
8.570	Dodecane	0.17	170	$C_{12}H_{26}$	+	+	-
9.614	2,7,10-Trimethyldodecane	0.12	212	$C_{15}H_{32}$	+	-	-
9.731	5-Hydroxymethylfurfural	6.89	126	$C_6H_6O_3$	-	-	+
10.038	Tridecane	0.13	184	$C_{13}H_{28}$	+	-	-
11.009	2-Bromo dodecane	0.07	248	$C_{12}H_{25}Br$	+	-	-
11.400	Tetradecane	0.24	198	$C_{14}H_{30}$	-	+	-
11.414	Pentadecane	1.37	212	$C_{15}H_{32}$	+	-	-
12.719	2,5-Difluorobenzoic acid, 6-tetradecyl ester	0.11	354	$C_{12}H_{32}F_2O_2$	-	-	+
13.910	Hexadecane	0.19	226	$C_{16}H_{34}$	-	+	-
14.931	Methyl α-d-galactopyranoside	3.93	194	$C_7H_{14}O_6$	-	-	+
16.159	Myo inositol	14.89	194	$C_7H_{14}O_6$	-	-	+
16.159	Octadecane	0.65	254	$C_{18}H_{38}$	+	+	-
16.432	Isopropyl myristate	1.24	270	$C_{17}H_{34}O_2$	+	-	-
16.549	Neophytadiene	0.03	278	$C_{20}H_{38}$	-	+	-
16.637	Hexahydrofarnesyl acetone	0.26	268	$C_{18}H_{36}O$	+	+	-
17.485	Palmitic acid methyl ester	0.11	270	$C_{17}H_{34}O_2$	-	+	+
17.500	Methyl palmitate	0.41	270	$C_{17}H_{34}O_2$	+	-	-
18.063	Hexadecanoic acid	14.02	256	$C_{16}H_{32}O_2$	+	+	+
18.213	Heneicosane	0.16	296	$C_{21}H_{44}$	+	-	-
19.129	Linoleic acid, methyl ester	1.65	294	$C_{19}H_{34}O_2$	+	+	+
19.205	Linolenic acid, methyl ester	1.01	292	$C_{19}H_{32}O_2$	+	-	+
19.342	Phytol	0.08	296	$C_{20}H_{40}O$	-	+	-
19.609	Palmidrol	1.17	299	$C_{18}H_{37}NO_2$	-	+	+

RT	Compound name	%Area	MW	MF	n-Hexane	DCM	Methanol
19.709	9,12-Octadecadienoic acid	10.64	280	$C_{18}H_{32}O_2$	-	-	+
19.765	(7z)-7-Tetradecenal	12.65	210	$C_{14}H_{26}O$	-	-	+
19.866	9,10-Dibromopentacosane	30.26	508	$C_{25}H_{50}Br_2$	+	-	-
19.852	10,12-Hexadecadien-1-ol	15.83	238	$C_{16}H_{30}O$	-	+	-
19.931	Stearic acid	1.31	284	$C_{18}H_{36}O_2$	+	+	+
20.531	Tributyl acetylcitrate	0.68	402	$C_{20}H_{34}O_8$	+	-	-
20.854	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.57	213	$C_{12}H_{23}NO_2$	-	-	+
20.874	Octanoic acid, 2-dimethylaminoethyl ester	0.11	215	$C_{12}H_{25}NO_2$	-	+	-
20.973	Glycidyl palmitate	0.27	312	$C_{19}H_{36}O_3$	-	-	+
21.143	1-hydroxy-2,2,6,6-tetramethyl-3-(1-piperidinylmethyl)-4-piperidinone	3.41	268	$C_{15}H_{28}N_2O_2$	+	-	+
21.163	Caprylic acid monoethanol amide	1.91	187	$C_{10}H_{21}NO_2$	-	+	-
21.417	Laurylamidoethanol	0.34	243	$C_{14}H_{29}NO_2$	-	+	-
21.658	9-Octadecenamide	0.45	281	$C_{18}H_{35}NO$	+	-	-
22.265	N,6-Dimethyl-5-hepten-2-amine	0.08	141	$C_9H_{19}N$	-	+	-
22.270	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.56	213	$C_{12}H_{23}NO_2$	-	-	+
22.615	Palmitoyl chloride	0.27	274	$C_{16}H_{31}ClO$	-	+	+
22.911	1,2-Benzenedicarboxylic acid, bis(ethylhexyl) ester	0.17	390	$C_{24}H_{38}O_4$	-	-	+
23.379	Tetratetracontane	0.28	618	$C_{44}H_{90}$	+	-	-
23.994	Trans, trans-9,12-Octadecadienoic acid, propyl ester	1.09	322	$C_{21}H_{38}O_2$	-	-	+
24.682	Benzyl linoleate	0.40	370	$C_{25}H_{38}O_2$	+	-	-
24.931	Carbonic acid, hexadecyl prop-1-en-2-yl ester	0.38	326	$C_{20}H_{38}O_3$	+	-	-
24.943	2-Ethylhexyl sebacate	4.99	426	$C_{26}H_{50}O_4$	-	+	-
26.055	Stigmasterol acetate	4.50	454	$C_{31}H_{50}O_2$	+	+	-
26.551	δ-Tocopherol	7.62	402	$C_{27}H_{46}O_2$	+	+	+
26.679	β-Sitosterol monomethyl ether	0.43	428	$C_{30}H_{52}O$	-	+	-
27.315	(22E)-Stigmasta-4,7,22-trien-3-ol	0.29	410	$C_{29}H_{46}O$	+	+	-
27.832	γ-Tocopherol	3.97	416	$C_{28}H_{48}O_2$	+	+	+
28.445	Stigmast-5-en-3-yl 9-octadecenoate	1.65	678	$C_{47}H_{82}O_2$	-	-	+
28.338	Tetracontane	0.81	562	$C_{40}H_{82}$	+	-	-
28.747	2-Methyltetracosane	0.63	352	$C_{25}H_{52}$	+	-	-
30.830	24-Epicampesterol	0.65	400	$C_{28}H_{48}O$	+	+	-
31.326	Stigmasterol	10.87	412	$C_{29}H_{48}O$	+	+	+
32.787	γ-Sitosterol	8.48	414	$C_{29}H_{50}O$	+	+	-
33.138	Fucosterol	0.79	412	$C_{29}H_{48}O$	+	+	-
33.679	β-Amyrin	10.22	426	$C_{30}H_{50}O$	+	+	+

From the data, it was notable that palmitic acid, dodecane, stearic acid, tetracontane, stigmasterol, δ-tocopherol, and γ-tocopherol were the common compounds detected in adzuki and mung bean extracts. Similarly, horse gram and rice bean extract shared several identical compounds, encompassing palmitic acid, dodecane, stearic acid, tetracontane, stigmasterol, δ-tocopherol, and γ-tocopherol.

Notably, compounds such as palmitic acid and stigmasterol have been previously reported for their antioxidant, antimicrobial, and anti-inflammatory activities, thereby suggesting the functional relevance of the samples studied. Palmitic acid and stearic acid are the saturated fatty acids that are consumed most frequently in the Western diet [134]. They aid in regulating glucose metabolism and developing insulin resistance in T2D [135]. The phytosterols like stigmasterol, γ-sitosterol, and β-sitosterol are plant sterols that are known to be beneficial for human health. According to the research, phytosterols could be a complementary treatment for obesity and diabetes [136]. Based on the GC-MS results, the most common volatile compounds in both legumes were hydrocarbons, fatty acids, and terpenes, which are comparable to the results of other researchers [137]. The findings highlight the presence of various secondary metabolites with potential nutraceutical and therapeutic applications.

4.7 UHPLC-QTOF-MS analysis

UHPLC-QTOF-MS is an advantageous technology that, when used as a targeted and non-targeted profiling approach in either negative or positive ionisation modes, may identify analytes by accurately quantifying their ionic mass and fragmentation patterns. Herein, this technique is used to identify the secondary metabolites in different extracts of the selected samples and is presented in Table 4.10 to Table 4.15. All the identified compounds were analysed on the basis of mass-to-charge (m/z) values of mass

spectrometry in positive ionization mode. To identify the maximum metabolites in the legumes, we analysed all the extracts using the targeted and non-targeted approach.

Non-targeted metabolites

The identified metabolites belong to several natural product classes including flavonoids, sugars, amino acids, fatty acids, fatty acid derivatives, and other organic acids. In adzuki bean extracts (as shown in Table 4.10), a total of 15 secondary metabolites were identified based on their mass fragmentation pattern. The data confirms the presence of phytosterols, phenolic compounds, and terpenoids. The mung bean extract contains 18 metabolites (as shown in Table 4.11), which are primarily phytosterols, flavonoids, and phenolic compounds. Oligosaccharides were also present in a slight amount. In the little millet extracts (as shown in Table 4.12), a total of 21 metabolites were identified, which mainly contain phytosterols, phenolic compounds, terpenoids, and flavonoids. Similarly, 13 secondary metabolites in horse gram extracts (as shown in Table 4.13) and 46 secondary metabolites in rice bean extracts (as shown in Table 4.14), predominantly flavonoids, phytosterols, and phenolic compounds, with oligosaccharides present in minor quantities.

This study represents one of the first reports utilizing a non-targeted approach via UHPL-QTOF-MS for the characterization of secondary metabolites in the selected samples. Polyphenols have the ability to possess anti-inflammatory and antioxidant properties. Flavonoids can show anti-tumor, anti-inflammatory, and antioxidant properties and are considered an essential component in cosmetics, nutraceuticals, and medicinal applications [138]. In this study, some carboxylic acids like para-coumaric acid, vernolic acid, gypsogenic acid were detected, which are beneficial for microbial growth by acting as a vitamin for microbial nutrition [139]. Kaempferol, a natural flavonoid identified in the methanol extract of little millet, has been shown positive impacts on reducing chronic diseases like liver injury, cancer, diabetes, and obesity [140].

Target metabolites

A total of 9 metabolites were detected in the positive ion mode, as shown in Table 4.15. We found that catechin-7-O-glucoside, catechin, epicatechin, quercetin, gallocatechin, gallic acid, caffeic acid, para-coumaric acid, and glycitein were significantly present in both the selected legumes. This concurred with previous research, which found that (+)-catechin was the predominant phenolic compound in Korean adzuki beans [141]. Several studies have reported that catechin nd its derivatives have been examined for their possible health advantages, particularly their function in the treatment of obesity [142].

Table 4.10: Identification of secondary metabolites using UHPLC-QTOF-MS in adzuki bean

Peak No.	Tentative metabolites	RT (min)	MF	MM	$[M-H]^+$	Error (ppm)	Compound ID
n-hexane extract							
1.	Stigmasterol	20.6780	$C_{29}H_{48}O$	412.3705	413.3789	2.7	CSID4444352
2.	Ergostan-3-ol	27.7371	$C_{28}H_{50}O$	402.6960	403.3596	2.8	CSID532070
3.	(20S)-Protopanaxadiol	27.7371	$C_{30}H_{52}O_3$	460.3897	461.3975	-2.7	CSID9388412
4.	22β-Hydroxycholesterol	27.0741	$C_{27}H_{46}O_2$	402.6530	403.3596	6.1	CSID146693
5.	γ-Butyrobetain	29.6280	$C_7H_{15}NO_2$	145.9765	145.9843	9.7	CSID705
6.	Lecithin	29.6009	$C_{44}H_{78}NO_8P$	783.5779	784.5857	0.7	CSID24766800
DCM extract							
7.	Stigmasterol	28.0249	$C_{29}H_{48}O$	412.3709	413.3787	2.3	CSID4444352
8.	1-Linoleoyl-2-α-linolenoyl-sn-glycero-3-phosphocholine	28.9651	$C_{44}H_{78}NO_8P$	779.5478	780.5556	2.3	CSID24766802
Methanol extract							
9.	Nystose	1.0400	$C_{24}H_{42}O_{21}$	666.0902	667.0980	5.1	CSID145907
10.	2,3-Dimethoxy-5-geranyl-6-methyl-1,4-benzoquinone	11.8810	$C_{19}H_{26}O_4$	318.1832	319.1910	2.2	CSID4444053
11.	Dopaxanthin quinone	18.4130	$C_{18}H_{16}N_2O_8$	388.0867	389.0945	-8.6	CSID30791578
12.	Hexadecenal	20.1950	$C_{16}H_{30}O$	238.2035	239.2113	3.4	CSID4444172
13.	Poriferasta-5,7-dien-3beta-ol	26.7430	$C_{29}H_{48}O$	412.3713	413.3791	3.0	CSID13183578
14.	Tyramine	28.4271	$C_8H_{11}NO$	137.0849	138.0927	9.7	CSID5408
15.	1-Linoleoyl-2-α-linolenoyl-sn-glycero-3-phosphocholine	28.9651	$C_{44}H_{78}NO_8P$	779.5470	780.5548	1.1	CSID24766802

Table 4.11: Identification of secondary metabolites using UHPLC-QTOF-MS in mung bean

Peak No.	Tentative metabolites	RT (min)	MF	MM	$[M-H]^+$	Error (ppm)	Compound ID
Methanol extract							
1.	1,5-Anhydro-D-fructose	2.3943	$C_6H_{10}O$	139.1513	140.1591	2.2	CSID112421
2.	Baycovin	2.3943	$C_6H_{10}O$	139.1513	140.1591	2.2	CSID2943
3.	L-Rhamno-1,4-lactone	2.3943	$C_6H_{10}O$	139.1513	140.1591	2.2	CSID4573835
4.	3,6-Anhydro-β-D-mannopyranose	2.3943	$C_6H_{10}O$	139.1513	140.1591	2.2	CSID58830027
5.	2-Methylbutannitril	19.9881	C_5H_9N	82.1242	83.132	7.9	CSID27284
6.	(2S)-2-Methylbutannitril	19.9881	C_5H_9N	82.1242	83.132	7.9	CSID557380
7.	Piperidin	19.9881	$C_5H_{11}N$	84.1392	85.147	6.1	CSID7791
8.	2-Methyl-2,5-cyclohexadien-1,4-diol	19.9881	$C_7H_{10}O_2$	125.1452	126.153	2.3	CSID58829837
9.	(5β,8α,9β,10α)-Kaurane-17,18-dioic acid	25.5481	$C_{20}H_{30}O_4$	271.1186	272.1264	-5.3	CSID24703318
10.	13-apo-β-carotenone	25.5481	$C_{18}H_{26}O$	218.0922	219.1000	5.3	CSID4516011
11.	p-cymene	25.5481	$C_{10}H_{14}$	110.1120	111.1198	7.4	CSID7183
12.	Butyl propanoate	25.5481	$C_7H_{14}O_2$	129.1772	130.185	5.7	CSID11045
13.	(4Z)-4-[(3S)-3-Hydroxybutylidene]-3,5,5-trimethyl-2-cyclohexen-1-one	25.5481	$C_{13}H_{20}O_2$	207.2892	208.297	4.6	CSID30825341
14.	g-Butyrobetaine	27.3812	$C_7H_{15}NO_2$	106.1007	107.1085	2.1	CSID705
15.	L-γ-Palmitoyl-α-lysolecithin	30.1764	$C_{24}H_{50}NO_7P$	472.3427	473.3505	2.6	CSID405287
16.	Oleic acid	30.1764	$C_{18}H_{34}O_2$	281.4532	282.461	6.2	CSID393217
17.	(6Z)-6-Octadecenoic acid	30.1764	$C_{18}H_{34}O_2$	281.4532	282.461	6.2	CSID4444569
18.	cis-Vaccenic acid	30.1764	$C_{18}H_{34}O_2$	281.4532	282.461	6.2	CSID4445888

Table 4.12: Identification of secondary metabolites using UHPLC-QTOF-MS in little millet

Peak No.	Tentative metabolites	RT (min)	MF	MM	$[M-H]^+$	Error (ppm)	Compound ID
n-hexane							
1.	Palmitic acid	2.0217	$C_{16}H_{32}O_2$	256.2402	257.2477	1.0	CSID960
2.	Stigmasterol	19.5681	$C_{29}H_{48}O$	412.3705	413.3789	2.5	CSID4444352
3.	1-Linoleoyl-glycero-3-phosphocholine	25.6577	$C_{26}H_{50}NO_7P$	519.3324	520.3399	1.2	CSID9181014
DCM							
4.	Gibberellin A44 diacid	12.9609	$C_{20}H_{26}O_6$	362.1740	363.1844	2.9	CSID24784737
5.	β-Stigmasterol	28.0249	$C_{29}H_{48}O$	412.3705	413.3785	2.3	CSID4444352
6.	Ergostan-3-ol	29.1246	$C_{28}H_{50}O$	402.3861	403.3941	4.9	CSID532070
Methanol							
7.	D-Verbascose	1.5164	$C_{30}H_{52}O_{26}$	828.2753	829.2831	0.7	CSID390167
8.	Quercetin	1.759	$C_{24}H_{42}O_{21}$	302.0427	303.0505	1.8	CSID4444051
9.	Fagopyritol a3	7.286	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID145907
10.	Kestotetraose	7.286	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID19128807
11.	1,6-Kestotetraose	7.286	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID24785159
12.	Lychnose	7.286	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID30785504
13.	A-1,4-tetraglucose	7.286	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID3493964
14.	Luteolin	7.286	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.7	CSID4444102
15.	Kaempferol	7.286	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.7	CSID4444395
16.	Scutellarein	7.286	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.7	CSID4445014
17.	Orobol	14.229	$C_{15}H_{10}O_6$	286.236	287.0548	-0.7	CSID4445113
18.	2'-Hydroxygenistein	14.229	$C_{15}H_{10}O_6$	286.236	287.0548	-0.7	CSID4445299
19.	Nystose	14.229	$C_{15}H_{10}O_6$	666.2225	667.2303	1.0	CSID145907
20.	Stachyose	16.399	$C_{19}H_{36}O_5$	666.2225	667.2303	1.0	CSID388624
21.	Fagopyritol b3	7.286	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID30777084

Table 4.13: Identification of secondary metabolites using UHPLC-QTOF-MS in horse gram

Peak No.	Tentative metabolites	RT (min)	MF	MM	$[M-H]^+$	Error (ppm)	Compound ID
n-Hexane extract							
1.	3α,21-Dihydroxy-D-homo-5β-pregn-17a (20)-en-11-one	19.4659	$C_{22}H_{34}O_3$	346.2507	347.2587	1.6	CSID10128389
2.	1,4a-Dimethyl-8-methylene gibbane-1,10-dicarboxylate	21.1374	$C_{20}H_{26}O_4$	330.1842	331.1922	2.8	CSID24784772
3.	8-Hydroxy-2-(3-hydroxy-4,5-dimethoxybenzyl)-7-methoxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepinium	22.9102	$C_{21}H_{28}NO_5$	374.1961	375.2041	1.0	CSID24784686
4.	1-Naphthylacetylspermine	24.2378	$C_{22}H_{34}N_4O$	370.2732	371.2812	1.8	CSID114826
5.	1'-Hydroxy-γ-carotene glucoside	25.4244	$C_{46}H_{68}O_6$	716.5015	717.5095	3.1	CSID30792006
6.	Dehydrosqualene	26.2531	$C_{30}H_{48}$	408.3756	409.3836	4.9	CSID26332853
7.	1-Palmitoyl-2-lysophosphatidylcholine	28.3023	$C_{24}H_{50}NO_7P$	495.3324	496.3404	3.2	CSID405287
DCM extract							
8.	α-Tocopherol	29.2322	$C_{29}H_{50}O_2$	430.3810	431.3890	4.1	CSID14265
9.	Stigmasterol	30.0348	$C_{29}H_{48}O$	412.3709	413.3787	2.3	CSID4444352
Methanol extract							
10.	Nystose	2.1436	$C_{24}H_{42}O_{21}$	666.0902	667.0980	4.9	CSID145907
11.	D-(+)-catechin	6.1344	$C_{15}H_{14}O_6$	290.0790	291.0868	1.2	CSID8711
12.	Hexadecenal	23.1850	$C_{16}H_{30}O$	238.2035	239.2113	3.4	CSID4444172
13.	1,2-Linoleoylphosphatidylcholine	24.8049	$C_{44}H_{80}NO_8P$	781.5750	782.5830	4.6	CSID10716780

Table 4.14: Identification of secondary metabolites using UHPLC-QTOF-MS in rice bean

Peak no.	Tentative metabolites	RT (min)	MF	MM	$[M-H]^+$	Error (ppm)	Compound ID
n-hexane							
1.	(+)-Secoisolariciresinol	13.6086	$C_{20}H_{26}O_6$	362.1729	363.1819	4.5	CSID28288853
2.	Palmitic acid	24.2378	$C_{16}H_{32}O_2$	256.2402	257.2477	0.72	CSID960
3.	1-Linoleoyl-glycero-3-phosphocholine	26.5970	$C_{26}H_{50}NO_7P$	519.3324	520.3399	0.14	CSID9181014
DCM							
4.	1-Linoleoyl-glycero-3-phosphocholine	26.5970	$C_{26}H_{50}NO_7P$	519.3324	520.3399	0.14	CSID9181014
5.	Palmitic acid	24.2378	$C_{16}H_{32}O_2$	256.2402	257.2477	0.72	CSID960
Methanol							
6.	6,7-(Methylenedioxy) coumarin	25.8557	$C_{10}H_6O_4$	190.0249	191.0327	-5.5	CSID2340777
7.	β-D-Fructofuranosyl-(2->1)- β-D-fructofuranosyl β-D-fructofuranosyl-(2->6)-alpha-D-glucopyranoside	2.0153	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID10190528
8.	Nystose	2.0153	$C_{24}H_{42}O_{21}$	666.0902	667.0980	5.1	CSID145907
9.	Fagopyritol A3	2.0153	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID145907
10.	Kestotetraose	2.0153	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID19128807
11.	Lychnose	2.0153	$C_{24}H_{42}O_{21}$	666.2218	667.2296	1.0	CSID30785504
12.	A-1,4-Tetraglucose	2.0153	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID388711
13.	3F-α-D-Galactosylraffinose	2.0153	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID389173
14.	Isolychnose	2.0153	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID389173
15.	Mediose	2.0153	$C_{24}H_{42}O_{21}$	666.2221	667.2299	1.1	CSID847
16.	2-[(1H-Indol-3-ylacetyl) amino]-4-methylpentanoate	2.0153	$C_{24}H_{42}O_{21}$	287.1392	288.1470	-1.2	CSID24784918
17.	(+)-Leucopelargonidin	5.9445	$C_{15}H_{14}O_6$	290.0788	291.0866	1.0	CSID389080
18.	Luteoforol	5.9445	$C_{15}H_{14}O_6$	290.0788	291.0866	1.0	CSID389678
19.	(â^')-Epicatechin	5.9445	$C_{15}H_{14}O_6$	290.0790	291.0868	1.0	CSID65230
20.	D-(+)-catechin	5.9445	$C_{15}H_{14}O_6$	290.0790	291.0868	1.0	CSID8711
21.	Cianidanol	9.2003	$C_{15}H_{14}O_6$	290.0785	291.0863	0.1	CSID1166
22.	P-Coumaroyl quinic acid	9.4230	$C_{16}H_{18}O_8$	338.1007	339.1085	3.3	CSID4945466

Peak no.	Tentative metabolites	RT (min)	MF	MM	$[M-H]^+$	Error (ppm)	Compound ID
23.	5,6-Dihydroxy-2-(4-hydroxyphenyl)-7-methoxy-2,3-dihydro-4H-chromen-4-one	9.4230	$C_{16}H_{14}O_6$	301.0797	303.0875	4.1	CSID58829719
24.	Cis-(-)-7,2'-dihydroxy-4',5'-methylenedioxy isoflavanol	9.4230	$C_{16}H_{14}O_6$	301.0797	303.0875	4.1	CSID58829833
25.	4-Methylumbelliferyl-β-D-glucoside	11.9033	$C_{16}H_{18}O_8$	302.0790	303.0868	1.7	CSID2015550
26.	2,6,7-Trihydroxy-3-(4-methoxyphenyl)-2,3-dihydro-4H-chromen-4-one	11.9033	$C_{16}H_{14}O_6$	302.0790	303.0868	1.8	CSID24785201
27.	Hesperitine	11.9033	$C_{16}H_{14}O_6$	302.0790	303.0868	1.8	CSID3467
28.	4'-Methoxy-2',3,7-trihydroxyisoflavanone	11.9033	$C_{16}H_{14}O_6$	302.0790	303.0868	1.8	CSID35015216
29.	Furcatin	12.0246	$C_{20}H_{28}O_{10}$	428.1656	429.1734	-4.8	CSID391122
30.	6,7,3',4'-Tetrahydroxyisoflavone	12.5572	$C_{15}H_{10}O_6$	286.0471	287.0549	-0.1	CSID4577544
31.	Indole-3-acetyl-glutamate-N-β-D-glucose	12.6785	$C_{21}H_{24}N_2O_{10}$	464.1453	465.1531	3.5	CSID24785300
32.	Homoeriodictyol	12.7659	$C_{16}H_{18}O_8$	302.0794	303.0872	3.0	CSID66296
33.	Solasodine	13.4198	$C_{27}H_{43}NO_2$	575.3821	576.3899	0.7	CSID58837434
34.	6-Hydroxy-2-(4-glucosyl-phenoxymethylene)-benzofuran-3-one	13.7974	$C_{21}H_{20}O_{10}$	432.1068	433.1146	4.0	CSID58837758
35.	Gypsogenic acid	14.0060	$C_{30}H_{46}O_5$	486.3346	487.3424	1.5	CSID10217372
36.	Calycosin	14.4711	$C_{16}H_{12}O_5$	284.0681	285.0759	0.7	CSID4444104
37.	Coreopsin	14.8150	$C_{21}H_{22}O_{10}$	434.1212	435.1290	0.9	CSID24784916
38.	8,3'-Dihydroxydaidzein	15.1250	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.4	CSID23255668
39.	Luteolin	15.1250	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.4	CSID4444102
40.	Scutellarein	15.7789	$C_{15}H_{10}O_6$	286.0470	287.0548	-0.4	CSID4445014
41.	3-Hydroxy-1,2-propanediyl bis(2-propylpentanoate)	21.0498	$C_{19}H_{36}O_5$	344.2554	345.2632	-1.1	CSID21163206
42.	Phytoceramide	21.2387	$C_{18}H_{39}NO_3$	317.2921	318.2999	-0.9	CSID108921
43.	1-Hydroxy-1,2-ethanediyl dioctanoate	21.6361	$C_{18}H_{34}O_5$	330.2398	331.2476	-0.9	CSID58829678
44.	1-Linoleoyl-2-Hydroxy-sn-glycero-3-PC	21.7037	$C_{26}H_{50}NO_7P$	519.3322	520.3400	-0.3	CSID9181014
45.	Vernolic acid	21.9800	$C_{18}H_{32}O_3$	296.2348	297.2426	1.3	CSID4512106
46.	12(13)-Epoxy-9Z,15Z-octadecadienoic acid	22.8425	$C_{18}H_{30}O_3$	294.2195	295.2273	1.6	CSID17220744

Table 4.15: Target Molecules identified by UHPLC-QTOF-MS in the selected legumes

S.No.	Compound name	Molecular weight	Adzuki bean	Mung bean	Rice bean	Horse gram	Little millet
1.	Catechin-7-O-glucoside	452.1319	+	+	+	+	+
2.	Catechin	290.0790	+	+	+	+	+
3.	Epicatechin	290.0790	+	+	+	+	+
4.	Quercetin	302.0427	+	+	+	+	+
5.	Gallocatechin	2037.4728	-	+	+	+	-
6.	Gallic acid	170.1210	+	+	+	+	+
7.	Caffeic acid	180.0423	+	+	+	+	+
8.	Para-coumaric acid	164.0473	+	+	+	+	+
9.	Glycitein	284.2610	+	+	+	+	+

4.8 Amino acid profiling

The nutritional potential of the selected cereal samples was assessed through amino acid profiling. The profile of amino acids plays a vital role in defining the nutritional quality of proteins, particularly because cereals/legumes are widely consumed as staple foods and represent a major source of dietary protein across various populations. The amino acid profile of the selected cereal samples revealed some interesting results, as shown in Tables 4.16 to 4.18. The selected samples contained essential, non-essential, and non-proteinogenic amino acids. All the essential amino acids, viz. arginine, leucine, histidine, lysine, methionine, tryptophan, phenylalanine, valine, threonine, and isoleucine, were found in significant amounts.

In adzuki bean, essential (47.60%), non-essential (46.99%), and non-proteinogenic amino acids (5.39%) were present in significant quantities. Arginine, leucine, and tryptophan accounted for 48.55% of the essential amino acids. The data revealed that the major essential and non-essential amino acids in adzuki bean were tryptophan and glutamic acid, respectively. In mung bean, essential amino acids (44.34%), non-essential amino

acids (53.80%), and non-proteinogenic amino acids (1.84%) were significantly present, in which leucine and phenylalanine account for 34.43% of the essential amino acids. The data revealed that the major essential and non-essential amino acids in mung beans were leucine and glutamic acid. In little millet, the essential amino acids (39.20%), non-essential amino acids (54.13%), and non-proteinogenic amino acids (6.66%) were in adequate quantity. Valine and leucine account for 40.72% of the essential amino acids. The most common and major essential and non-essential amino acids were leucine and glutamic acid, respectively. Similarly, both rice bean and horse gram exhibited a balanced distribution of amino acids, with rice beans containing 42.66% essential amino acids, 51.11% non-essential amino acids, and 6.22% non-proteinogenic amino acids. Similarly, horse gram comprised 43.41% essential amino acids, 50.56% non-essential amino acids, and 6.01% non-proteinogenic amino acids. Notably, leucine and phenylalanine constituted a substantial proportion of the essential amino acid content - 33.21% in rice bean and 36.95% in horse gram. These amino acids have distinct health advantages e.g., leucine promotes protein biosynthesis, glutamic acid plays a significant role in brain disorders such as schizophrenia, Parkinson's disease, and epilepsy [143-144]. Leucine, an essential amino acid present in both legumes, plays a crucial role in several physiological functions, including protein synthesis, energy production, neurotransmitter formation, glucose metabolism, immune response, liver cell apoptosis, and regeneration [143-145]. On the other hand, glutamic acid, a non-essential amino acid, serves as a key excitatory neurotransmitter in the brain, influencing neural development by supporting differentiation, migration, and survival [144].

Moreover, several non-proteinogenic amino acids were also present, out of which hydroxyproline was the major among adzuki bean, little millet, and rice bean while 1-

methylhistidine was the major non-proteinogenic amino acid in mung beans and horse gram, consistent with previous findings reporting abundant non-proteinogenic amino acids in Fabaceae seeds [146].

β-Alanine is the precursor of pantothenic acid (vitamin B5), and it has been widely used as a precursor for many significant industrial chemicals in food, medicine, and environmental applications [147]. Recently, branched chain essential amino acids and non-proteinogenic amino acids like taurine, orinithine are known to have a positive effect on the regulation of body weight, muscle protein synthesis, and glucose homeostasis [148]. Taurine and ornithine are reported to play an anti-obesity role [149-150]. β-Amino isobutyric acid, which is present in little millet, favourably affects lipid metabolism, decreases inflammatory reactions, and increases insulin sensitivity. According to some recent studies, it can protect against diet-induced obesity in animal models [151]. Thus, these results support the exceptional nutritional value of these cereals/legumes, which serve as abundant sources of essential, non-essential, and non-protein amino acids, with the non-protein compounds further elevating their comprehensive nutritional benefits.

Table 4.16: Essential amino acids composition of selected samples

Essential Amino Acids (g/100 g dry sample)	**Sample**				
	Adzuki bean	**Mung bean**	**Rice bean**	**Horse gram**	**Little millet**
Arginine	1.43 ± 0.012	0.86 ± 0.008	0.99 ± 0.013	0.94 ± 0.015	0.40 ± 0.006
Histidine	0.41 ± 0.001	0.37 ± 0.002	0.41 ± 0.005	0.44 ± 0.006	0.10 ± 0.001
Isoleucine	0.87 ± 0.002	0.92 ± 0.019	0.98 ± 0.009	0.99 ± 0.009	0.40 ± 0.002
Leucine	1.42 ± 0.006	1.51 ± 0.021	1.63 ± 0.018	1.57 ± 0.012	0.90 ± 0.005
Lysine	1.03 ± 0.011	1.02 ± 0.015	1.15 ± 0.025	1.12 ± 0.011	0.10 ± 0.002
Methionine	0.197 ± 0.001	0.11 ± 0.001	0.07 ± 0.001	0.11 ± 0.003	0.10 ± 0.004
Phenylalanine	1.061 ± 0.013	1.18 ± 0.012	1.21 ± 0.015	1.77 ± 0.019	0.50 ± 0.003
Threonine	0.774 ± 0.001	0.71 ± 0.004	0.87 ± 0.008	0.92 ± 0.008	0.40 ± 0.002
Tryptophan	2.210 ± 0.022	0.07 ± 0.00	0.08 ± 0.00	0.08 ± 0.000	0.03 ± 0.002
Valine	1.009 ± 0.01	1.07 ± 0.009	1.15 ± 0.013	1.08 ± 0.015	0.50 ± 0.006

Table 4.17: Non-essential amino acids composition of selected samples

Non-Essential Amino Acids (g/100 g dry sample)	**Samples**				
	Adzuki bean	**Mung bean**	**Rice bean**	**Horse gram**	**Little millet**
Alanine	0.78 ± 0.002	0.79 ± 0.005	0.88 ± 0.006	0.88 ± 0.002	0.70 ± 0.005
Aspartate	2.21 ± 0.027	2.26 ± 0.028	2.54 ± 0.032	1.83 ± 0.019	0.50 ± 0.004
Cysteine	1.14 ± 0.018	0.08 ± 0.00	0.09 ± 0.000	0.28 ± 0.001	0.05 ± 0.001
Glutamic acid	2.92 ± 0.025	3.34 ± 0.106	3.34 ± 0.103	3.70 ± 0.103	1.80 ± 0.009
Glycine	0.79 ± 0.005	0.75 ± 0.014	0.87 ± 0.008	0.98 ± 0.006	0.20 ± 0.013
Proline	0.94 ± 0.008	0.87 ± 0.013	1.06 ± 0.005	1.09 ± 0.016	0.70 ± 0.011
Serine	0.97 ± 0.007	1.02 ± 0.010	1.07 ± 0.018	1.23 ± 0.006	0.50 ± 0.005
Tyrosine	0.51 ± 0.003	0.37 ± 0.001	0.40 ± 0.009	0.54 ± 0.002	0.20 ± 0.001

Table 4.18: Non-proteinogenic amino acids composition of selected samples

Non-proteinogenic Amino Acids (g/100 g dry sample)	**Sample**				
	Adzuki beans	**Mung beans**	**Rice beans**	**Horse gram**	**Little millet**
Phosphoserine	0.069 ± 0.001	0.021 ± 0.002	0.030 ± 0.001	0.040 ± 0.00	0.02 ± 0.001
Taurine	0.047 ± 0.002	0.026 ± 0.003	0.037 ± 0.002	0.044 ± 0.00	0.04 ± 0.001
Phospho ethanol amine	0.002 ± 0.00	ND	0.002 ± 0.00	0.004 ± 0.00	ND
α Amino adipic acid	ND	0.054 ± 0.006	ND	ND	ND
β-Alanine	0.064 ± 0.001	0.045 ± 0.002	0.064 ± 0.00	0.058 ± 0.00	0.005 ± 0.00
Ethanol amine	0.013 ± 0.001	0.008 ± 0.00	ND	0.001 ± 0.00	0.002 ± 0.00
Ornithine	0.003 ± 0.00	0.004 ± 0.00	0.006 ± 0.00	0.003 ± 0.00	0.001 ± 0.00
1-Methylhistidine	ND	0.089 ± 0.002	0.095 ± 0.00	0.107± 0.001	0.05 ± 0.001
Hydroxyproline	0.839 ± 0.009	0.0790 ± 0.001	1.018 ± 0.016	0.999 ± 0.005	0.4 ± 0.002
Cystathionine	ND	ND	ND	ND	0.03 ± 0.002
β-Amino isobutyric acid	ND	ND	ND	ND	0.003 ± 0.00

4.9 Antioxidant activity (DPPH assay)

The antioxidant activity of the selected cereal extracts was assessed to evaluate their potential in neutralizing free radicals and reducing oxidative stress, which is closely linked to the prevention of chronic diseases. DPPH free radical scavenging activity is a widely used screening method for antioxidants in most plant extracts. It is a free-radical compound that has an absorbance in its oxidized form at 515-520 nm [152]. The data of the antioxidant activity of rice bean, horse gram, and little millet extracts at different concentrations are shown in Figures 4.2, 4.3, and 4.4.

The antioxidant analysis of different solvent extracts revealed distinct differences among the tested cereal samples. In horse gram and rice bean, the methanol extracts consistently showed the highest radical scavenging activity compared to their n-hexane and dichloromethane extracts, highlighting the role of polar phytochemicals such as phenolics and flavonoids. The antioxidant assay results of horse gram indicate that the methanol extract of both the beans showed the highest antioxidant activity, with a sample concentration of 300 µL/mL. The antioxidant activity of horse gram's methanolic extract was slightly higher than that of rice bean. For little millet, only the methanol extract was assessed, and the highest antioxidant activity was observed at a sample concentration of 200 µL/mL (as shown in Figure 4.4). From the antioxidant data of all the selected cereal methanol extracts, it can be inferred that horse gram showed the highest radical scavenging activity compared to rice bean and little millet extract. This suggests that horse gram has a greater concentration or variety of antioxidant phytochemicals, particularly phenolics and flavonoids, than rice bean and little millet. The results also highlight methanol as an efficient solvent for extracting these bioactive compounds.

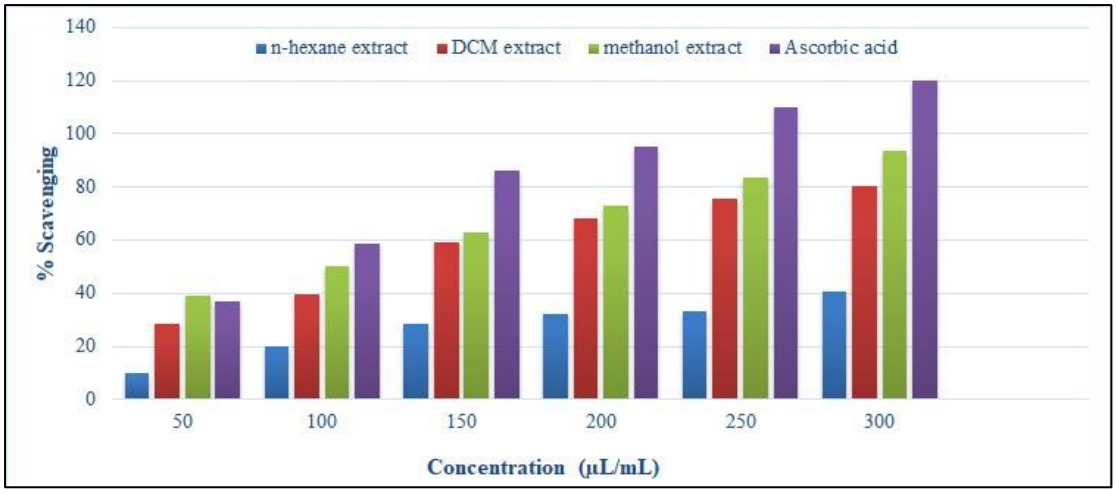

Figure 4.2: Antioxidant activity of different extracts of horse gram

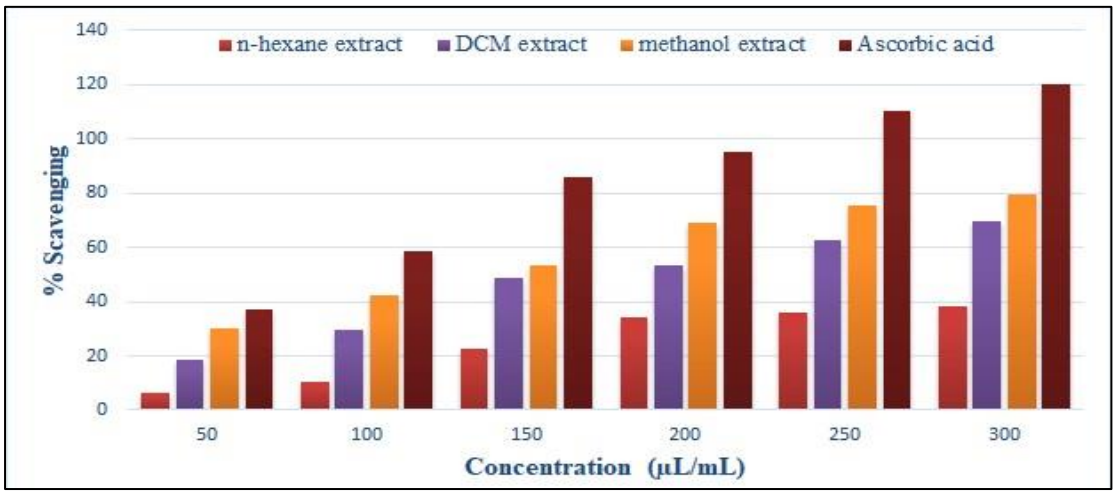

Figure 4.3: Antioxidant activity of different extracts of rice bean

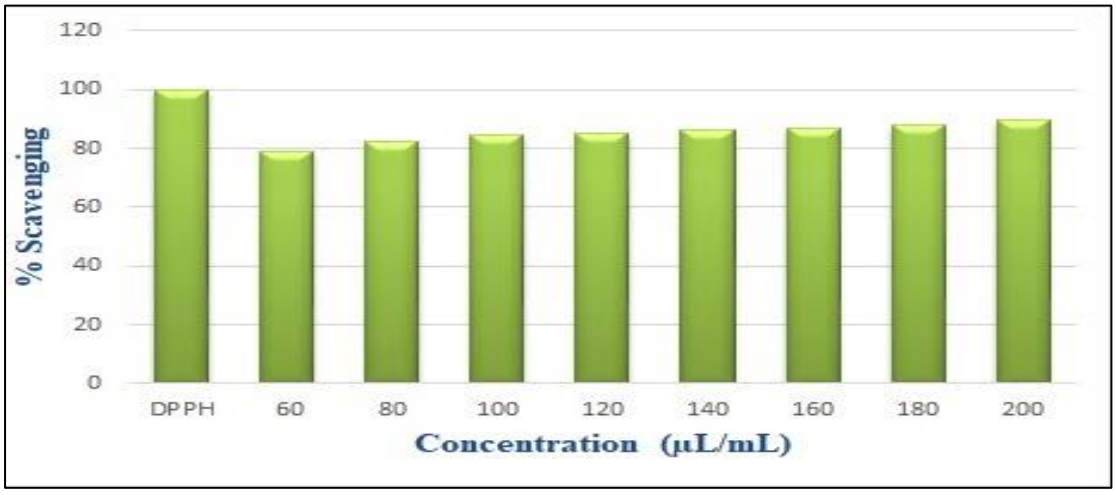

Figure 4.4: Antioxidant activity of the methanol extract of little millet

4.10 Antimicrobial activity (Disc-Diffusion method)

The antimicrobial potential of the selected cereal extracts was assessed to determine their ability to inhibit the growth of pathogenic microorganisms. The results demonstrated that the extracts exhibited varying degrees of inhibitory activity against the tested bacterial strains, indicating the presence of bioactive phytochemicals capable of suppressing microbial growth.

The antimicrobial assay demonstrated a distinct variation in activity among the methanolic extracts of the selected cereal samples. The extract of horse gram exhibited the strongest inhibitory effect, showing a 17 mm zone of inhibition against a Gram-negative bacterium (*E. coli*) and a 15 mm zone against Gram-positive bacterium (*S. aureus*). In comparison, the methanolic extract of rice bean exhibited only mild activity, with a 14 mm inhibition zone against *E. coli,* but no detectable effect against *S. aureus* (Figure 4.5).

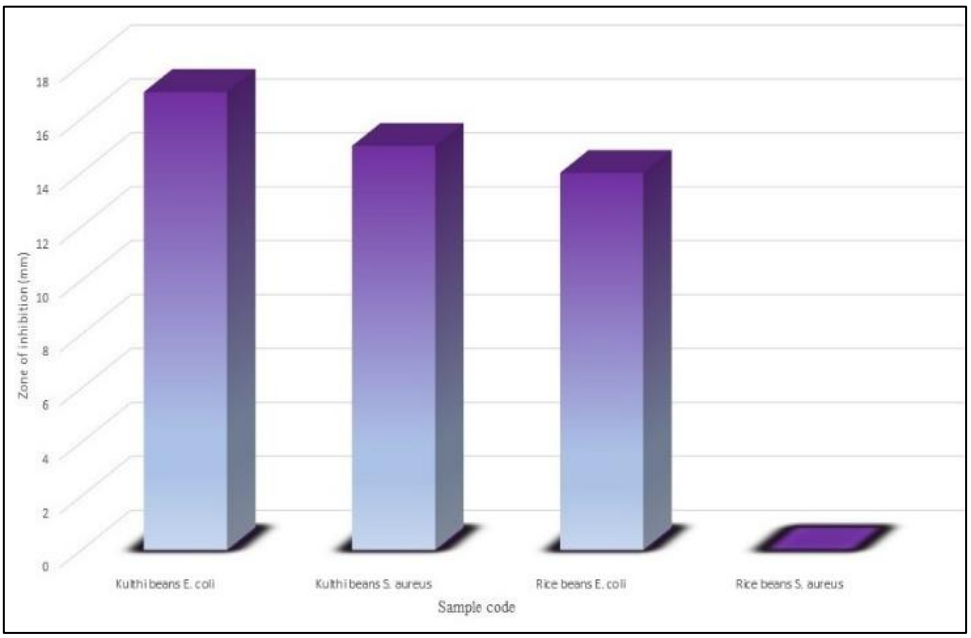

Figure 4.5: Antimicrobial activity of horse gram and rice bean against *E. coli* and *S. aureus*

The methanolic extract of little millet also showed pronounced antimicrobial potential, yielding a 16 mm zone of inhibition against *E. coli* and 14 mm against *S. aureus*. Collectively, these observations suggest that among the tested extracts, horse gram demonstrates superior antimicrobial efficacy, highlighting their potential as a promising natural source of antimicrobial agents.

4.11 Cytotoxicity activity (Neutral red uptake assay)

The potential safety and biological activity of the selected underutilized cereals were evaluated through cytotoxicity analysis. Among the five cereal samples, two representative extracts (adzuki and mung bean methanol extract) showing promising phytochemical and bioactivity profiles were selected for detailed cytotoxicity assessment. This evaluation is important to determine whether the bioactive compounds present in these extracts exert any toxic effects on the respective cell line, thereby ensuring their suitability for nutraceutical/functional food applications.

To evaluate cytotoxicity (cell viability), the NRU assay was utilized due to its widespread acceptance, reliability, and sensitive ability to determine cell viability. This assay evaluates how effectively living cells absorb and retain neutral red dye within lysosomes, serving as a reliable marker of membrane integrity and overall cellular health.

The results of the cytotoxicity assay at different concentrations of methanol extract of adzuki bean and mung bean are shown in Figure 4.6. It can be observed that both the samples exhibited cell viability values of more than 80%, which indicate good biocompatibility of both the methanol extracts with the 3T3-cell line. The IC_{50} values for adzuki bean and mung bean extract were found to be 921.7 µg/mL and 174.1

µg/mL, respectively. The decrease in cell viability is generally proportional to the extent of cytotoxicity. From the dose-response curve, it is clear that the extent of cytotoxicity is lower in both extracts. Also, the lower the IC_{50} value of the extract, the higher the cytotoxicity. From the IC_{50} value data, the mung bean methanol extract is lower than the adzuki bean methanol extract. Thus, mung bean extract showed maximum cytotoxicity on 3T3- cell line. Hence, it may be interpreted that the methanol extract of adzuki bean is less cytotoxic and more cyto-compatible than the mung bean methanol extract.

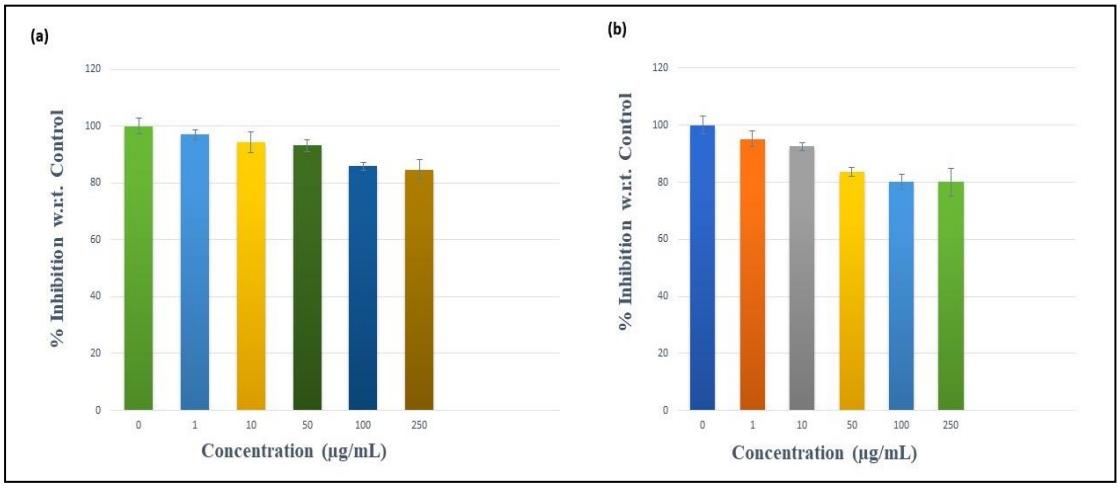

Figure 4.6: Cytotoxicity study of methanol extract of (a) adzuki bean (b) mung bean

4.12 HMG-Co-A reductase enzyme activity

To investigate the cholesterol-lowering/anti-obesity potential of the selected cereal extracts (adzuki and mung bean), the inhibitory effect of the methanol extract of adzuki bean and mung bean on the HMG-Co-A reductase enzyme was assessed by in vitro assay. HMG-CoA (3-hydroxy-3-methyl-glutaryl-coenzyme A) reductase is the key enzyme in the biosynthesis of cholesterol. The marked down-regulation of hepatic HMG-CoA reductase can contribute to hypocholesteremia [153-155]. Each extract sample could inhibit the HMG-Co-A reductase enzyme in a dose-dependent manner.

Among the methanol extracts of adzuki bean and mung bean, adzuki bean showed an inhibitory effect of more than 50% on HMG-Co-A reductase activity. The inhibitory effect of adzuki bean extract was 60%, as shown in Figure 4.7. The results indicate that adzuki bean methanol extract may have the potential to lower cholesterol levels and have the potential for anti-obesity activity.

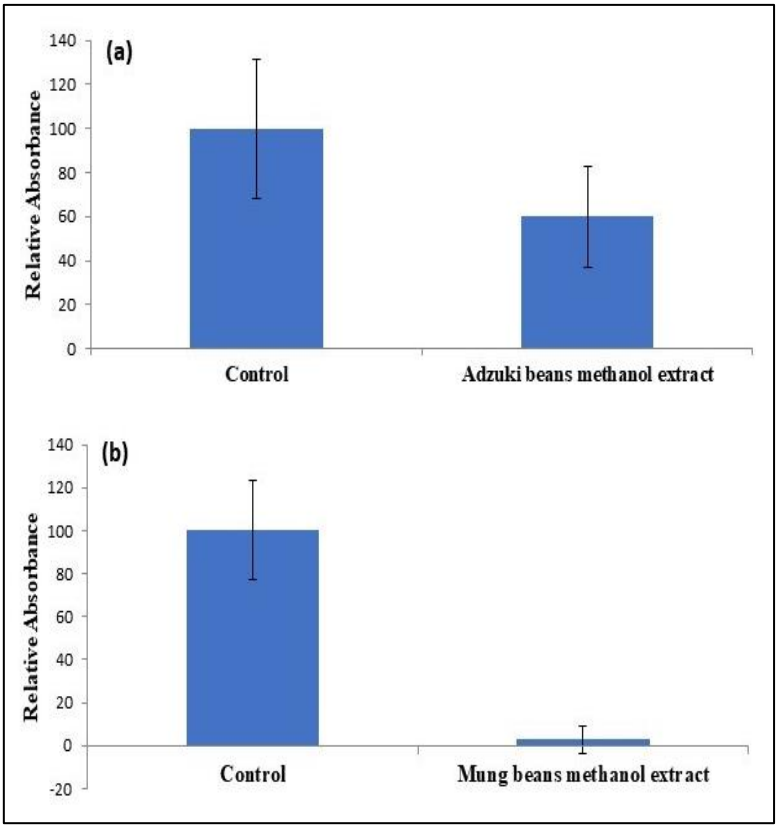

Figure 4.7: Inhibitory effect of (a) adzuki bean methanol extract, (b) mung bean methanol extract, against HMG-Co-A reductase enzyme

4.13 Pancreatic lipase inhibitory activity

In addition to cholesterol regulation, the inhibition of pancreatic lipase is an important mechanism for controlling obesity. For this, the lipase inhibitory activity of the cereal extracts was evaluated. In this study, adzuki and mung beans extract were evaluated for their lipase inhibitory activity. Pancreatic lipase, synthesized and secreted by the pancreas,

is an enzyme that is responsible for the digestion of body lipids. The inhibitory activity of both the extracts against pancreatic lipase is presented in Figure 4.8. Concentration is the key factor in the inhibition rate of bean extracts and the standard drug orlistat (positive control). As Figure 4.8 shows, the inhibition of enzyme activity has increased as the extract concentration has increased. The adzuki bean extract showed the maximum inhibition of 75.45% as compared to mung bean extract (72.72%). The IC_{50} values of adzuki and mung bean extract were 37.16 ± 0.15 µg/mL and 44.93 ± 0.04 µg/mL, respectively, as compared to standard orlistat (IC_{50} = 24.83 ± 0.16 µg/mL). Lower the IC_{50} value ($p < 0.05$), higher will be the lipase inhibitory activity. Overall, adzuki bean extract showed lower IC_{50} value and higher pancreatic lipase activity, suggesting it as an excellent source of lipase inhibitor.

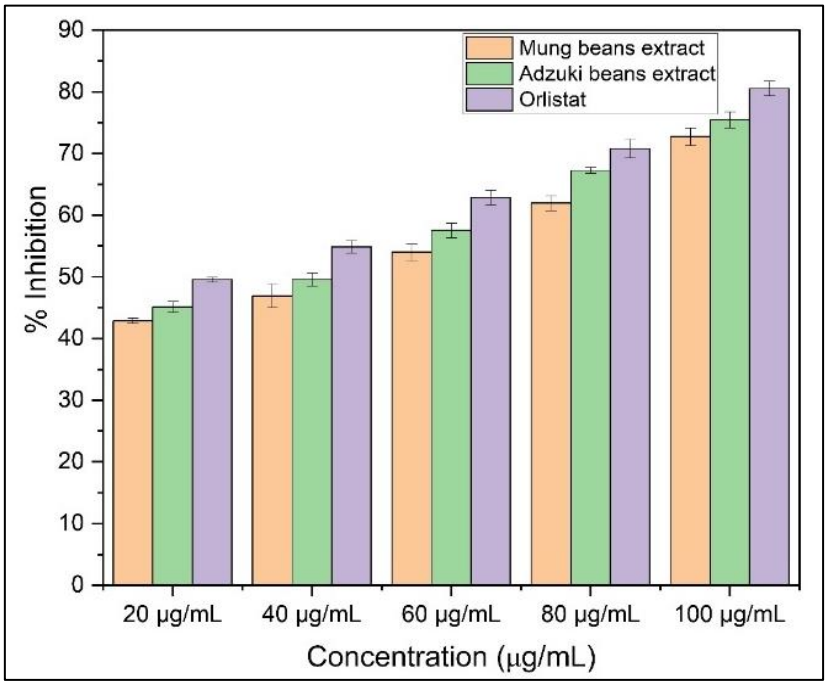

Figure 4.8: In-vitro pancreatic lipase inhibitory activity of adzuki and mung bean extract; the data is presented as the mean ± standard deviation ($p < 0.05$)

CHAPTER 5
APPLICATIONS

5.1 Introduction

This chapter explores the diverse applications of underutilized cereals, underscoring their promise for transformation into value-added novel food products and functional biomaterials. Their abundant nutritional content, presence of bioactive compounds, and distinctive functional attributes make them a compelling basis for innovations that go beyond conventional dietary applications. This chapter is subdivided into two sections for better understanding.

Section-5A: The focus of this section is on developing novel food products (nutritional cookies and breakfast cereals) that utilize underutilized cereals, which serve as ready-to-eat, nutritionally enhanced alternatives to conventional cereal-based food products. Such innovations not only promote dietary diversification and improve nutritional intake but also align with the growing consumer demand for healthier and sustainable foods.

Section-5B: This section explores the potential of mucilage extracted from underutilized cereals and investigates its application through the synthesis of mucilage-based hydrogels. Plant-derived mucilage serves various functional roles, including as a binder, stabilizer, emulsifier, thickener, and gelling agent. To this end, four novel mucilage sources-adzuki bean, amaranth, proso millet, and little millet-were studied. Hydrogels were developed using the free radical co-polymerization method to assess the utility of these mucilages. The findings suggest that mucilage from these lesser-known cereals could serve as a promising raw material for hydrogel production, with potential applications in the food, cosmetic, and pharmaceutical sectors.

Section-5A

Novel cereals and cookies to combat obesity using adzuki and mung bean

5A.1 Introduction

Underutilized legumes hold immense potential as sustainable and nutrient-dense resources for addressing global food security and health challenges. Despite their rich nutritional profiles, including high-quality proteins, dietary fiber, essential amino acids, and bioactive compounds, many of these legumes remain underexploited in modern food systems [104]. Due to health-related issues of individuals, consumers are looking for more functional, nutritious, and convenient food products. Interest has been sparked in developing healthy alternatives to typical cookies and breakfast flakes. Adzuki bean (*Vigna angularis*) and mung bean (*Vigna radiata*) are a few of the many legumes that are little explored for functional food formulations. The reason they have become so popular is because of their excellent nutritional profile and positive health effects. The presence of bioactive compounds in these legumes, due to their antioxidant, anti-inflammatory, anti-diabetic, anti-obesity, and cardioprotective benefits, offers great nutraceutical potential [156],[8]. Since they have these properties, which position them as good ingredients for developing functional foods for preventing and controlling chronic diseases like diabetes, cardiovascular diseases, and obesity, they are of great interest. It is possible to incorporate these versatile ingredients in convenient formats such as breakfast flakes and cookies, offering a unique opportunity to make them mainstream health-oriented products. By increasing the value of underutilized crops and improving dietary diversity, sustainability, and nutrition security, this approach adds to other public and economic benefit approaches. This research explores the functional

properties and nutraceutical potential, processing characteristics, and sensory acceptance of these legumes to assess their potential to develop innovative and consumer-friendly products that bridge the gap between the traditional and modern aspects of nutritional needs.

Adzuki bean, a dietary legume crop commonly consumed in East Asian diets, are recognized for their antioxidant properties, attributed to the presence of phenolic compounds and flavonoids. It is appraised as a valuable crop because of its nutritional profile. They are also a significant source of dietary fiber, contributing to improved digestive health and glycemic control [157]. Similarly, mung beans, a staple in many Asian cuisines, are lauded for their protein content, bioactive peptides, and low glycemic index, which collectively support cardiovascular health, weight management, and metabolic regulation [68]. Despite their established nutritional and functional potential, incorporating these legumes into ready-to-eat food formats like breakfast flakes and cookies remains underexplored.

Incorporating legumes such as adzuki and mung bean into flakes and cookies offers a practical and innovative way to enhance the nutritional value of these popular food products. However, adzuki and mung bean have not been extensively explored as primary ingredients in breakfast flakes and cookies. Limited studies employing these underutilized legumes restrain our understanding of their potential in nutraceutical food products.

Therefore, this study evaluated the pancreatic lipase inhibiting activity of adzuki and mung beans and their feasibility to develop breakfast flakes and nutritional cookies. It investigated the functional properties of the formulated products, like the water solubility

and water absorption indices, nutritional profile, and sensory evaluation. Exploration of the potential legumes' flour will contribute to developing novel, nutritive, and health-benefiting breakfast options. Over and above that, this could promote the broader utilization of the underutilized and affordable crops, benefiting the dearth of food regions.

5A.2 Materials and methods

5A.2.1 Selection and preparation of raw materials

Adzuki bean was purchased from SOS Organics, Uttarakhand, India. Mung bean was purchased from the Patanjali store in Delhi, India. Dr. Sunita Garg, CSIR-NIScPR, Raw Materials Herbarium, and Museum, Delhi (RHMD), India, authenticated the selected beans. Adzuki and mung bean flour were obtained by grinding the beans in a local flour mill. Adzuki bean flour (ABF) and mung bean flour (MBF) were used as such, as no processing technique was applied before their use. Ragi and jowar flour were purchased from Interlink Foods Pvt. Ltd., National Institute of Food Technology, Entrepreneurship and Management (NIFTEM), Haryana, India.

5A.2.2 Preparation of extracts

For the preparation of ethanol extract of adzuki and mung bean, 15 g of coarsely ground sample was mixed with 50 mL of ethanol and soaked for 48 h by hot percolation method [158]. After incubation, both extracts were filtered using muslin cloth. Filtered extracts were labelled and stored at 4 ºC until further use.

5A.2.3 Product formulations

Traditional techniques were used to prepare different products (breakfast cereals and cookies). The complete formulations of the products are shown in Table 5A.1. The amount of ABF and MBF additions was selected based on prior work.

5A.2.3.1 Preparation of breakfast cereals/flakes

To prepare breakfast flakes, 6 Kg ABF and 4 Kg ragi flour were mixed in a mixing machine. A small-scale industry, Interlink Foods Pvt. Ltd., pre-optimized this ratio. Water (2 L) and oil (250 mL) were added, followed by beating for 10 mins at 60-70 rpm (Figure 5A.1). The batter was then transferred to a screw conveyor and hot extruder to form small balls of the material. Then, it passed through a flaker to form uniform-sized flakes. Finally, flakes were baked in a five-layered oven at 210 °C for 5 min. Different formulations were made using the same procedure (Table 5A.1).

Table 5A.1: Composition of breakfast flakes and cookies

Ingredients	**Breakfast flakes**		**Cookies**	
	Adzuki bean	**Mung bean**	**Adzuki bean**	**Mung bean**
ABF (Kg)	6	-	0.5	-
Ragi flour (Kg)	4	-	-	-
Jowar flour (Kg)	-	4	-	-
MBF (Kg)	-	6	-	0.5
Wheat flour (Kg)	-	-	1.5	1.5
Oil (mL)	250	250	-	-
Water (mL)	2000	2000	10	10
Sugar (Kg)	-	-	1	1
Ghee (Kg)	-	-	1	1
Skim milk powder (g)	-	-	50	50
Baking powder (g)	-	-	10	10
Custard powder (g)	-	-	50	50
Baking soda (g)	-	-	10	10
Vanilla essence (mL)	-	-	2	2

5A.2.3.2 Preparation of cookies

Cookies were also prepared with the help of Interlink Foods company, based on their recipe of typical Indian cookies (Figure 5A.1). The ingredients used for cookie preparation included: wheat flour, 1.5 Kg; adzuki bean flour, 500 g; sugar, 1 Kg; baking powder, 10 g; baking soda, 10 g; skim milk powder, 50 g; ghee, 1 kg; custard powder, 50 g; vanilla essence, 2 mL. Firstly, ghee and powdered sugar were mixed for 15 min, followed by the addition of baking powder, baking soda, vanilla essence, and custard powder. After that, different flours were added (Table 5A.1), followed by the addition of water. The prepared dough was dropped into a cookie-making machine. A conventional baking oven was employed to bake the prepared dough at 170°C for around 15 min, and then it was allowed to cool down to room temperature. The prepared cookies were then packed in aluminum-laminated sealed bags and stored at 25 °C for further analysis.

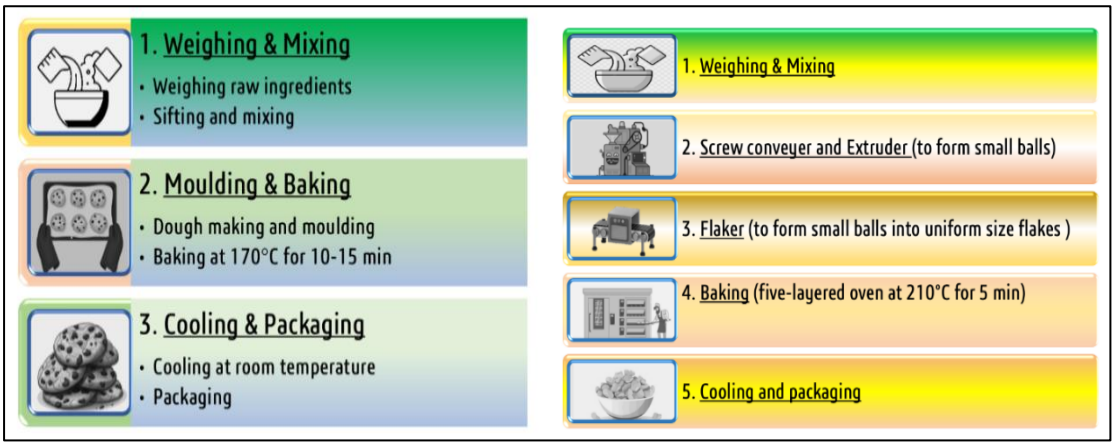

Figure 5A.1: Flowchart for legume-based cookies and flakes preparation

5A.2.4 Physical properties

5A.2.4.1 Weight, diameter, and thickness

Cookies and flakes from each formulation were weighed using digital balance. The height and diameter of cookies and flakes were measured using a digital vernier caliper

(Digimatic, Mitutoyo). To check the reproducibility of this study, the experiment was performed in triplicate, n=3.

5A.2.4.2 Spread ratio

Spread ratio of both formulations of cookies and flakes was determined by taking the ratio of thickness and width, following the below-mentioned formula. The study was performed in triplicate, n=3.

$$\text{Spread ratio} = \frac{\text{Diameter of sample}}{\text{Thickness of sample}}$$

5A.2.4.3 Bulk density

The bulk density of prepared cookies and flakes was determined using the mass to volume ratio. The study was performed in triplicate, n=3.

5A.2.5 Functional properties

5A.2.5.1 Water solubility index

The water solubility index (WSI) of prepared formulations of cookies and flakes sample were determined according to the method described by Mugabi et al., with some modifications [159]. 2.5 g milled cookies and flakes (separately) were suspended in 25 mL of distilled water and stirred for 30 min at room temperature (26 ºC). After successful stirring, the solution was kept undisturbed for 20 min for better solubility. The solution was then centrifuged at 3000 rpm for 10 min. The supernatant was transferred into a petri dish with a known weight and dried until it was consistent in weight. WSI (%) was measured as per the below-mentioned formula. The study was performed in triplicate, n=3.

$$\text{WSI (\%)} = \frac{\text{Weight of dried supernatant (g)}}{\text{Initial weight of sample (g)}} \text{ x } 100$$

5A.2.5.2 Water absorption index

Water absorption index of prepared formulations of cookies and flakes samples was determined according to the method described in IS (Indian Standard) 12516 (P-3) 1988. The study was performed in triplicate, n=3.

5A.2.6 Chemical properties

5A.2.6.1 Total phenolic and flavonoid content

The total phenolic and flavonoid content present in prepared formulations (cookies and flakes) was analyzed following AFLPL/SOP/CH/INH/60 (Avon Food Lab Private Limited/Standard Operating Procedure/Chemical/Instrumentation) and AFLPL/SOP/ CH/INH/319 method of Avon Food Lab, Delhi. The study was performed in triplicate, n=3.

5A.2.7 Nutritional analysis

The formulated products were characterized for protein, moisture, carbohydrate, fat, dietary fiber, and ash content following the AOAC (Association of Official Analytical Chemists) :991.20 (2019), IS:1656:2007 (2018), IS:4684:1975 (2020), IS:11062 (2019), AOAC (2016), and AOAC (2019) methods, respectively [160-164]. The mineral contents like potassium, calcium, iron, and sodium in the formulated products were determined by the method described in IS:12760:2012 (2018), AFLPL/SOP/CH/INH/163, AFLPL/SOP/CH/INH/162, and AFLPL/SOP/CH/INH/204, respectively. The vitamin content was also determined following the AFLPL/SOP/ CH/INH/206, EN (European Standard):14122:2014, EN:14152:2014, AFLPL/SOP/CH/

INH/01, AOAC:2004.07:2019 methods, respectively. The AFLPL/SOP/CH/INH/376 method investigated the products' folic acid content. All the analyses were performed in triplicate, n=3.

5A.2.8 Sensory evaluation

Sensory evaluation of the formulated products was conducted at Delhi Technological University (Delhi, India) by 30 panelists. The five-point hedonic scale is a suitable and efficient method for sensory tests, resulting in informative results [165]. In five-point hedonic scaling, 1= I don't like it very much; 2 = I moderately dislike it; 3 = I don't like or dislike it; 4 = I moderately like it; 5 = I like it very much. All the panelists were seated in an isolated area at room temperature (25°C) and were provided with samples packed inside of a digit-codified plastic container and with a glass of normal water to rinse between each tasting. All the samples were evaluated for different attributes (taste, texture, appearance, and overall acceptability). The products' acceptability index was calculated using the previously described method and the following equation [166]. The product formulations with an AI% higher than 70% were accepted well. To check the reproducibility of the analysis, this study was performed in triplicate, n=3.

$$\text{Acceptability index (\%)} = \frac{\text{Mean score}}{\text{Maximum score}} * 100$$

5A.2.9 Statistical analysis

The results are presented as mean ± standard error, with statistical significance defined as $p < 0.05$. All the analyses were conducted with a sample size of n = 3, and the experimental data was analyzed by the t-test, which was performed using Microsoft Excel and OriginPro 2025.

5A.3 Results and Discussion

5A.3.1 Physical properties

The physical properties of the formulated cookies and flakes using adzuki and mung bean legume flours are shown in Table 5A.2. Significant differences were found between the physical properties data of prepared formulations.

The weight of cookies developed from adzuki and mung bean legume flour was 9.70 ± 0.2 g and 11.80 ± 0.1 g, while for flakes it was found to be 0.13 ± 0.01 g and 0.19 ± 0.1 g, respectively. Both adzuki bean cookies and flakes had lower weight compared to mung bean cookies and flakes, which may be attributed to differences in their water holding capacity (WHC). According to the reported literature, cookie formulations with higher WHC can absorb and retain more water during dough preparation, resulting in heavier cookies [167]. The higher WHC observed in mung bean flour is likely due to its richer content of dietary fiber and protein, which enhances its ability to bind and retain water. So, it can be inferred that the cookies and flakes have different weights due to different WHC.

The thickness of adzuki and mung bean cookies was 6.25 ± 0.01 mm and 8.21 ± 0.2 mm, while for flakes it was 0.72 ± 0.1 mm and 0.91 ± 0.2 mm, respectively. From the data, it can be inferred that mung bean cookies had maximum thickness.

The diameter of adzuki and mung bean cookies was 49.46 ± 0.1 mm and 42.32 ± 0.1 mm, while for flakes it was 16.31 ± 0.02 mm and 14.04 ± 0.1 mm, respectively. The difference in diameter of formulated products could be attributed to their different protein content [48]. The previous report indicates that the diameter and protein content are inversely related. The protein gluten present in the flour undergoes glass transition

when heated during baking, leading to increased mobility. This mobility enables them to interact and create a network structure, which increases the dough's viscosity and ultimately halts the spread of cookie dough [168].

Spread ratio of adzuki bean cookies was found to be more than mung bean cookies (as shown in Table 5A.2). It is restricted by dough viscosity as dough with lower viscosity causes cookies to spread faster. According to Okpala et al., cookies having higher spread ratios are considered the most desirable than those with lower values [169].

In cookies, bulk density is an important quality criterion as consumers prefer less dense cookies. Table 5A.2 shows that the bulk density of adzuki bean cookies was lower, which suggests its higher potential for expansion compared to mung bean cookies. A lesser bulk density also implies a lesser packaging space requirement [159].

Table 5A.2: Physical properties of formulated breakfast flakes and cookies

Formulation	**Weight (g)**	**Thickness (mm)**	**Diameter (mm)**	**Spread ratio**	**Bulk density (g/cm^3)**
Adzuki bean cookies	9.70 ± 0.2	6.25 ± 0.01	49.46 ± 0.1	7.91 ± 0.01	0.11 ± 0.01
Mung bean cookies	11.80 ±0.1	8.21 ± 0.2	42.32 ± 0.1	5.15 ± 0.12	0.14 ± 0.01
Adzuki bean flakes	0.13 ± 0.01	0.72 ± 0.1	16.31 ± 0.02	22.65 ±0.15	0.12 ± 0.02
Mung bean flakes	0.19 ± 0.1	0.91 ± 0.2	14.04 ± 0.1	15.42 ± 0.03	0.20 ± 0.01

All the data are presented by the mean ± standard deviation ($p < 0.05$).

5A.3.2 Functional properties

Table 5A.3 shows the functional properties of cookies and flakes. WAI gives insight into how a product interacts with water, which directly affects texture and processing behavior. It demonstrates that starch polymers can absorb water and expand in excess water. The different values ($p < 0.05$) of WAI suggest the different structural

properties and hydration capacity of the products. The lower value for adzuki bean cookies and flakes compared to mung bean cookies and flakes suggests that the former is less capable of absorbing water. Lower values of WAI indicate a crisper texture and reduced moisture uptake during storage, underscoring their potential to improve storage stability by limiting moisture absorption. In comparison with recently reported data on cassava and corn flakes, adzuki and mung bean flakes exhibited lower water absorption. This suggests that they take up less moisture, making both formulated products more suitable for storage stability due to their reduced tendency to absorb water [159].

WSI evaluates starch degradation or conversion during extrusion, emphasizing the quantity of soluble polysaccharides released from the starch. This contributes significantly to the assessment of digestibility and product stability. The results indicate that adzuki bean cookies and flakes exhibit higher WSI values compared to their mung bean counterparts, suggesting that adzuki bean products may dissolve more effectively, thereby potentially improving their digestibility. The WSI of both the formulated products was higher than that of corn flakes, indicating that both the formulated products could dissolve more easily and are easily digestible [159].

Table 5A.3: Functional properties of formulated breakfast flakes and cookies

Sample	**Parameter**	
	Water absorption index (%)	**Water solubility index (%)**
Adzuki bean flakes	1.22 ± 0.2	34.01 ± 0.1
Mung bean flakes	2.03 ± 0.1	25.89 ± 0.3
Adzuki bean cookies	4.57 ± 0.1	18.21 ± 0.05
Mung bean cookies	5.92 ± 0.4	12.31 ± 0.1

All the data is presented by the mean ± standard deviation, ($p < 0.05$).

5A.3.3 Chemical properties

The total phenolic content in adzuki and mung bean cookies was 23.59 ± 0.11 (%) and 20.09 ± 0.30 (%), while for flakes it was 10.10 ± 0.32 (%) and 5.23± 0.41 (%) respectively. Adzuki bean products showed higher phenolic content. The results may suggest that the antioxidant potential of both products is enhanced due to the presence of naturally occurring phenolics, which is in accordance with our previous study [48]. The incorporation of legume flour instead of wheat flour improves the nutritional quality of the functional food.

The total flavonoid content in adzuki and mung bean cookies was 2.59 ± 0.35 (%) and 1.09 ± 0.52 (%), while for flakes it was 2.03 ± 0.21 (%) and 1.22 ± 0.31 (%) respectively. From the data, it can be interpreted that both formulated adzuki bean products (cookies and flakes) are rich in phenolic and flavonoid content, making them valuable nutraceutical-rich ingredients for enhancing the nutritional value of everyday foods.

5A.3.4 Nutritional composition of cookies

Table 5A.4 shows the nutritional composition of the formulated cookies of adzuki and mung beans. Legumes flour-based cookies (as shown in Figure 5A.3) were found to be more nutritious than regular whole-wheat cookies. The data showed that mung bean cookies generally exhibited higher proximate values (protein, fat, sugar, and energy content). In contrast, carbohydrates, vitamins, and folic acid content were higher in adzuki bean cookies. The nutritional data revealed that both cookies are a good source of minerals like potassium, calcium, iron, and sodium. The prepared cookies demonstrated a high folic acid content, positioning them as a nutritionally enriched functional food product. Folic acid (vitamin-B9) is an essential nutrient that offers numerous health benefits, such as preventing birth defects (neural tube defects),

neurological health, reducing the risk of cancer, and supporting the production of red blood cells [170]. Both cookies contain a good amount of folic acid, which supports the recommended dietary allowance by FSSAI and ICMR [171], [172].

Our previous study also showed that both these legumes are great sources of bioactive compounds and exhibited excellent anti-obesity activity, which is why we used their flour to supplement wheat flour [48]. Comparing our results with the recent studies on wheat-based cookies, we found that the prepared cookies had higher values of energy, carbohydrate, and protein [173],[174]. Compared with other legume-based cookies. such as broad beans, chickpeas, and corn, it was found that both adzuki and mung bean flour cookies had higher carbohydrate and total fat content and nearly similar amounts of total fat and dietary fiber. The protein content in prepared cookies was higher than in corn-based cookies [175]. The nutritional profiling data suggests that these legume-based cookies are good alternatives to wheat-based cookies and can be incorporated into our daily diet.

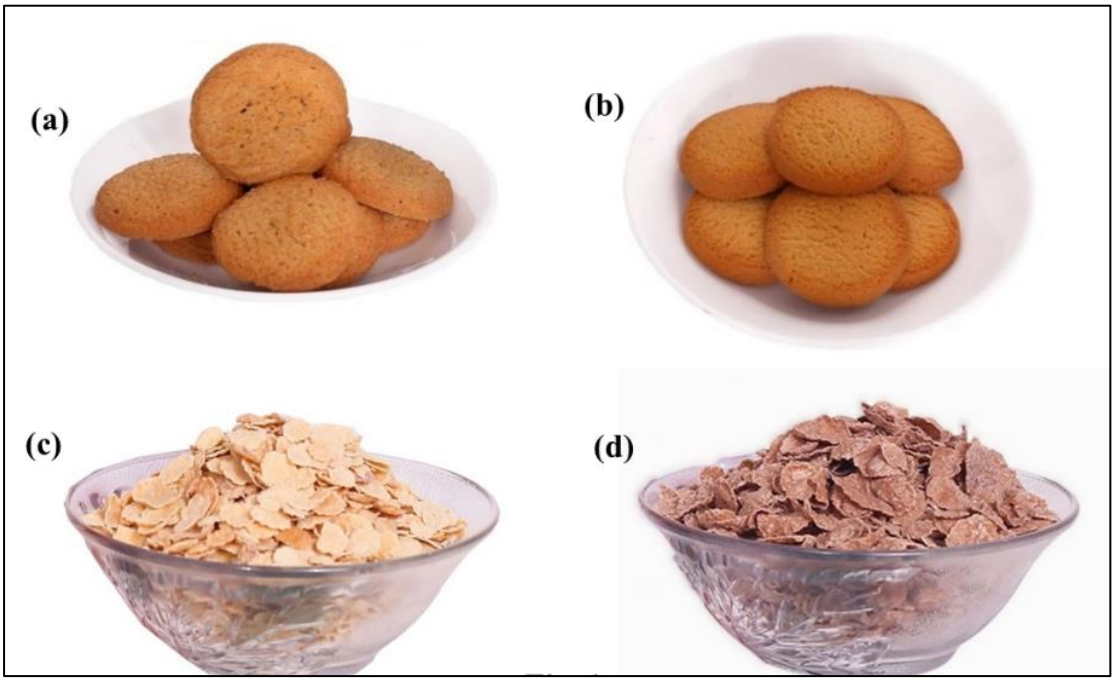

Figure 5A.2: Appearance of formulated product (a) mung bean cookies (b) adzuki bean cookies (c) mung bean breakfast flakes (d) adzuki bean breakfast flakes

Table 5A.4: Nutritional composition (on a dry basis) of adzuki bean and mung bean cookies

S.No.	Parameters	Test Method	Mung bean cookies	Adzuki bean cookies
1	Energy (Kcal/100g)	IS: 14433: 2007 (2018)	479.17 ± 0.11	473.99 ± 0.14
2	Protein (g/100g)	AOAC:991.20:2019	10.30 ± 0.01	10.04 ± 0.15
3	Total Carbohydrate (g/100g)	IS: 1656: 2007 (2018)	68.97 ± 0.12	70.05 ± 0.23
4	Total Fat (g/100g)	IS: 4684: 1975 (2020)	18.01 ± 0.13	17.07 ± 0.01
5	Sugar (g/100g)	IS: 2650: 1975 (2020)	30.18 ± 0.15	27.38 ± 0.21
6	Added Sugar (g/100g)	IS: 3884: 1993 (2015)	29.07 ± 0.32	26.26 ± 0.02
7	Dietary Fiber (g/100g)	IS: 11062: 2019	2.20 ± 0.01	2.20 ± 0.34
8	Saturated Fatty Acids (g/100g)	AOAC: 996.06: 2019	1.56 ± 0.13	0.90 ± 0.12
9	Trans Fatty Acids (g/100g)	AOAC: 996.06: 2019	BLQ	BLQ
10	Cholesterol (mg/kg)	AOAC: 994.10: 2019	BLQ	BLQ
11	Vitamin D (µg/100g)	AFLPL/SOP/CH/INH/206	BLQ	BLQ
12	Thiamine (Vitamin B1) (mg/100g)	EN:14122: 2014	0.59 ± 0.15	0.61 ± 0.20
13	Riboflavin (Vitamin B2) (mg/100g)	EN: 14152: 2014	0.63 ± 0.11	0.64 ± 0.12
14	Niacin (Vitamin B3) (mg/100g)	AFLPL/ SOP/CH/INH/01	7.66 ± 0.05	7.45 ± 0.12
15	Pyridoxine (Vitamin B6) (mg/100g)	AOAC: 2004.07: 2019	0.44 ± 0.23	0.42 ± 0.21
16	Folic Acid (Folate) (µg/100g)	AFLPL/SOP/CH/INH/376	65.32 ± 0.12	74.22 ± 0.21
17	Vitamin B12 (µg/100g)	AFLPL/SOP/CH/INH/03	0.23 ± 0.12	0.24 ± 0.13
18	Potassium (µg/100g)	IS: 12760: 2012 (2018)	136.85 ± 0.11	101.59 ± 0.22
19	Calcium (µg/100g)	AFLPL/SOP/CH/INH/163	74.62 ± 0.13	107.30 ± 0.03
20	Iron (µg/100g)	AFLPL/SOP/CH/INH/162	7.09 ± 0.01	3.62 ± 0.22
21	Sodium (µg/100g)	AFLPL/SOP/CH/INH/204	47.57 ± 0.04	47.03 ± 0.13

All the data is presented by the mean ± standard deviation, ($p < 0.05$).

5A.3.5 Nutritional composition of breakfast flakes

The nutritional composition of adzuki and mung bean breakfast flakes is shown in Table 5A.5. The mung bean flakes (as shown in Figure 5A.2) generally exhibited higher energy, protein, fat, sugar, and mineral values. In contrast, the carbohydrate, dietary fiber, and vitamin contents of the adzuki bean flakes were higher. Folic acid content was higher in the case of mung bean flakes. This study found that both these legume-based flakes are nutritionally richer than cornflakes [176]. However, the fat content was lower

in the case of adzuki and mung bean flakes as compared to cornflakes [177]. Both flakes are good sources of potassium, calcium, and sodium.

Table 5A.5: Nutritional composition (on a dry basis) of adzuki bean and mung bean flakes

S.No.	Parameters	Test Method	Adzuki bean flakes	Mung bean flakes
1	Energy (Kcal/100g)	AFLPL/SOP/CH/INH/253	381.62 ± 0.01	397.14 ± 0.02
2	Protein (g/100g)	IS: 7219: 1973 (2020)	14.44 ± 0.13	21.69 ± 0.11
3	Total Carbohydrate (g/100g)	IS: 1656: 2007 (2018)	77.77 ± 0.03	71.34 ± 0.04
4	Total Fat (g/100g)	IS: 4684: 1975 (2020)	1.42 ± 0.21	2.78 ± 0.04
5	Sugar (g/100g)	IS: 2650: 1975 (2020)	2.55 ± 0.02	3.07 ± 0.01
6	Added Sugar (g/100g)	IS: 2650: 1975 (2020)	BLQ	BLQ
7	Dietary Fiber (g/100g)	IS: 11062: 2019	15.13 ± 0.01	7.54 ± 0.02
8	Saturated Fatty Acids (g/100g)	AOAC: 996.06: 2019	BLQ	BLQ
9	Trans Fatty Acids (g/100g)	AOAC: 996.06: 2019	BLQ	BLQ
10	Cholesterol (mg/kg)	AOAC:994.10:2019	BLQ	BLQ
11	Vitamin D (mg/100g)	AFLPL/SOP/CH/INH/206	BLQ	BLQ
12	Thiamine (Vitamin B1) (mg/100g)	EN: 14122:2014	1.02 ± 0.31	0.98 ± 0.12
13	Riboflavin (Vitamin B2) (mg/100g)	EN: 14152: 2014	1.14 ± 0.01	1.21 ± 0.02
14	Niacin (Vitamin B3) (mg/100g)	AFLPL/ SOP/CH/INH/01	11.25 ± 0.21	10.69 ± 0.11
15	Pyridoxine (Vitamin B6) (mg/100g)	AOAC: 2004.07: 2019	1.67 ± 0.02	2.01 ± 0.11
16	Folic Acid (Folate) (µg/100g)	AFLPL/SOP/CH/INH/376	60.23 ± 0.01	66.39 ± 0.02
17	Vitamin B12 (µg/100g)	AFLPL/SOP/CH/INH/03	0.32 ± 0.05	0.36 ± 0.12
18	Potassium (mg/100g)	IS: 12760: 2012 (2018)	53.25 ± 0.02	110.32 ± 0.01
19	Calcium (mg/100g)	AFLPL/SOP/CH/INH/163	103.68 ± 0.11	144.01 ± 0.02
20	Iron (mg/100g)	AFLPL/SOP/CH/INH/162	7.34 ± 0.11	6.72 ± 0.02
21	Sodium (mg/100g)	AFLPL/SOP/CH/INH/204	46.27 ± 0.12	55.15 ± 0.07

All the data is presented by the mean ± standard deviation, ($p < 0.05$).

5A.3.6 Sensory evaluation

The hedonic scale is a neutral-focused balanced bipolar scale with phrase-labeled categories representing various degrees of likability. Its ability to capture likability data has been extensively tested in consumer research. The results of different sensory attributes of color, flavor, odor, texture, aftertaste, hardness, and overall acceptability for both cookies are shown in Figure 5A.3. ABF cookies received the lowest values for appearance, color, and texture but the highest value for flavor, odor, and aftertaste. The slight dark color of ABF cookies may be responsible for their lowest values of appearance and color. The panelists observed that the mung bean cookies were slightly nutty and had a lousy aftertaste, which was similar to the reported study on cowpea legume-based cookies [178] . In addition to that, panelists liked the beany flavor in the case of adzuki bean cookies. The difference in texture is maybe due to the differences in gluten levels [179]. The overall acceptability score was highest for ABF cookies.

Table 5A.6 presents the acceptability index (%) of the prepared products, calculated by dividing the average score of each evaluated parameter by the highest possible score, then multiplying the result by 100 to express it as a percentage. The index ranges from a minimum acceptable value of 20% to a maximum of 100%. The product had to achieve an acceptability index greater than 60% to be considered acceptable. The data shows that all the products have an AI% greater than 60%, indicating good acceptance of the products. Overall, the AI% of adzuki bean products was higher than that of mung bean products.

Table 5A.6: Acceptability index (%) of the formulated products

Product name	**Appearance**	**Color**	**Flavor**	**Odor**	**Texture**	**After taste**	**Hardness**
Adzuki bean cookies	92.83 ± 2.01	86.82 ± 2.301	92.92 ± 1.70	94.51 ± 1.99	90.19 ± 0.59	79.14 ± 2.11	85.99 ± 2.41
Mung bean cookies	91.94 ± 1.99	84.90 ± 2.01	79.92 ± 1.98	91.55± 0.97	90.87 ± 2.10	75.92 ± 1.52	85.35 ± 1.44
Adzuki bean flakes	92.83 ± 1.20	86.82 ± 1.23	92.92 ± 4.98	94.51 ± 3.98	90.19 ± 2.32	79.14 ± 1.70	85.99 ± 1.02
Mung bean flakes	95.63 ± 2.65	91.46 ± 2.33	84.80 ± 1.97	93.81 ± 1.54	90.39 ± 1.89	77.97 ± 2.89	85.99 ± 1.88

All the data is presented by the mean ± standard deviation, ($p < 0.05$).

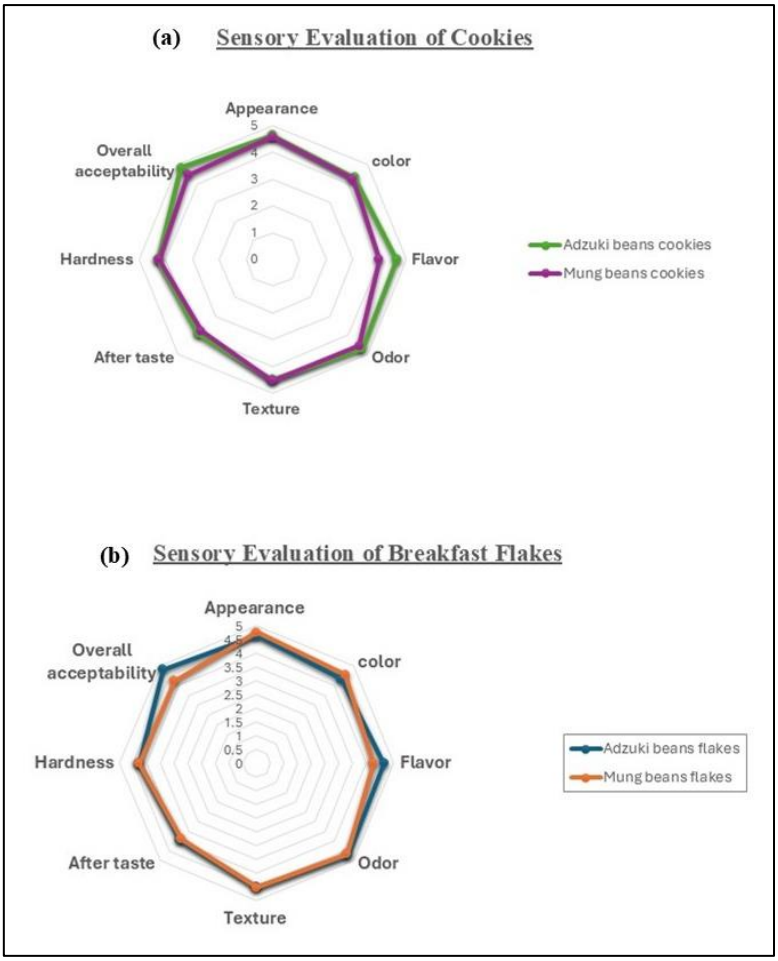

Figure 5A.3: Sensory evaluation of innovative legume-based formulated products (a) cookies (b) breakfast flakes

5A.4 Conclusion

In summary, this study highlights the immense potential of two underutilized legumes of India - adzuki and mung beans, as a potent lipase inhibiting agent and as a key ingredient in developing novel nutritionally enriched legume-based breakfast cereal and nutritional cookies which are not yet available in the market for consumers to combat obesity and metabolic disorders. Both extracts were highly effective in inhibiting pancreatic lipase as compared to the standard drug orlistat, highlighting great potential for managing obesity. This study also demonstrated the possible use of legume flour to partially substitute wheat flour in producing cookies and flakes with acceptable physical

characteristics. Incorporating these legumes enhances the formulations' protein, fiber, and micronutrient content and introduces bioactive compounds that offer significant health benefits like weight loss and cholesterol-lowering benefits. The sensory evaluation also suggests panelists' acceptance of the formulated products. Further studies could explore the potential of adzuki and mung beans in developing a diverse range of innovative breakfast cereals, cookies, and other functional food products in the future.

Section-5B

Hydrogels based on mucilage of underutilized cereals: Synthesis and characterization

5B.1 Introduction

In recent years, there has been a lot of interest in plant-derived mucilage owing to their non-toxicity, eco-friendliness, cost-effectiveness, and biodegradable nature. To meet the growing need, new sources are being investigated on a regular basis. Chemically, mucilage is a natural polysaccharide composed of highly branched structures of carbohydrates such as L-arabinose, D-xylose, and D-galactose monomer units [180]. They can be obtained from different parts of the plant like seeds, leaves, roots, and stems. The process of producing mucilage from the plant part is known as Myxospermy. Mucilage is partially soluble when it comes in contact with water [181]. These polysaccharides are composed of ten or more monosaccharide units. Mucilages obtained from different sources exhibit varied functional properties due to differences in the monosaccharide units, type of glycosidic bond, and conformation of the chains [182]. Due to their hydrophilic nature, these polysaccharides swell in water and form a gel-like solution, which has excellent and diverse uses as a binding agent, stabilizing agent, emulsifying agent, thickening agent, etc.

Mucilage isolated from different plant materials has been extensively used in the pharmaceutical and food industries. Recently, the isolation and characterization of new sources of plant-derived mucilage (chia mucilage, okra seed mucilage, marshmallow mucilage, and Chinese yam mucilage) and their applications have been investigated. Herein, we report four newer sources of mucilage, these are underutilized cereals of India i.e., adzuki bean, amaranth, proso millet, and little millet.

Adzuki bean (*Vigna angularis*) is a legume belonging to the Fabaceae (Leguminosae) family. It is widely cultivated in countries like China, Japan, and Korea. It is widely utilized as an ingredient in desserts [36]. Amaranth (*Amaranthus*) belongs to the family Amaranthaceae. It is commonly known as Ramdana or Rajgira in India and is a nutritious pseudo-cereal. It is widely cultivated in different countries as a cereal, vegetable, weed, or crop. Proso millet (*Panicum miliaceum*) belongs to the Poaceae family. It is cultivated in India, China, Nepal, Africa, Turkey, Romania, and Russia. It is gluten-free and rich in proteins, vitamins, and minerals [183]. Little millet (*Panicum sumatrense*) belongs to the family Poaceae. It is widely cultivated across India, China, and Africa. It is considered "cool food" in light of its cooling impact on the human body when consumed in summer [184].

According to Deore et al., mucilage possesses swelling property because of the presence of distinct functional and polar groups that exhibit their hydro- gelling potential [185]. This swelling ability of mucilage can be further enhanced by synthesizing hydrogels based on mucilage. Hydrogels are three-dimensional polymeric networks that contain hydrophilic or polar functional groups to hold water in them. They are known for their high absorption capacity. Hydrogels are typically crosslinked with physical or chemical crosslinking, preventing them from dissolving in water [186]. They are suitable for various biomedical, agricultural, food, and cosmetic applications due to their biocompatibility and harmless nature. As natural polymers show several advantages over synthetic polymers, they have gained the interest of researchers as a potential source for hydrogel formation [187].

The aim of the current study is for the isolation of mucilage from four underutilized cereals of India, namely adzuki bean (*Vigna angularis*), amaranth (*Amaranthus*), proso millet (*Panicum miliaceum*), and little millet (*Panicum sumatrense*) and to develop mucilage-co-Acrylic acid (M-co-AAc) graft copolymeric hydrogels by free radical polymerization. The study also focused on their physicochemical, morphological, and structural characterization by various instrumental analyses. This is the first report on the isolation of mucilage from these underutilized cereals and their hydrogel synthesis to the best of our knowledge.

5B.2 Materials and methods

Adzuki beans (A_b) were purchased from Himjoli Products, Delhi, India. Proso millet (P_r), and Little millet (L_m) were gifted from ICAR, Hyderabad, India. Amaranth (A_m) was purchased online from Amazon, India. The grain samples were authenticated by CSIR- NIScPR, RHMD, India. All samples were prepared in Milli-Q grade water.

5B.2.1 Extraction of mucilage

The extraction was done following the method given by Nuria et al. with slight modifications [188]. Each cereal sample was sieved to remove any foreign particles and then sun-dried. 50 g of each dried and powdered sample was mixed with deionized water in a separate Erlenmeyer flask. The pH was maintained at 8 with 0.1 M sodium hydroxide (NaOH) solution. The temperature of the solution was kept at 70 ± 2 ºC under constant stirring until a viscous solution was obtained. The solution was cooled at an ambient temperature and then passed through a muslin cloth to separate the mucilage from the cereals. Then the solution was centrifuged at 10,000 rpm, 25ºC for 20 min. The

supernatant obtained after centrifugation was collected and used for further purification. A pictorial representation of the extraction method of mucilage is shown in Figure 5B.1.

5B.2.2 Purification of mucilage

Isolated mucilage was purified, according to the method described by Morales-Tovar et al. with slight changes [189]. The supernatant obtained in the previous step was mixed with acetone in a 1:3 (sample: acetone) ratio and left undisturbed for 3 h. The precipitated mucilage was then filtered and dried in an oven below 50 ºC overnight. The dried mucilage was powdered using mortar and a pestle and stored in a desiccator for further use. The percentage yield of pure mucilage isolated from 50 g of powdered cereals was recorded and calculated using the below-mentioned formula [190].

$$\% \text{ Yield} = \frac{\text{Weight of dried mucilage obtained}}{\text{Weight of powdered cereal material used}} \times 100$$

5B.2.3 Physicochemical characterization

5B.2.3.1 pH and solubility

To measure the pH of the isolated mucilage, 1% (w/v) aqueous solution was prepared and stirred for 30 min. The pH was measured using a calibrated digital pH meter. The solubility of the extracted mucilage was studied using different solvents like deionized water, acetone, ethanol, methanol, and chloroform [191].

5B.2.3.2 Swelling index

Swelling index (SI) was obtained by the method reported by Archana et al. with slight modifications [124]. 1 g dried mucilage was taken in a stopper-graduated cylinder. 2 mL ethanol (95%) was added for better dispersion, and then 10 mL deionized water was added. After a gentle shaking, it was kept at room temperature till a constant weight was

observed. The volume raised was observed and recorded. SI was calculated by the following formula.

$$\%SI = \frac{V_f - V_i}{V_i} \times 100$$

Where, V_f is the final volume after hydration (mL); V_i is the initial volume before hydration (mL).

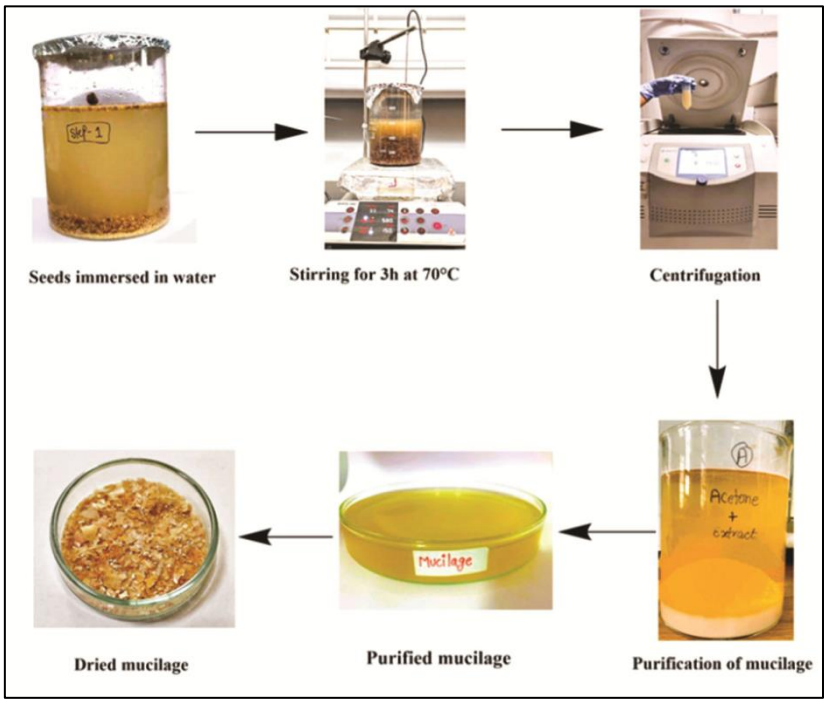

Figure 5B.1: Pictorial representation of the extraction of mucilage

5B.2.3.3 Organoleptic characterization

For organoleptic characterization, the mucilage was analyzed for various parameters like odour, colour, appearance, fracture, and taste [124].

5B.2.4 Phytochemical investigation

To confirm the chemical nature of the isolated mucilage, various identification tests like Molisch's test (carbohydrates), Ninhydrin test (proteins and amino acids), Ruthenium

Red test (mucilage), Iodine test (starch), Ferric chloride test (tannins), and Wagner's test (alkaloids) were performed [191].

5B.2.5 Exploration of cereal-based mucilage as a hydrogel

The isolated polysaccharide exhibited the ability to form a viscous solution with increasing concentration. The mucilage obtained from all four cereals demonstrated hydrophilic behavior and appropriate swelling capacity. Hence, it was planned to prepare hydrogels (A_bH, A_mH, P_rH & L_mH) based on isolated mucilages.

5B.2.5.1 Synthesis of M-co-AAc hydrogels

The hydrogels were synthesized following the method described by Hussain et al. with slight modifications [186]. The hydrogels were synthesized using a free radical copolymerization mechanism by taking N,N'-methylenebisacrylamide and potassium persulphate as a crosslinker and initiator, respectively. The first step was to dissolve the desired amount of dried mucilages in 10 mL of hot deionized water. To this, AAc, NaOH, MBA, and KPS were mixed in desired ratios and continuously stirred for 2 h at room temperature using a magnetic stirrer. Each mixture was sonicated for 5 min to remove any air bubbles. The solutions were poured into the test tube and placed in a water bath for an hour. Prepared hydrogels were cut into small discs. The hydrogels were then air-dried, followed by drying in an oven at 60 °C until a constant weight was observed. Formulations of all four M-co-AAc hydrogels are given in Table 5B.1.

5B.2.5.2 Swelling index

Swelling studies were conducted using deionized water at an ambient temperature. The dried hydrogels were weighed with the help of a weighing balance. The dried hydrogels were weighed and then immersed in deionized water till a constant weight was

observed [192]. At regular intervals, the swollen hydrogels were removed, and any excess surface water was gently blotted using tissue paper. The SI for each sample was then determined using the following formula.

$$\%SI = \frac{W_{SH} - W_{DH}}{W_{SH}} \times 100$$

where, W_{SH} is the weight of the swollen hydrogel and W_{DH} is the initial weight of the dried hydrogel.

5B.2.6 Characterizations

All the mucilages and their respective hydrogels were characterized by FTIR (Perkin Elmer, spectrum version two) in ATR mode, thermogravimetric analysis (Perkin Elmer, TGA 4000) in N_2 atmosphere with a heating rate of 10 °C/min from 25 to 800°C, X-ray diffraction analysis (Bruker D8 Avance) with an angle ranging from 10° to 80° with a scan speed of 0.5 sec/step, scanning electron microscopy (Carl Zeiss, EVO 18) with 30 kV accelerating voltage and 2.519 A beam current, ^{1}H-NMR (Bruker Avance-III) and ^{13}C-NMR (JEOL Resonance ECX-400) at an operating frequency of 500 MHz.

Table 5B.1: Formulations of all four M-co-AAc hydrogels

S.No.	Formulation	Mucilage (g)	Monomer (AAc) (mL)	NaOH (g)	Initiator (KPS/Distilled water) (g/mL)	Crosslinker (MBA) (mg)
1.	A_bH	0.05	7.1	4.2	0.09/10	60
2.	A_mH	0.05	7.1	4.2	0.09/10	60
3.	P_rH	0.05	7.1	4.2	0.09/10	60
4.	L_mH	0.05	7.1	4.2	0.09/10	60

5B.3 Results and Discussion

5B.3.1 Isolation of mucilage

Mucilage from all four underutilized cereals was isolated effectively. The extraction method and species variation can affect the percentage yield of the polysaccharide obtained. The percentage yield of all four mucilages is shown in Table 5B.2. Among all, A_m showed the highest yield of 27.61%. A_b, A_m, P_r, and L_m were observed as light brown, white, creamy white, and light green mucilage powder, respectively, as shown in Figure 5B.2(A).

Table 5B.2: The percentage yield of mucilage extracted from four underutilized cereals

Mucilage samples	**% Yield**
Adzuki bean	12.86 %
Little millet	7.24 %
Proso millet	20.01 %
Amaranth	27.61 %

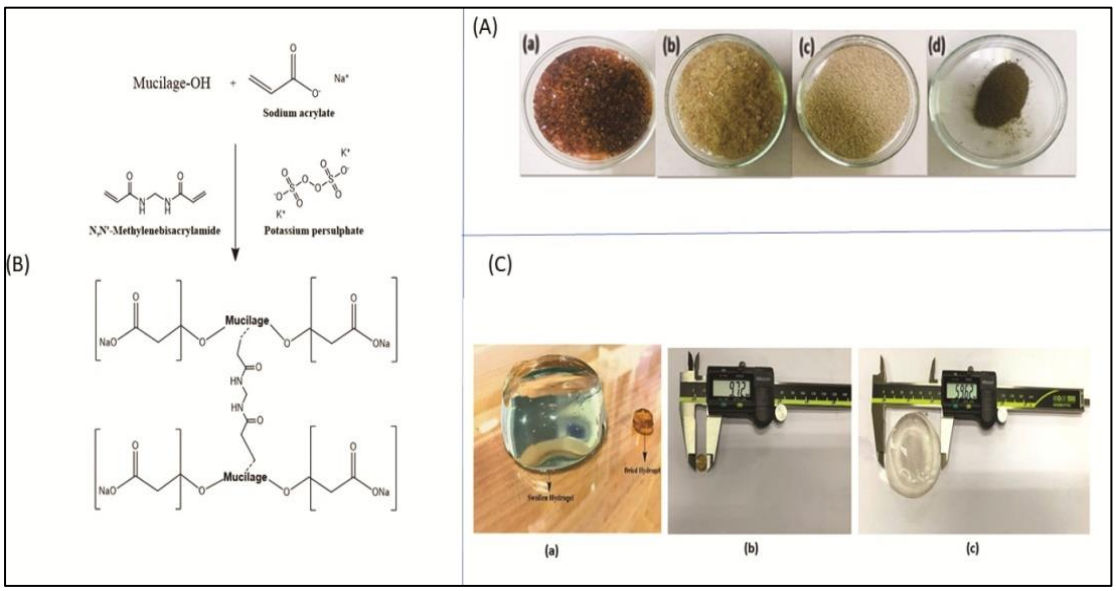

Figure 5B.2: (A)-Dried and powdered mucilage of (a) A_b (b) A_m (c) P_r (d) L_m], (B) schematic diagram for the formation of M-co-AAc graft copolymeric hydrogels and (C) images of hydrogels (a) dried and swollen hydrogel (b) diameter of dried hydrogel (c) diameter of swollen hydrogel

5B.3.2 Physicochemical characterization

The solubilities of all four mucilages were studied using different solvents like water, acetone, ethanol, methanol, and chloroform. They were partially soluble in cold water, soluble in hot water, and insoluble in acetone, methanol, and chloroform. From the data mentioned in Table 5B.3, it is observed that mucilage is insoluble in all organic solvents. However, due to the presence of hydrophilic moieties, it forms a viscous solution when combined with water [193].

The pH of the mucilage is one of the important factors in determining its suitability as an excipient in various pharmaceutical industries. The results shown in Table 5B.4 indicates that all four mucilaginous polysaccharides are nearly neutral. This suggests that it will be less irritating to the gastrointestinal tract. Hence, they can be used as an excipient in various pharmaceutical preparations [194].

Table 5B.3: Solubility of isolated mucilage in different solvents

Solvents	**Solubility**			
	A_b	**A_m**	**P_r**	**L_m**
Cold water (2°C)	Partially Soluble	Partially Soluble	Partially Soluble	Partially Soluble
Hot water (90°C)	Soluble	Soluble	Soluble	Soluble
Methanol	Insoluble	Insoluble	Insoluble	Insoluble
Acetone	Insoluble	Insoluble	Insoluble	Insoluble
Benzene	Insoluble	Insoluble	Insoluble	Insoluble
Chloroform	Insoluble	Insoluble	Insoluble	Insoluble
Dimethylsulphoxide	Insoluble	Insoluble	Insoluble	Insoluble

The swelling index of the mucilage is shown in Table 5B.4. The data indicates that all four mucilage have a hydrophilic character and hence swell when they absorb water due

to the hydroxyl groups present in them. Among all, L_m has the highest value of swelling index, which may confirm that it has more hydroxyl groups than others [195].

Table 5B.4: Swelling index and pH of the isolated mucilage

S. No.	Property	Mucilage			
		A_b	**A_m**	**P_r**	**L_m**
1.	pH	6.80	6.68	6.98	7.46
2.	% Swelling index	125	137.5	100	150

5B.3.3 Organoleptic characterization

The dried and powdered mucilage are shown in Figure 5B.2 (A) and the organoleptic characterization is tabulated in Table 5B.5. The quality of mucilage depends on the method of extraction. A_b, P_r, and L_m mucilage powder was rough, while A_m mucilage powder was shiny and flaky in nature. All four mucilage were colored and tasteless.

Table 5B.5: Organoleptic characterization of extracted mucilage

Property	**A_b**	**A_m**	**P_r**	**L_m**
Appearance	Amorphous powder	Lustrous crystalline flakes	Amorphous powder	Amorphous powder
Odor	Odorless	Odorless	Odorless	Odorless
Color	Light brown	White	Creamy white	Light green
Taste	Tasteless	Tasteless	Tasteless	Tasteless
Fracture	Rough	Smooth	Rough	Smooth

5B.3.4 Phytochemical characterization

The qualitative identification tests were done for the phytochemical investigation of the mucilage. The results obtained are shown in Table 5B.6. Phytochemical analysis

confirmed the presence of mucilage and carbohydrates by performing the Ruthenium Red test and the Molisch's test, respectively. The results also indicate the absence of starch, tannin, proteins, and alkaloids in all the mucilages. Therefore, it can be concluded that mucilages were pure and free from any impurities and other phytoconstituents of the seeds.

Table 5B.6: Phytochemical screening of the isolated mucilage

S. No.	Active constituent	Identification Test	Inference			
			A_b	A_m	P_r	L_m
1.	Carbohydrate	Molisch's test	+	+	+	+
2.	Protein	Ninhydrin test	-	-	-	-
3.	Tannin	Ferric chloride test	-	-	-	-
4.	Mucilage	Ruthenium Red test	+	+	+	+
5.	Starch	Iodine test	-	-	-	-
6.	Alkaloids	Wagner's test	-	-	-	-

5B.3.5 Instrumental analysis of mucilages

5B.3.5.1 FTIR-ATR spectroscopy

FTIR-ATR is used to study the molecular structure of the mucilages. This technique helps in identifying the functional groups attached to the polymeric structure. The mucilage isolated from the different underutilized cereals shows characteristic peaks in the range 4000-400 cm^{-1} that correspond to different stretching and bending vibrations. The spectra in Figure 5B.3(A) show typical bands and peaks corresponding to the polysaccharides. The broad absorption band at 3272 cm^{-1} corresponds to the presence of hydroxyl groups. The peak obtained at 2916 cm^{-1} corresponds to the C-H stretching vibration [196]. The band near 2109 cm^{-1} corresponds to C-C stretching bonds [197]. The peak at 1627 cm^{-1} is due to the

deformation of amide I / C=O asymmetric stretching mode [124]. The sharp peak at 1032 cm^{-1} and 1000 cm^{-1} is attributed to C-O-C and C-O-H vibrations of the glycosidic bond in the polysaccharide [191]. All mucilages showed characteristic absorption bands between 1200-800 cm^{-1} in the fingerprint region for carbohydrates. From the IR characteristic peaks, we can say that the isolated mucilages contain a carbohydrate moiety.

5B.3.5.2 Thermogravimetric analysis (TGA)

Thermal stability is a significant factor in determining whether a polysaccharide is suitable for use in the pharmaceutical industry. TGA gives information regarding the decomposition pattern and thermal stability of the polysaccharide. Figure 5B.3(B) represents the stability profile of all four isolated mucilage's which shows three weight loss events. The initial phase of weight loss was obtained below 200°C in all the mucilage samples. This may be associated with the desorption of moisture present in the mucilage. The second weight loss was obtained in the range of 200-500°C in each polysaccharide. According to Silveira et al., the breaking of polysaccharide branches is caused by the decomposition of mucilage. The last phase occurs between 500-800 °C. This is because of the degradation of the polysaccharide backbone [198]. From the decomposition pattern of all four mucilages, L_m is thermally more stable as it has 24% of residual weight at 800°C than the others. The observed thermal stability order of mucilages is $L_m > A_b > A_m > P_r$.

5B.3.5.3 Powder X-ray diffraction analysis (XRD)

XRD is done to analyze the spatial arrangement of the atoms and molecules within the sample. It enables us to determine whether the sample is amorphous or crystalline in

nature. The powder XRD analysis data of different mucilages is shown in Figure 5B. 3(C). In the XRD diffractogram, there was no sharp peak obtained in all the mucilage samples. This indicated the amorphous nature of all the mucilage. According to Ujwaldip et al., the absence of any intense peak indicates that mucilage is completely amorphous [191]. The broad peak obtained in the same region in all four mucilages indicated the similarity in the structure. Ma et al. obtained the X-ray diffractogram for Chinese yam mucilage and observed the amorphous nature similar to this study [199]. The broad peaks spanning 2θ values ranging from 15° to 25° indicate the amorphous nature of all four mucilages.

5B.3.5.4 Scanning electron microscopy (SEM)

To study the surface morphology of the polysaccharide obtained and to confirm the microstructure, SEM analysis was done and is represented in Figure 5B.3(D). The data revealed that the mucilage material is amorphous in nature. In the microphotograms, there is a high degree of irregularity with the dimensions and shapes of all the samples, and the surface looks rough. According to Silva et al., choosing the right method for the extraction and purification of the mucilage is very important, as it can change the structure, shape, and topography of the mucilage obtained [200]. Singh et al. obtained the SEM data for Diospyros melonoxylon Roxb. where they obtained the same micrographs indicating the amorphous nature of the mucilage [201]. They reported that rough surfaces and irregular particle sizes can affect the hydration behavior of the mucilage.

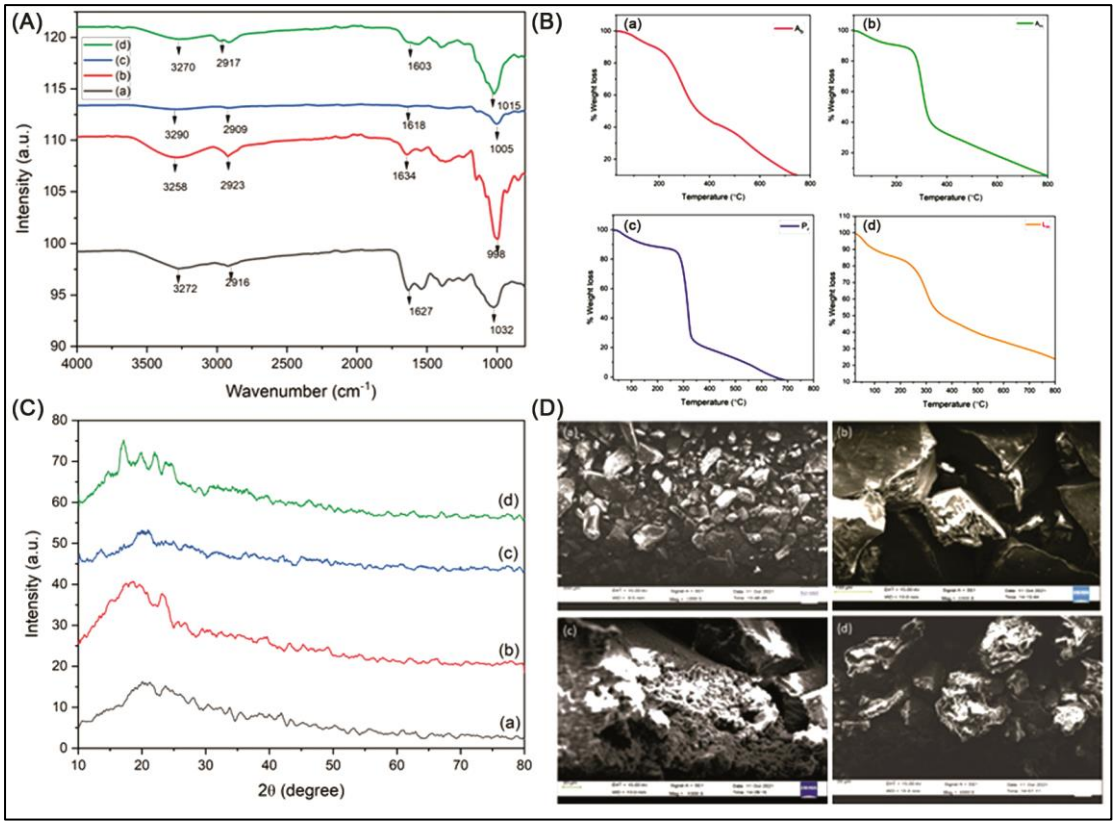

Figure 5B.3: (A) FTIR-ATR spectra, (B) thermograms, (C) XRD plots and (D) SEM micrographs (at 1000 magnification) of the mucilage (a) A_b (b) A_m (c) P_r (d) L_m

5B.3.5.5 1D Nuclear magnetic resonance studies (NMR)

NMR spectroscopy is one of the essential techniques used for the structural determination of the polysaccharide. The liquid state ^{1}H and ^{13}C-NMR of all the mucilage isolated from the different underutilized cereals was recorded and is shown in Figure 5B. 4. From the ^{1}H-NMR, it was confirmed that the protons in the up-field region that may be present in the mucilage might be attributed to the aliphatic protons. The peak around δ1.32 ppm is due to the methyl groups. The spectra of all the mucilages show the crowded region near δ3.1-5.3 ppm, indicating the polysaccharide region and the presence of various similar sugar units [202]. According to Kaushik et al., the peaks in the range of δ3-4.3 ppm are due to non- anomeric protons and δ4.5-5.5 ppm are due to anomeric protons [203]. The ^{1}H signals observed near δ3.35-3.58 ppm were due to the $-CH_2$ and -OH group of

arabinose. The signal between δ3.43-3.69 ppm was due to the -CH and -OH group of mannose. The presence of anomeric protons has been assigned to α-sugar residue (δ5-6 ppm) and β-sugar residues (δ4-5 ppm) as reported earlier by Singh et al. [201]. So, the signals in the range of δ5.0- 5.3 ppm can be assigned due to α-anomeric protons and from δ4.30- 4.96 ppm were assigned due to β-anomeric protons. The overall study of ^{1}H NMR of different mucilages revealed that mucilage may contain arabinose, mannose, and various sugar units. In the ^{13}C-NMR of mucilages, the data revealed that the peak near δ72.37 ppm indicates the presence of the -CH group of mannose, and δ70- 71.66 ppm indicates the presence of the -CH of arabinose.

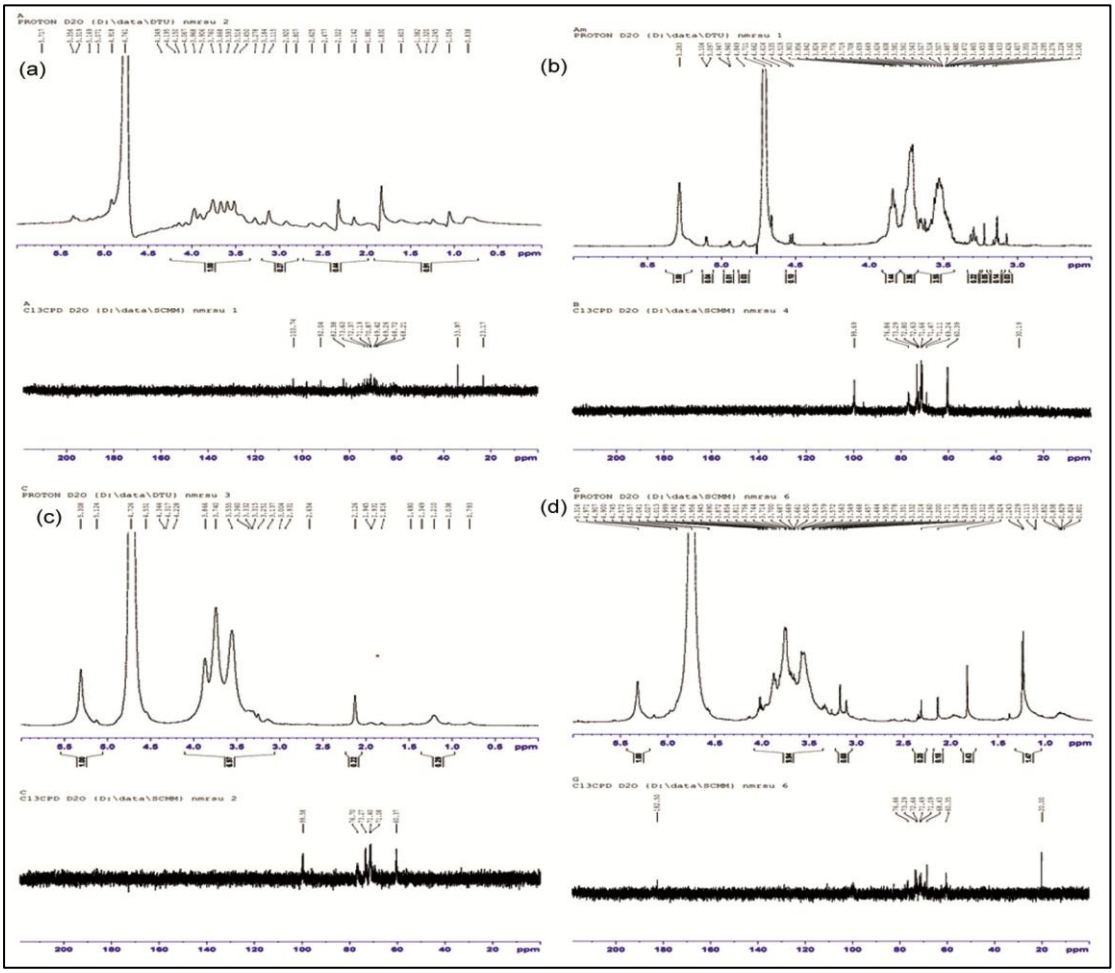

Figure 5B.4: ^{1}H and ^{13}C-NMR spectra of (a) A_b (b) A_m (c) P_r (d) L_m

5B.3.6 Exploration of mucilage as hydrogel

5B.3.6.1 Synthesis of M-co-AAc hydrogels

The hydrogels were successfully synthesized by free radical polymerization and are mentioned in Table 5B.1. The proposed mechanism for the formation of M-co-AAc graft copolymeric hydrogels is shown in Figure 5B.2(B). According to Sennakesavan et al., first, KPS turns into persulfate radical by thermal decomposition [204]. The radicals formed attack the hydrophilic groups present in the mucilage and make it a reactive species. Sodium acrylate makes a covalent bond with the hydroxyl groups of the mucilage and hence makes poly(sodium acrylate). Then, termination of the polymerization is carried out using MBA by the formation of cross-linking junctions. This results in the formation of a 3D polymeric structure of the hydrogels.

5B.3.6.2 Swelling index

Swelling studies of the hydrogels in deionized water at 25°C are shown in Table 5B.7. It is well-known fact that hydrogels, when immersed in water, tend to absorb it, and the swelling ratio increases with time until equilibrium is attained. The interaction between the hydrophilic groups of the polysaccharide hydrogel and water molecules led to an increase in swelling. As the hydrophilicity of the hydrogel is increased, the swelling index increases. Thus, M-co-AAc hydrogels show a significantly higher swelling index than the mucilage itself. According to the swelling studies, L_mH was observed to have a maximum swelling index in deionized water; hence, it can be predicted that it is more hydrophilic in nature as compared to others [205]. The dried and swollen hydrogel is shown in Figure 5B.2(C).

Table 5B.7: Swelling index of the formulated hydrogels

S. No.	Hydrogel formulation	% Swelling index
1.	A_bH	26854
2.	A_mH	28134
3.	P_rH	14794
4.	L_mH	35199

5B.3.6.2 Instrumental analysis of hydrogels

5B.3.6.2.1 FTIR-ATR

FTIR-ATR analysis was done to confirm the AAc grafting on mucilage isolated from all four cereals. FTIR-ATR data of all the hydrogels is shown in Table 5B.8, and the spectra are shown in Figure 5B.5(A). The stretching band at 3305 cm^{-1} indicated the presence of the -OH group present in the polysaccharide. The observed wavenumber near 2904 cm^{-1} was due to aliphatic C-H stretching vibration. The absorption peak at 1722 cm^{-1} showed C=O stretching vibration, which is attributed to the absorption shown due to the linking of poly(sodium acrylate) to the mucilage depicting (C=O) functional group [206]. Asymmetric stretching vibration of carboxylate ion at 1579 cm^{-1}. The confirmation of hydrogel formation is also evaluated by absorption bands near 1340-900 $cm^{-1,}$ which show C-N stretching due to crosslinking of MBA and poly(sodium acrylate). The appearance of the wavenumber at 1041 cm^{-1} indicated the presence of C-O-C stretching vibration [207].

Table 5B.8: FTIR-ATR data of all four hydrogels

S. No.	Functional group	Vibration	A_bH (cm^{-1})	A_mH (cm^{-1})	P_rH (cm^{-1})	L_mH (cm^{-1})
1.	O-H group	Stretching	3305	3300	3282	3295
2.	C-H group	Stretching	2904	2942	2925	2931
3.	C=O group	Stretching	1722	1717	1718	1717
4.	COO^- group	Asymmetric stretching	1579	1557	1556	1560
5.	C-N group	Stretching	1402	1406	1402	1401
6.	C-O-C group	Stretching	1041	1050	1048	1040

5B.3.6.2.2 SEM analysis

To evaluate the morphological features of the prepared hydrogels, scanning electron microscopy was done. The SEM images of dried hydrogels are shown in Figure 5B.6. The SEM images showed the porous morphology and rough surface of the super absorbent hydrogels. The pores in the hydrogel allow more liquid to seep into the voids. The large number of pores is responsible for the swelling behaviour of synthesized hydrogels. From the SEM data, it is evident that L_mH showed a greater number of pores as compared to other hydrogels, which supports its highest swelling index [208].

5B.3.6.2.3 TGA analysis

The stability profiles of hydrogels were studied by TGA, as shown in Figure 5B.5(B). All four thermograms showed four-phase degradation: 30-200°C, 200- 360°C, 360-570°C, and 570- 700°C. The first phase degradation is due to moisture removal; the second phase represents the decomposition of the polysaccharide chains, and the latter two phases represent the complete breakdown of the polysaccharide backbone and sodium acrylate chain, respectively [206]. Among all the hydrogels, L_mH is thermally more stable as it has 45% of residual weight at 800°C. The thermal stability order of hydrogels is $L_mH > A_bH > A_mH > P_rH$. The TGA data clearly proved that thermal stability has increased in the case of all hydrogels as compared with their native mucilaginous polysaccharides.

5B.3.6.2.4 XRD

Figure 5B.5(C) represents the diffractograms of all four hydrogels obtained from XRD. The XRD diffractogram data revealed that there was no sharp peak obtained in all the hydrogel samples. This indicates the amorphous nature of all four hydrogels [209].

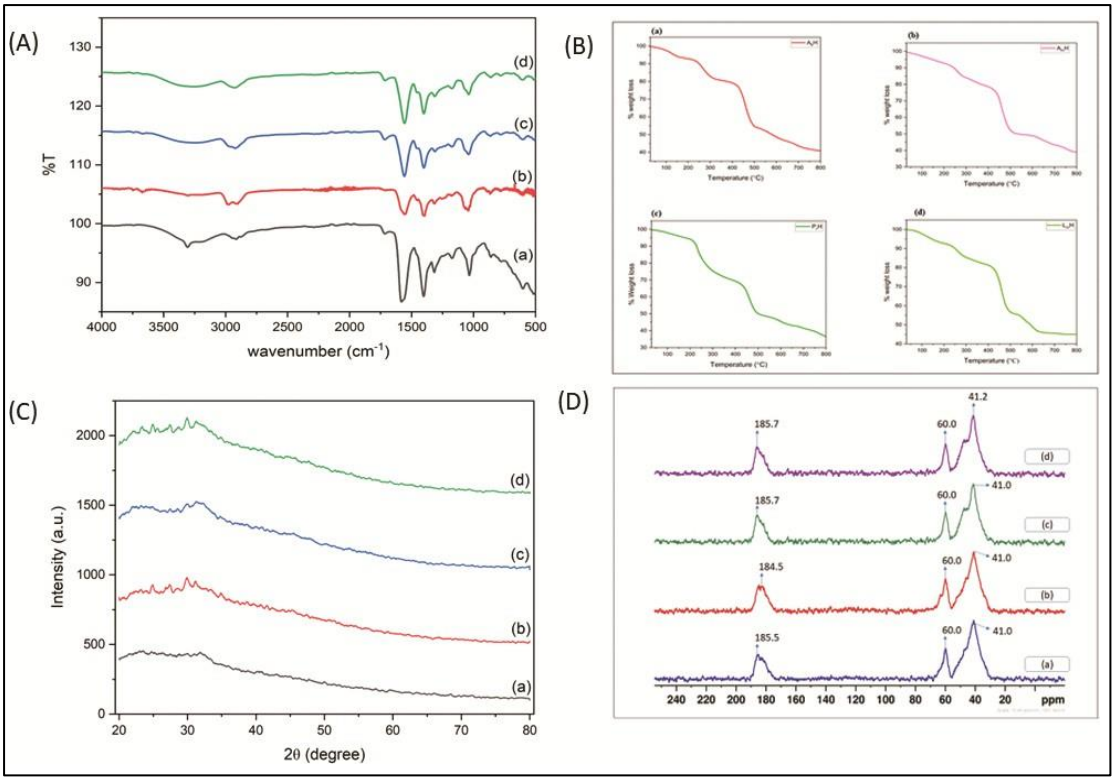

Figure 5B.5: (A)-FTIR spectra, (B) TGA plots, (C)-XRD plots and (D) ^{13}C-NMR spectra of the hydrogels (a) A_bH (b) A_mH (c) P_rH (d) L_mH

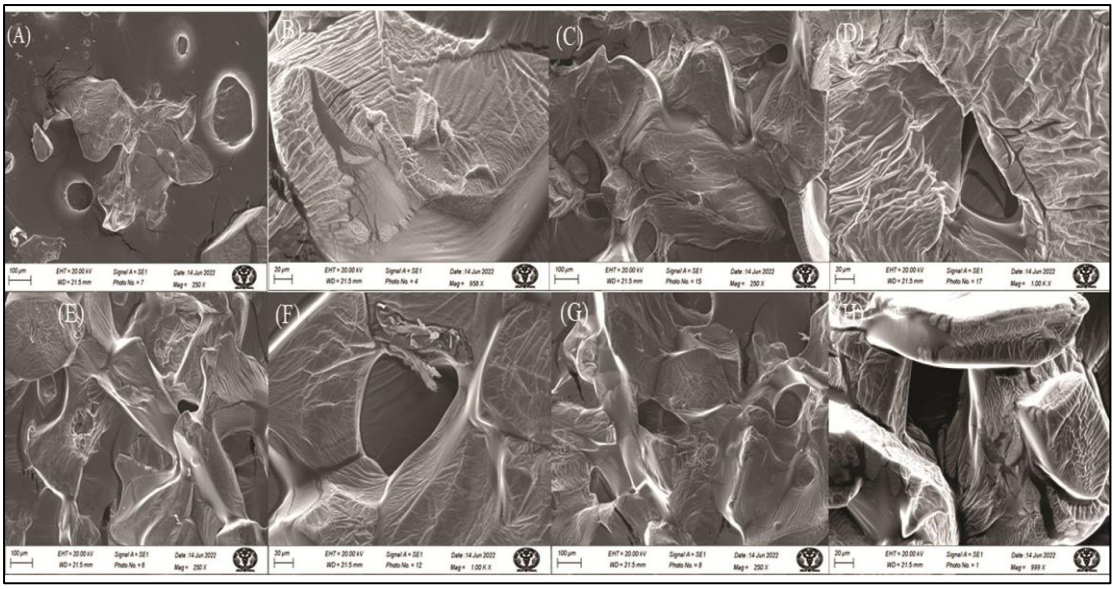

Figure 5B.6: SEM micrographs of the hydrogels (a) A_bH sample at approx.250 X, (b) A_bH sample at approx. approx.1000 X, (c) A_mH sample at 250 X, (d) A_mH sample at approx.1000 X, (e) P_rH sample at approx.250 X, (f) P_rH sample at approx.1000 X, (g) L_mH sample at approx.250 X and (h) L_mH sample at approx.1000 X

5B.3.6.2.5 ^{13}C-NMR

The solid state ^{13}C NMR spectra of all four hydrogels are shown in Figure 5B.5(D). The ^{13}C-NMR peaks are nearly similar in the case of all four hydrogels. The broad peak at δ185.7 ppm can be attributed to the carboxylate group of poly(sodium acrylate), δ60.0 ppm depicted the -CH_2OH group present in the mucilage, and δ41.0 ppm represented the methylene carbon (-CH_2-) which is due to the introduction of MBA crosslinker in the formation of hydrogels [210]. The peaks observed in all four spectra confirmed the synthesis of hydrogels by chemical crosslinking of poly(sodium acrylate) on the mucilage with the help of MBA as a crosslinking agent. Hence, ^{13}C-NMR data confirmed that all four mucilaginous polysaccharides have been crosslinked to form hydrogels.

5B.4 Conclusion

The present study concludes that mucilage isolated from the seeds of underutilized cereals of adzuki beans (*Vigna angularis*), Amaranth (*Amaranthus*), proso millet (*Panicum miliaceum*), and little millet (*Panicum sumatrense*) would be useful in pharmaceutical and bio-medical applications. Spectroscopic analysis (FTIR-ATR and ^{1}H & ^{13}CNMR) shows the presence of polysaccharides as carbohydrate residues in the mucilage which confirms the mucilage's' excipient property. The mucilages characteristics and applicability in advanced technical fields are enhanced by the modification of mucilages by graft copolymerization with sodium acrylate. The prepared hydrogels exhibited porous structure and a remarkable swelling index along with enhanced thermal properties with respect to their mucilages. So, from a technical perspective, these functionalized polymers can play a significant role in various fields.

CHAPTER 6

CONCLUSION, FUTURE PROSPECTS, AND SOCIAL IMPACT

6.1 Conclusion

Despite their rich nutritional and therapeutic potential, underutilized cereals of India have long remained neglected due to the dominance of rice, wheat, and maize in modern agriculture and diets. This overreliance on major cereals has contributed to dietary monotony, hidden hunger, and reduced agricultural biodiversity, leaving several traditional cereal crops underutilized and undervalued. Addressing this critical gap, the present study undertook a comprehensive phytochemical, nutritional, and biological investigation of selected underutilized cereals, while also assessing their functional and product development potential and demonstrating their value in health-oriented food applications.

The present thesis work focused on the comprehensive phytochemical, nutritional, and biological investigation of a few underutilized cereals of India namely, mainly adzuki bean (*Vigna angularis*), rice bean (*Vigna umbellata*), mung bean (*Vigna radiata*), horse gram (*Macrotyloma uniflorum*), and little millet (*Panicum sumatrense*). The successful extraction of bioactive compounds from these selected cereals was carried out using Soxhlet extraction, which is a reliable extraction method that enables efficient isolation of a wide spectrum of phytochemicals through solvents of varying polarity. Different solvents such as n-hexane, DCM, and methanol were used to ensure the comprehensive recovery of phytochemicals. The phytochemical analysis of different extracts of selected cereal samples was performed using both quantitative and qualitative analyses, such as

GC-MS (volatile and semi-volatile compounds), UHPLC-QTOF-MS (non-volatile compounds), ICP-MS (elemental analysis), FAME analysis (fatty acid profiling), and amino acid analysis (amino acid profile). The selected cereal samples were subjected to biological profiling such as HMG-CoA-reductase enzyme inhibitory activity for anti-obesity, pancreatic lipase enzyme inhibitory activity for anti-obesity, antioxidant activity (DPPH assay), and antimicrobial activity (disc-diffusion method).

From the advanced phytochemical analysis using GC-MS analysis, it was observed that adzuki bean extracts (n-hexane, DCM, & methanol) possessed 95 bioactive compounds having SI greater than 90%. The major classes identified were fatty acids, phenolic compounds, terpenes, tocopherols & hydrocarbons, and the predominant compounds were palmitic acid, glycerol, z-7-tetradecenal, and stigmasterol. For mung beans, a total of 38 bioactive compounds were identified, with fatty acids, phenolic compounds, terpenes, tocopherols, and hydrocarbons as major identified classes. The predominant compounds were palmitic acid, ethyl benzoate, and linoleic acid. Horse gram contained 70 bioactive compounds, with phenolic compounds, terpenes, tocopherols, and hydrocarbons being the primary classes. The predominant compounds were myo-inositol, palmitic acid, 9,12-linoleic acid, and caprylic acid monoethanol amide. For rice beans, a total of 70 compounds were identified, with phenolic compounds, terpenes, tocopherols & hydrocarbons as the major classes. The predominant compounds were myo-inositol, hexadecanoic acid, 9,12-octadecadienoic acid, (7z)-7-tetradecenal, 9,10-dibromopentacosane, stigmasterol, and β-amyrin. For little millet, 84 compounds were identified, with hydrocarbons, fatty acids, and terpenes as the major classes. The predominant compounds in little millet were cis-9-Hexadecenal and palmitic acid. From the data of UHPLC-QTOF-MS, it was observed that a total of 15, 18, 13, 46, and 21

secondary metabolites were identified in the adzuki beans, mung beans, horse gram, rice beans, and little millet, respectively, based on their mass fragmentation pattern. The major classes identified in these selected cereal samples were phytosterols, flavonoids, phenolic compounds & terpenoids. Additionally, 9 targeted metabolites - catechin-7-O-glucoside, catechin, epicatechin, quercetin, gallocatechin, gallic acid, caffeic acid, para-coumaric acid, and glycitein were also identified in the selected samples.

From the nutritional profiling results, proximate analysis confirmed that these underutilized cereals are valuable sources of proteins, dietary fiber, carbohydrates, and essential minerals. The elemental analysis confirmed that these cereals are a good source of potassium, molybdenum, and magnesium. Also, higher mineral content supports the use of these cereals in functional foods and nutraceuticals, especially for populations prone to mineral deficiencies. FAME analysis confirmed that these cereals were rich sources of palmitic acid, linoleic acid, and linolenic acid. Therefore, the results support the use of these cereals in daily diets as they contain high levels of polyunsaturated fatty acids which indicate potential cardioprotective and anti-inflammatory properties and highlight their nutritional importance. Amino acid profiling confirmed that all these cereals are nutritional as they are a rich source of both essential and non-essential amino acids. Moreover, the nutritional values of these selected cereals were enhanced by the presence of non-proteinogenic amino acids. Biological assays further established their therapeutic relevance, particularly their anti-obesity potential through significant inhibition of HMG-CoA reductase and pancreatic lipase activity.

Beyond experimental analyses, the successful integration of these underutilized cereals into nutritional cookies and breakfast flakes proved not only their suitability for food

processing, nutritional benefits, and consumer acceptability, but also highlighted their potential for industrial application. A significant achievement of this research was its successful commercialization through a partnership with Kalsubai Purest Company (Maharashtra). Leveraging the formulations developed during the study, their R&D team produced a trial batch and is actively preparing for its market launch as a functional and health-promoting food product. The company further recognized the practical relevance of this work by issuing an appreciation letter, underscoring its real-world impact. This real-world application illustrates how these neglected cereals can play a vital role in the development of innovative functional foods, bridging traditional agricultural biodiversity with modern dietary needs and offering opportunities for the development of health-oriented, ready-to-eat food products.

Overall, this work bridges a major research gap by scientifically validating the nutritional and functional value of underutilized cereals of India. The findings of this thesis work reinforce the potential of diversifying the food basket, promoting sustainable agriculture, and improving public health. This study offers a platform for using these underutilized cereals as a source of nutraceuticals for controlling various non-communicable diseases.

6.2 Future Prospects

The current thesis work has highlighted the functional and nutritional potential of underutilized cereals of India and their potential in developing novel food products. These findings open up multiple pathways for future research and innovation aimed at unlocking the full therapeutic and market potential of these cereals.

Key Future Directions:

- Isolation and characterization of individual bioactive compounds from cereal extracts to understand their therapeutic mechanisms.
- Conducting in-vivo and in-silico studies to validate the biological efficacy of identified bioactive compounds.
- Development of other value-added functional foods or nutraceuticals to promote their inclusion in daily diets and their commercialization.
- Investigation of different processing methods and their effects on nutraceutical content and other biological activities.

6.3 Social Impact

The social implications of this research extend across multiple dimensions of sustainable development and public welfare. Exploring underutilized cereal crops such as horse gram, rice bean, mung bean, adzuki bean, and little millet offers a possible approach to improving nutritional security and public health, particularly in rural areas. Integrating these nutrient-rich cereals into daily diets through functional foods and nutraceuticals can play a vital role in addressing micronutrient deficiencies, lowering the incidence of lifestyle-related conditions like diabetes, obesity, and heart disease, and fostering overall health and wellness.

Key social impacts:

a) **Nutritional security:** Boosts consumption of protein and micronutrient-rich underutilized cereals.

b) **Preservation of traditional knowledge and biodiversity:** Encourages the utilization of diverse, indigenous cereal crop varieties and lessens the dependency on certain staple crops.

c) **Promotes public health:** The development of functional foods from these cereals has the potential to decrease the frequency of non-communicable diseases.

d) **Sustainable agriculture:** Promotes the cultivation of underutilized cereal crops.

e) **Food innovations:** Development of novel nutrient-rich food products utilizing these cereals.

APPRECIATION LETTER

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21st July 2025

Prof. Rajinder K. Gupta
Department of Applied Chemistry,
Delhi Technological University, Shahbad Daulatpur,
Bawana Road, Delhi -110042

Dear Prof. Gupta:

Our company liked the cookies idea of your student, Ms. Ritu Sharma, and we have already started making these Azuki and Mung-based cookies, which contain our brand of natural and healthy jaggery. We, and our customers, are impressed by the taste and happy with the customer feedback. Now, we are in the process of commercial-scale production and marketing to our consumers to alleviate obesity and high cholesterol & other associated metabolic problems. We are thankful to you, Ms. Ritu, and your university, DTU, for this product.

Our best wishes to the entire team and DTU.

Best Regards,

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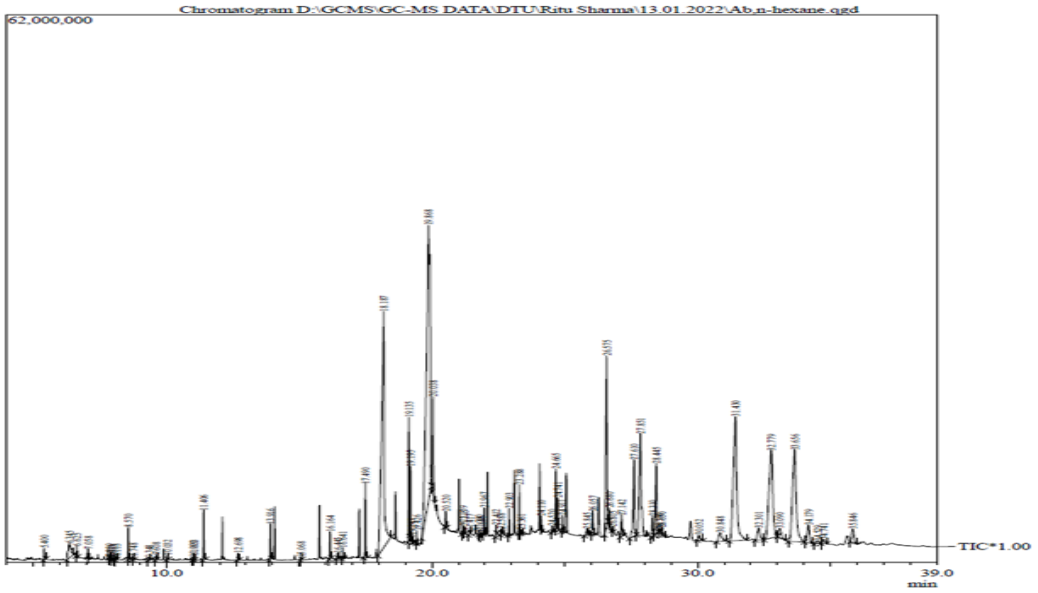
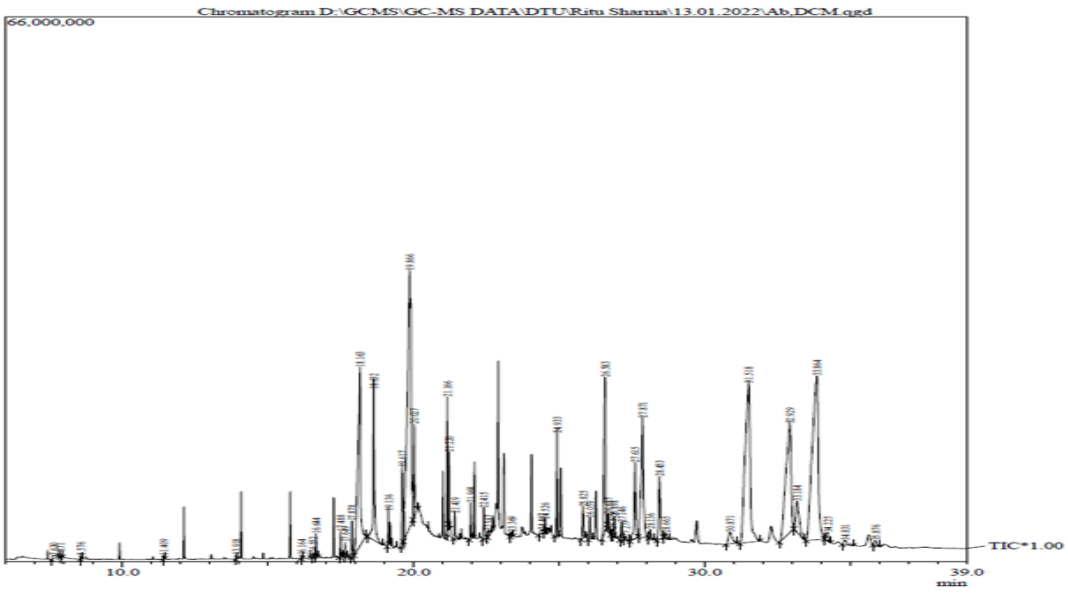
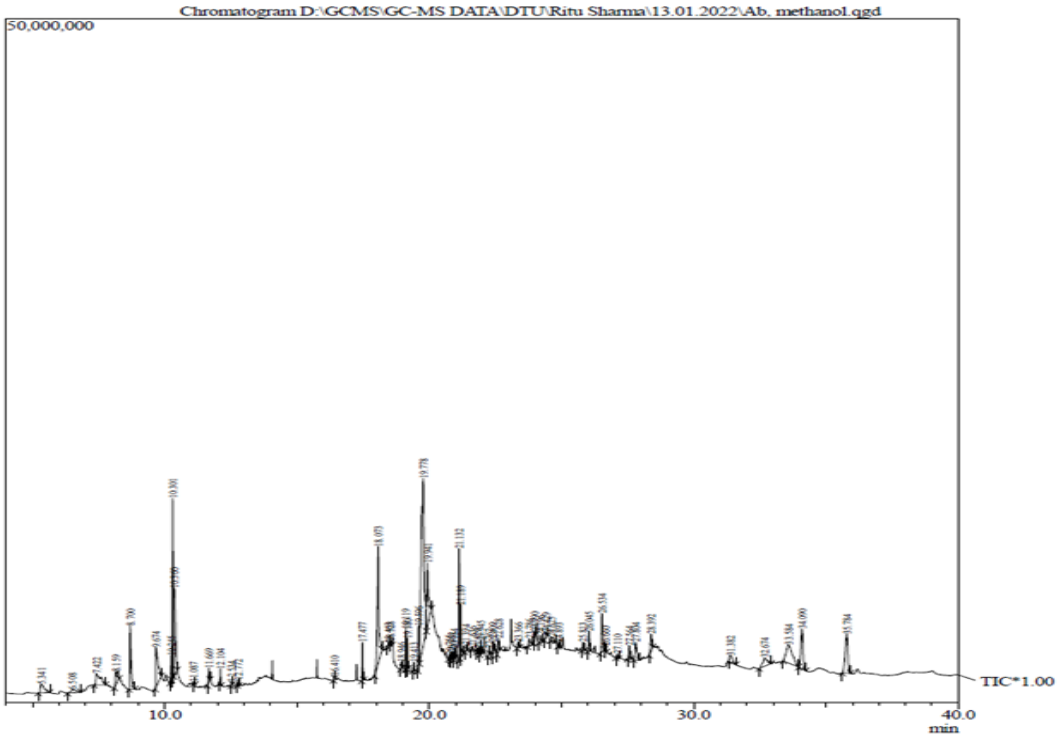
APPENDIX



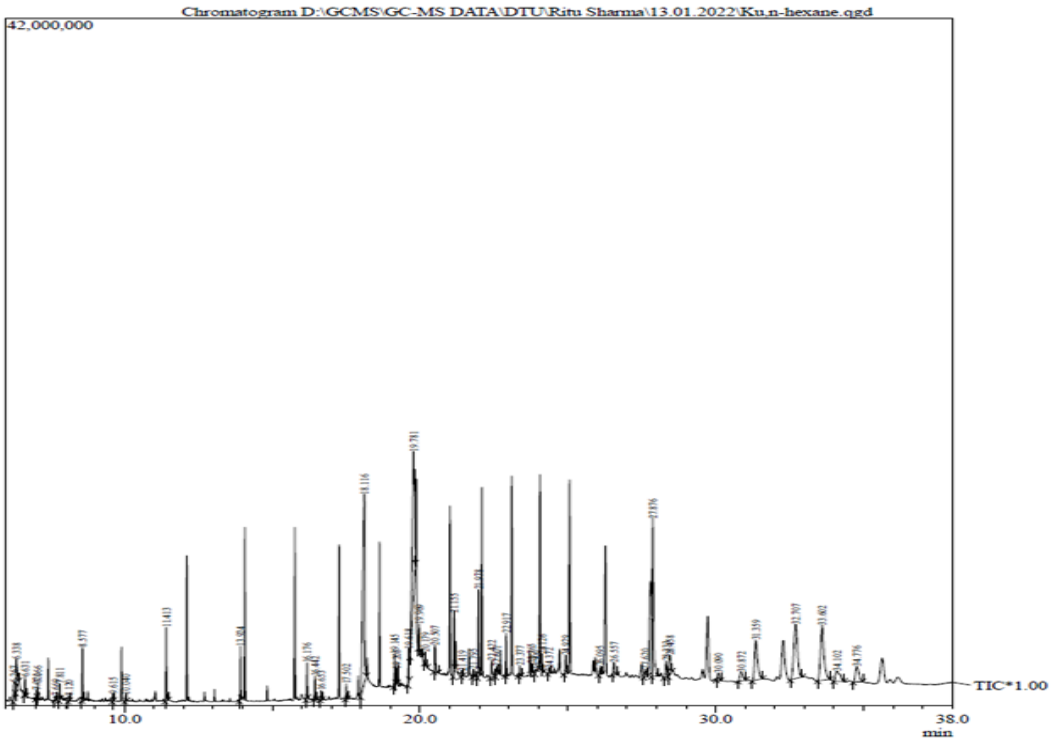
Appendix A1: GC-MS chromatogram of n-hexane extract of adzuki beans



Appendix A2: GC-MS chromatogram of DCM extract of adzuki beans



Appendix A3: GC-MS chromatogram of methanol extract of adzuki beans



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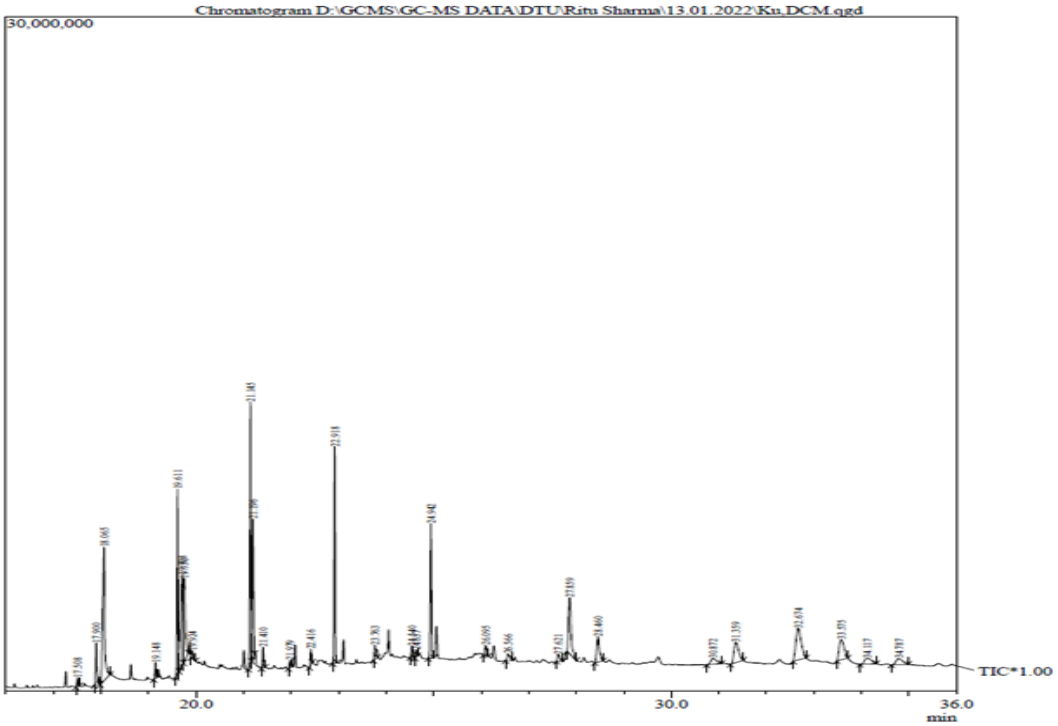
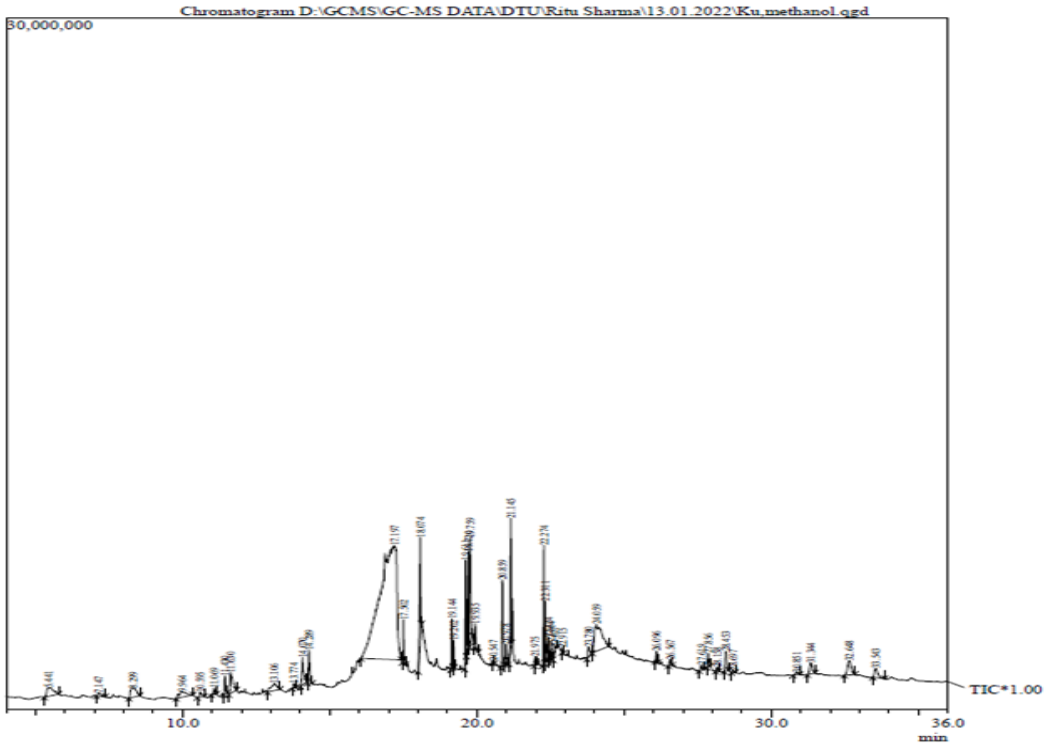
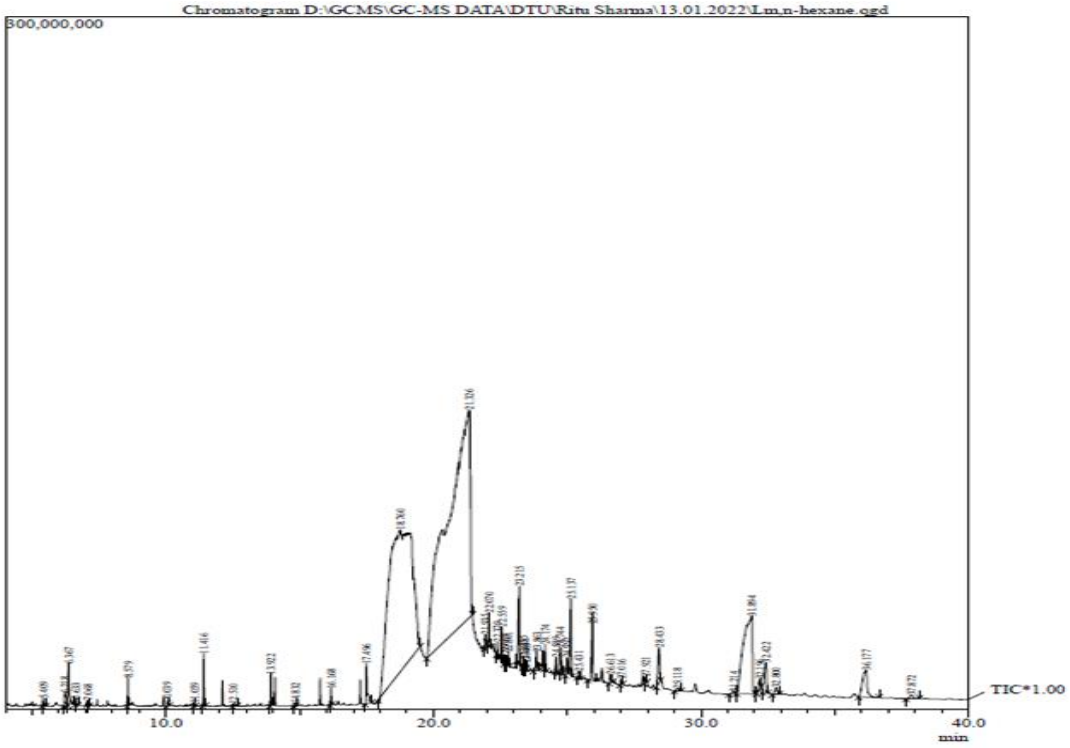


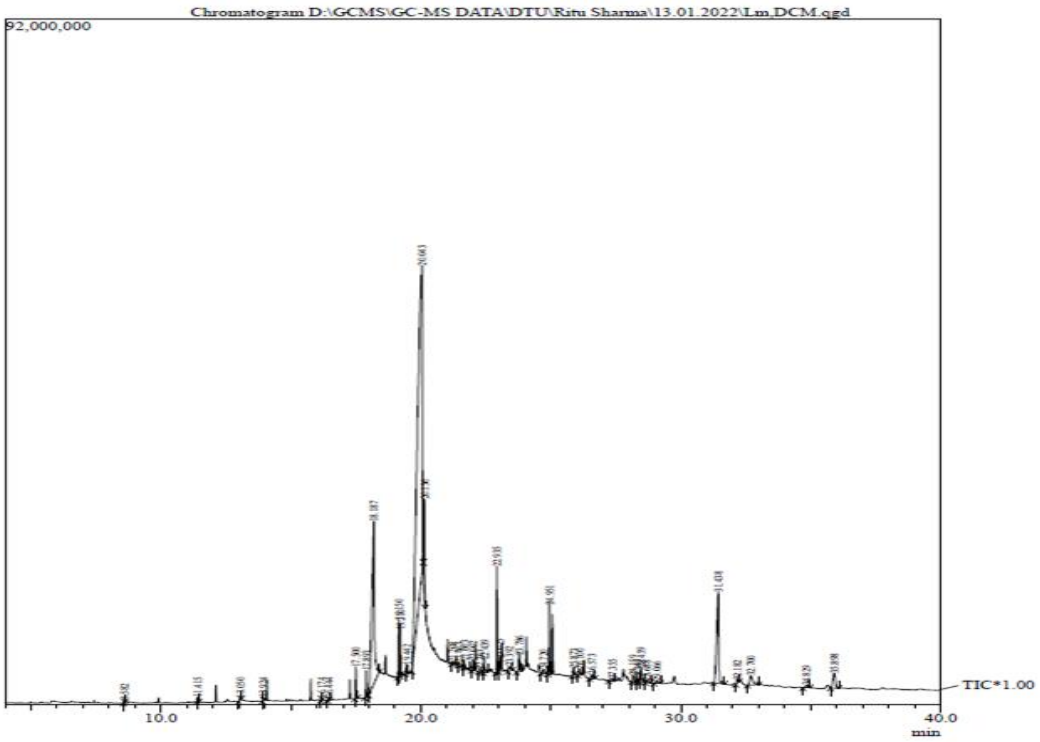
Appendix A5: GC-MS chromatogram of DCM extract of horse gram



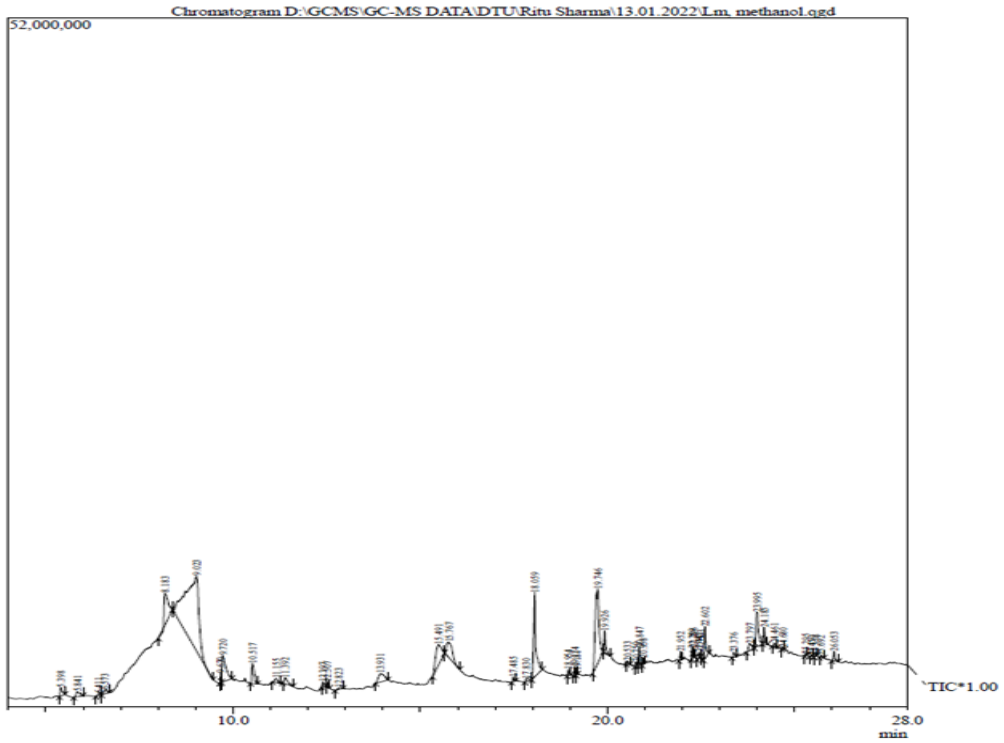
Appendix A6: GC-MS chromatogram of methanol extract of horse gram



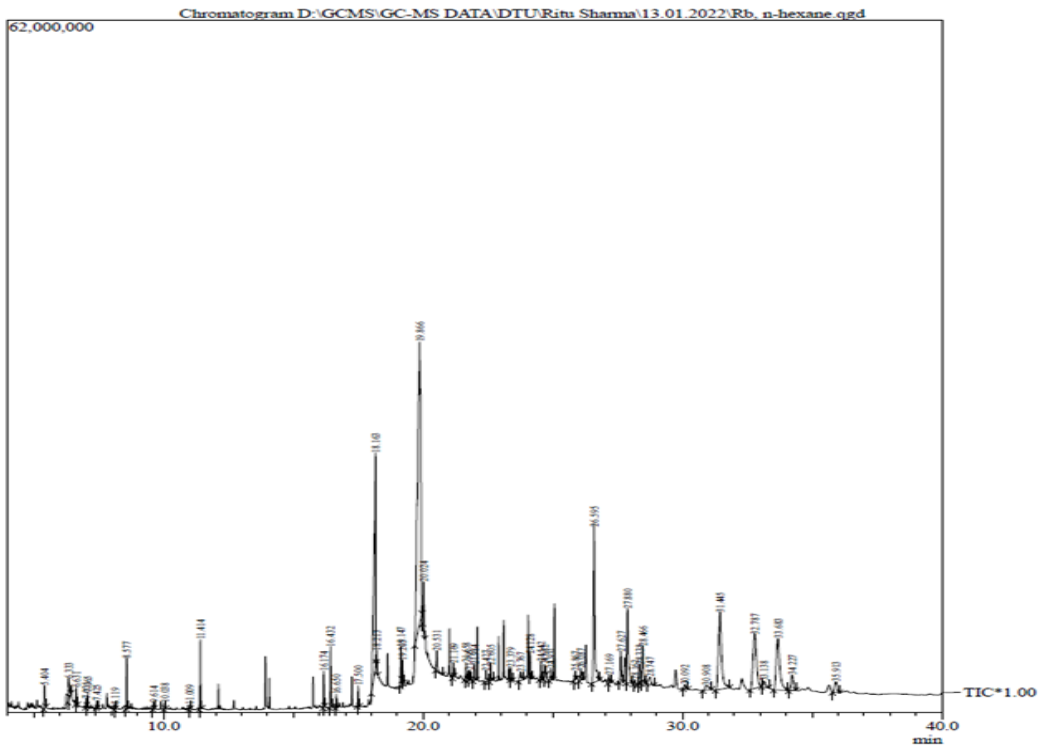
Appendix A7: GC-MS chromatogram of n-hexane extract of little millet



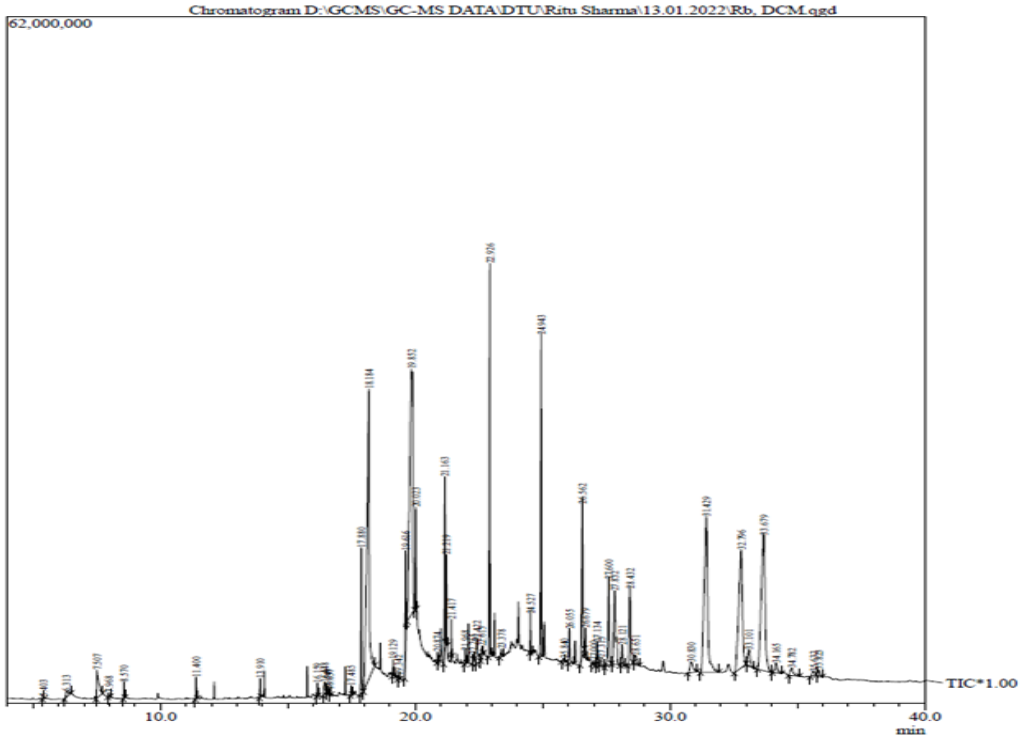
Appendix A8: GC-MS chromatogram of DCM extract of little millet



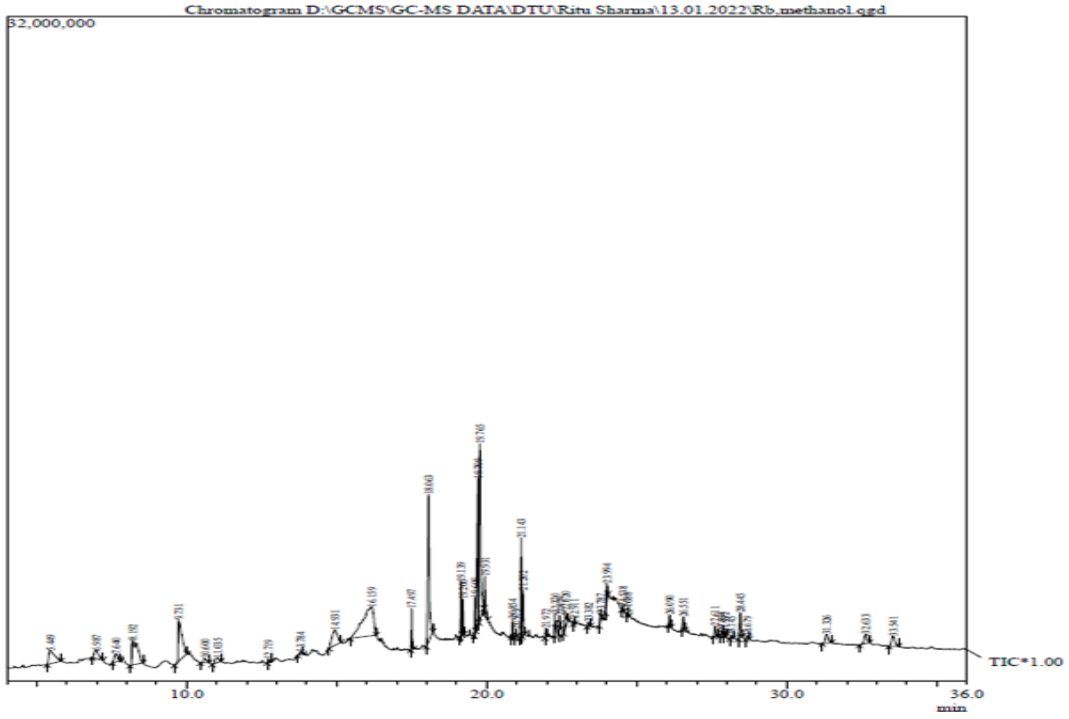
Appendix A9: GC-MS chromatogram of methanol extract of little millet



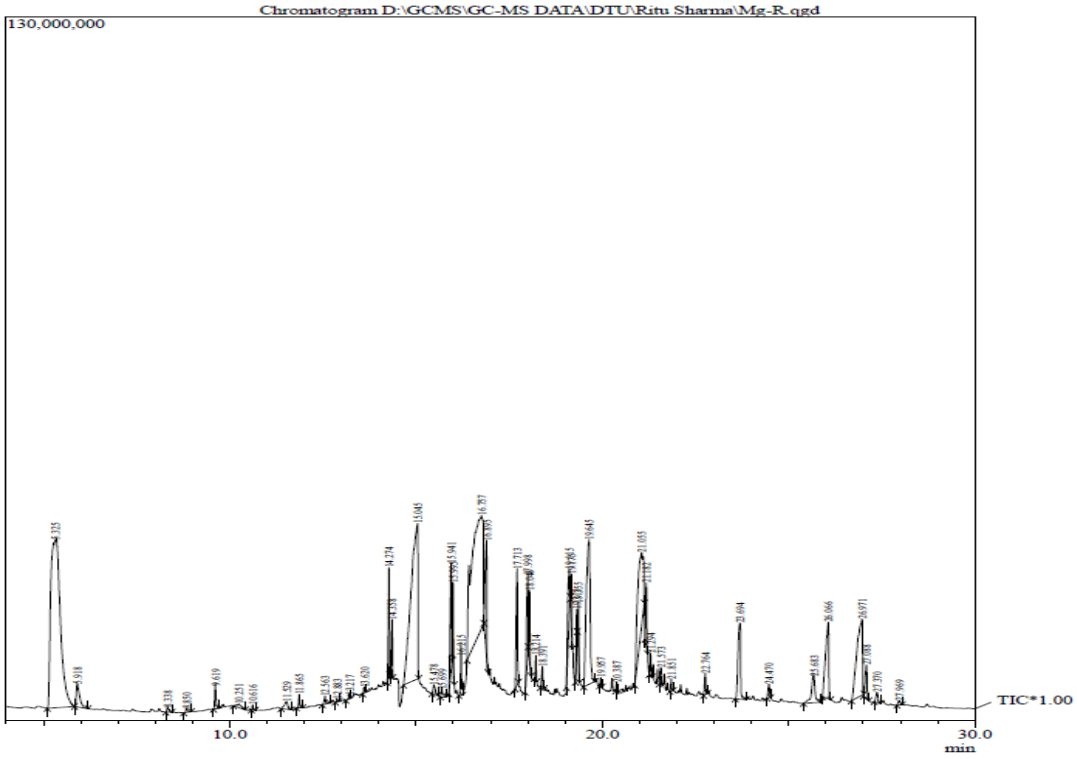
Appendix A10: GC-MS chromatogram of n-hexane extract of rice beans



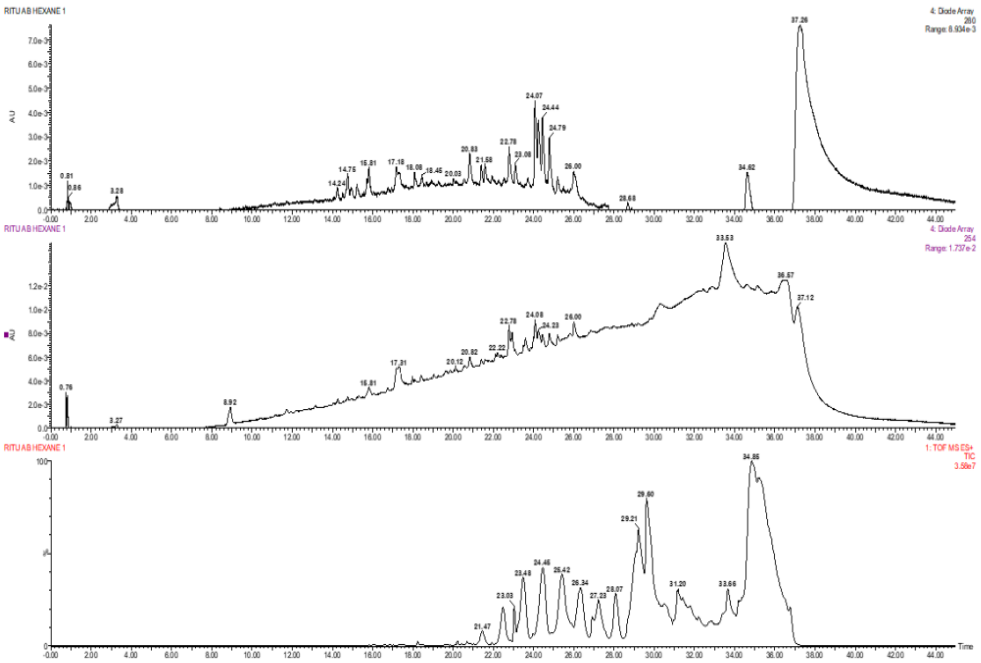
Appendix A11: GC-MS chromatogram of DCM extract of rice beans



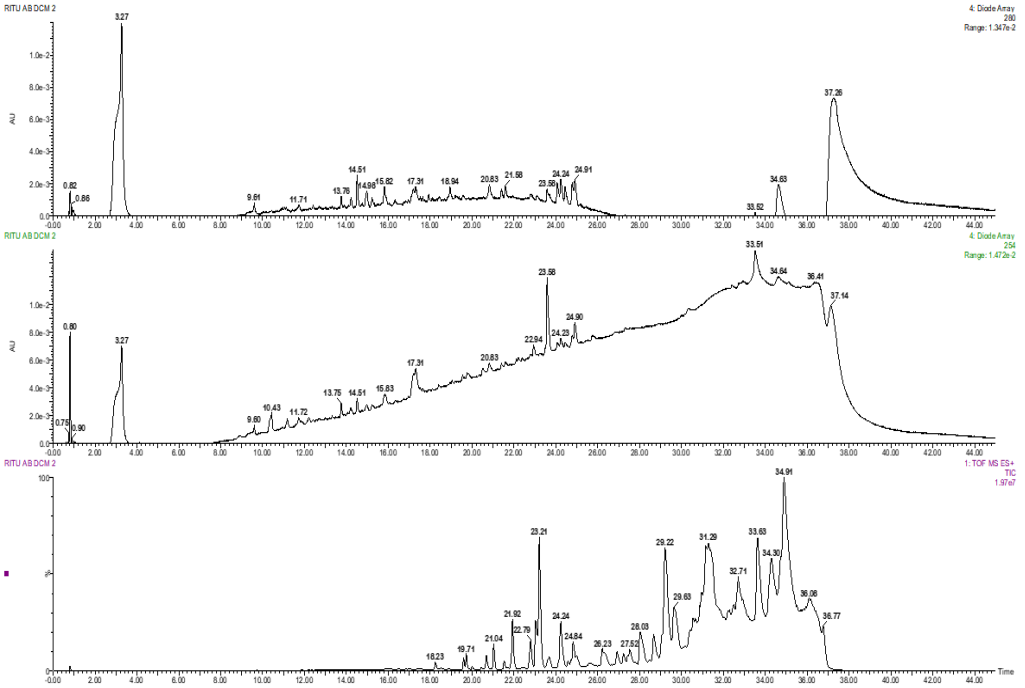
Appendix A12: GC-MS chromatogram of methanol extract of rice beans



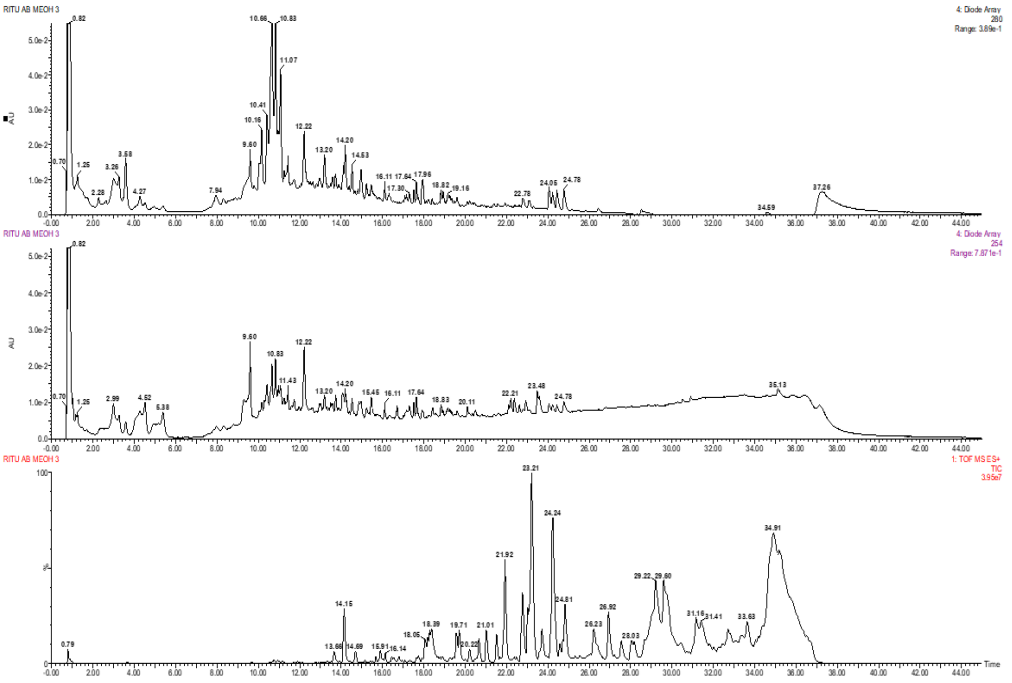
Appendix A13: GC-MS chromatogram of methanol extract of mung beans



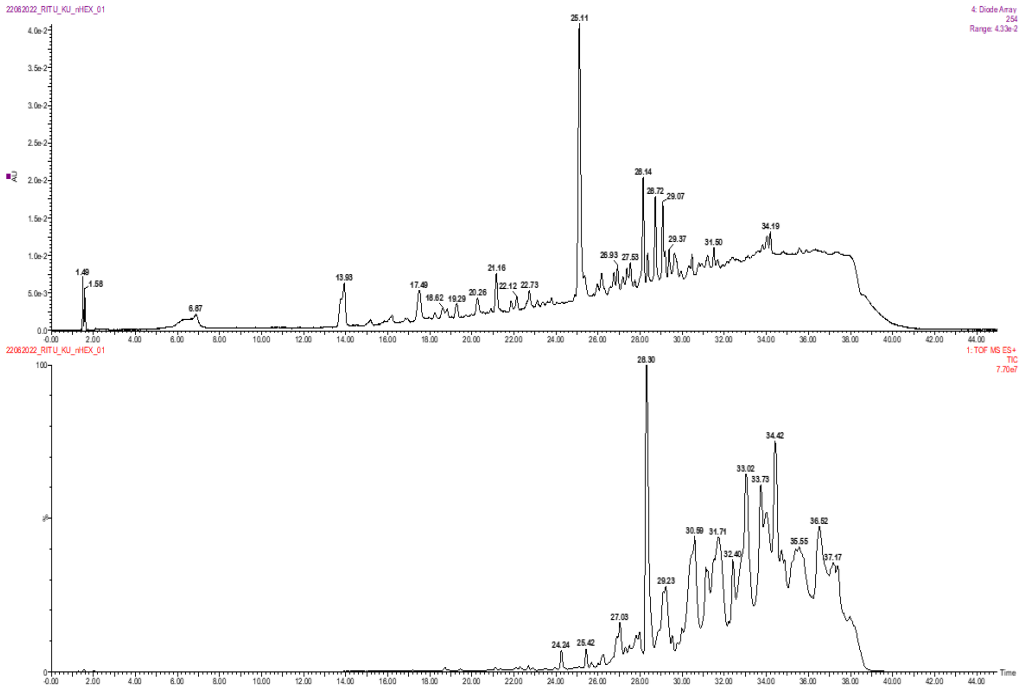
Appendix A14: UHPLC-QTOF-MS chromatogram of n-hexane extract of adzuki beans



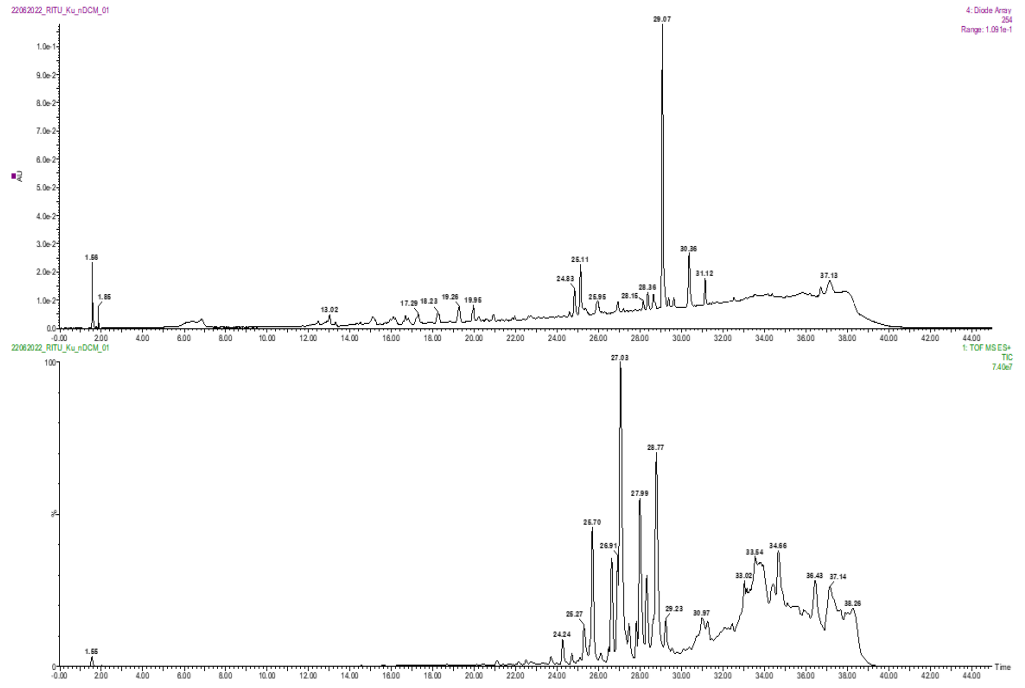
Appendix A15: UHPLC-QTOF-MS chromatogram of DCM extract of adzuki beans



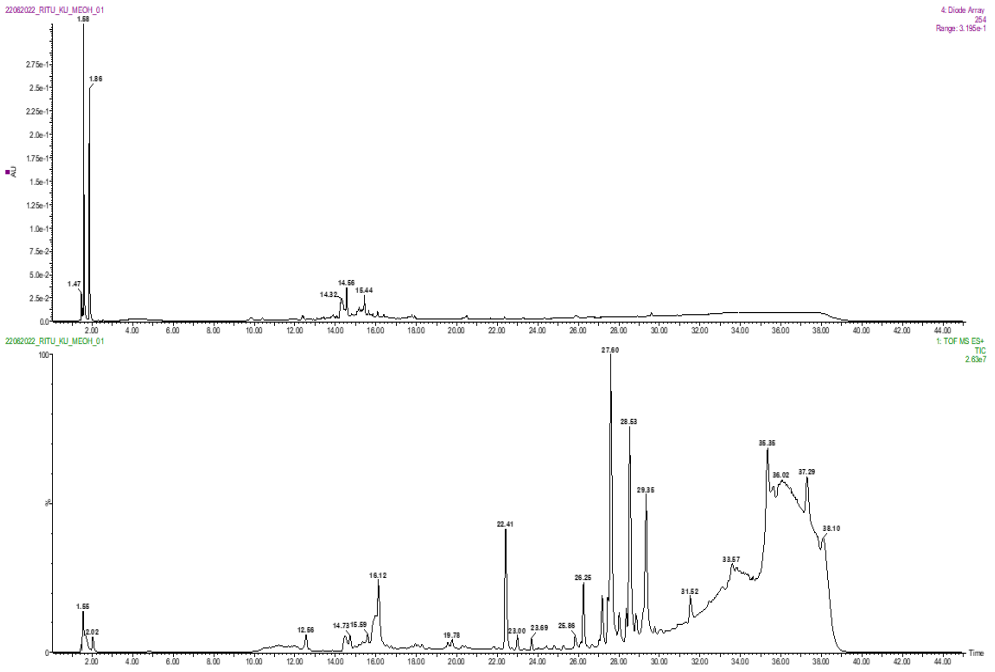
Appendix A16: UHPLC-QTOF-MS chromatogram of methanol extract of adzuki beans



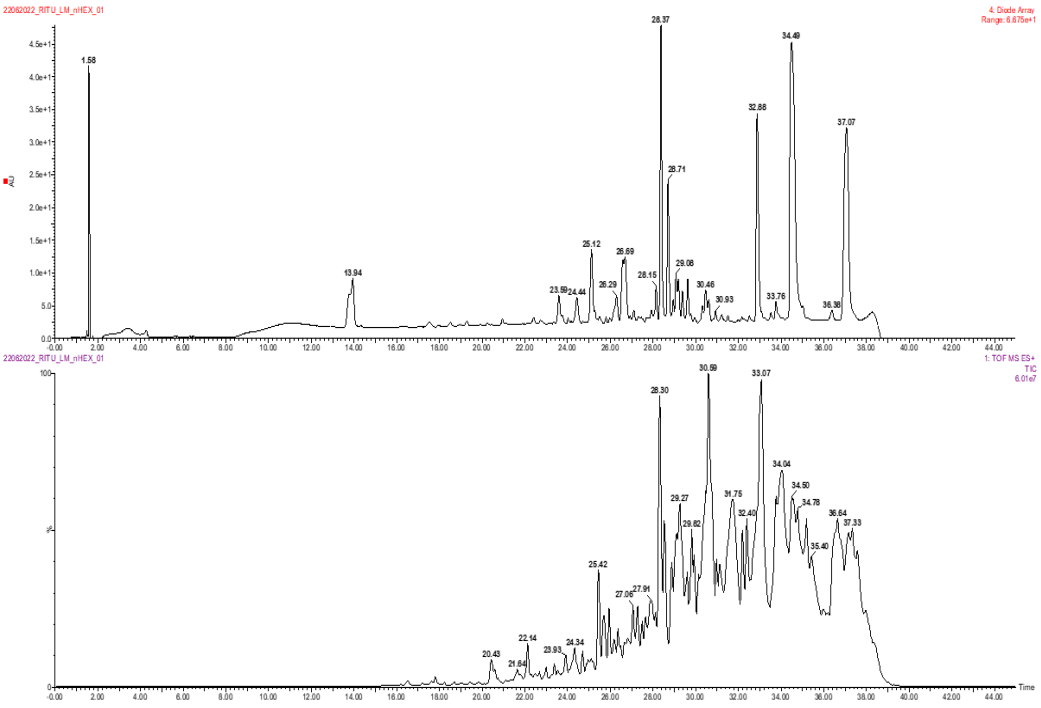
Appendix A17: UHPLC-QTOF-MS chromatogram of n-hexane extract of horse gram



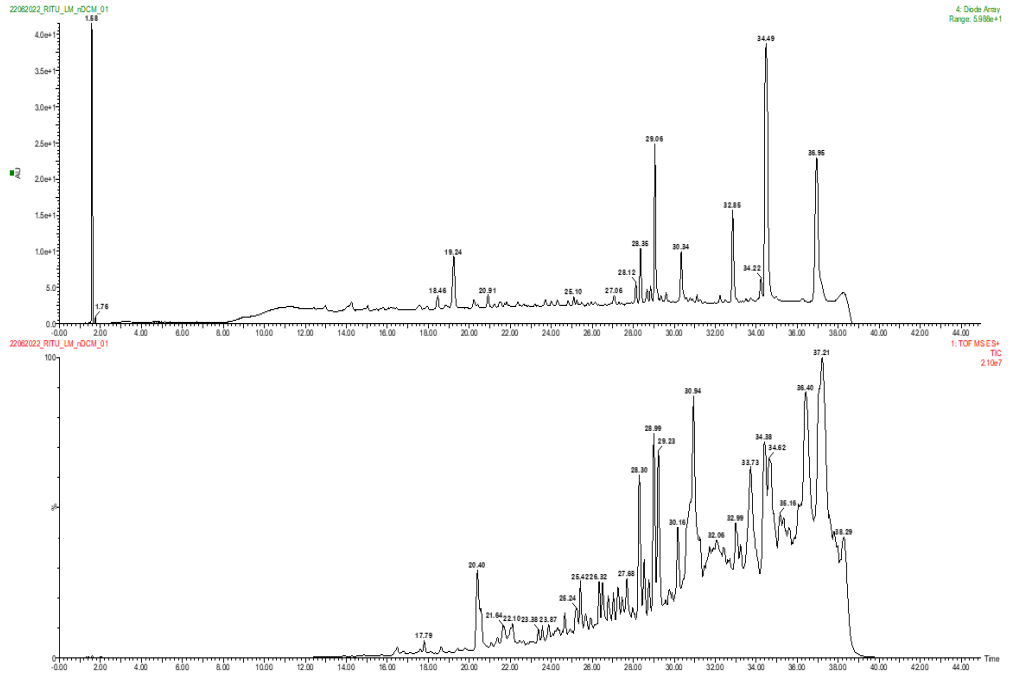
Appendix A18: UHPLC-QTOF-MS chromatogram of DCM extract of horse gram



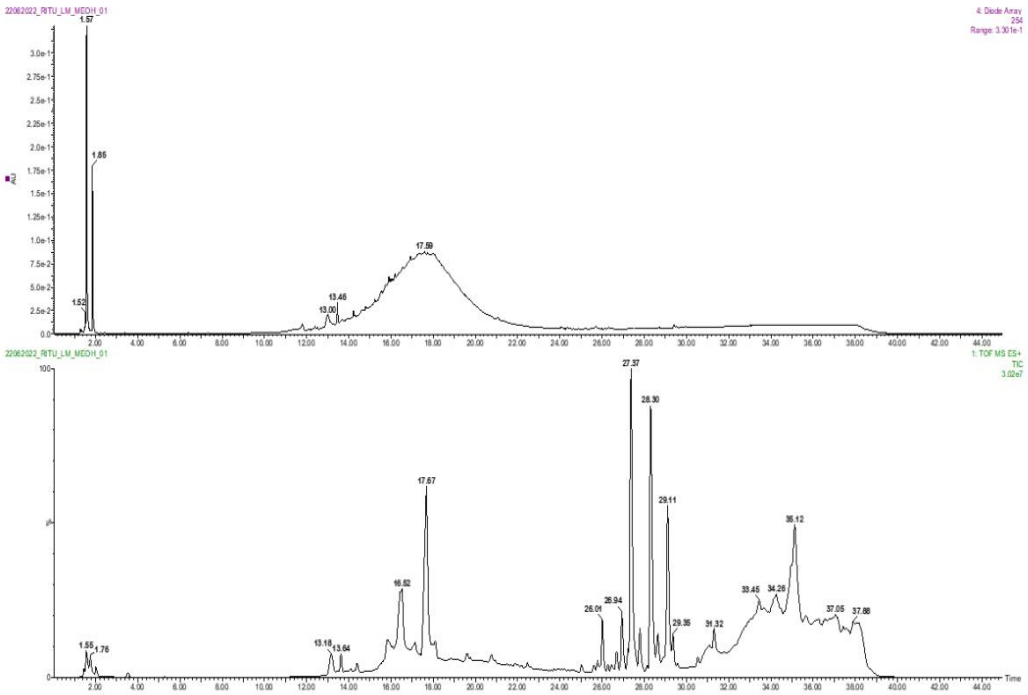
Appendix A19: UHPLC-QTOF-MS chromatogram of methanol extract of horse gram



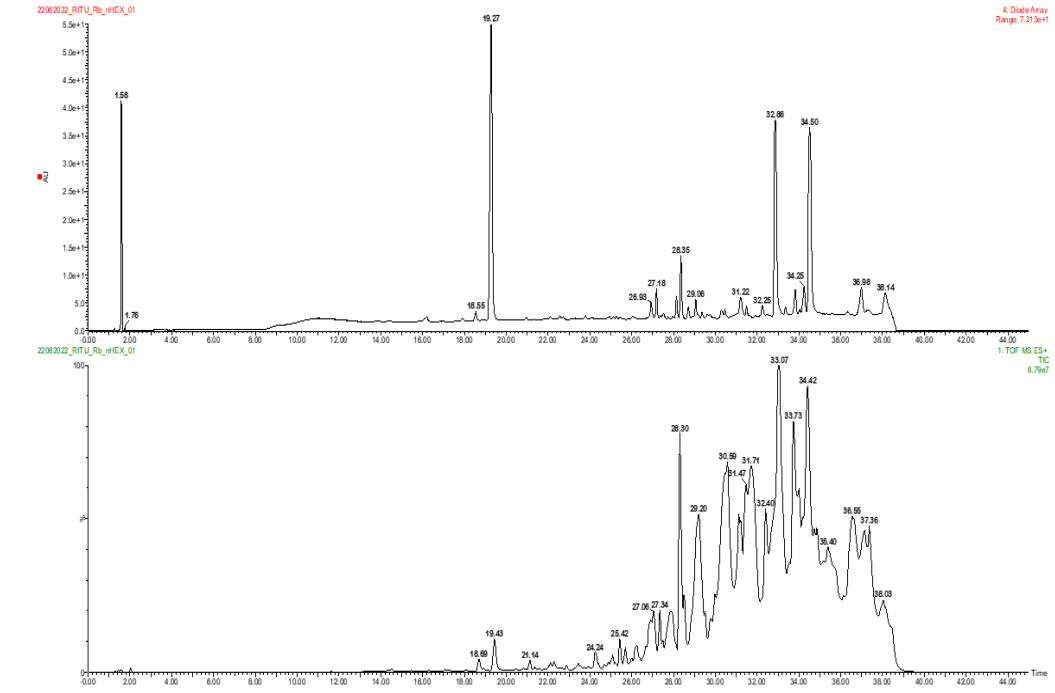
Appendix A20: UHPLC-QTOF-MS chromatogram of n-hexane extract of little millet



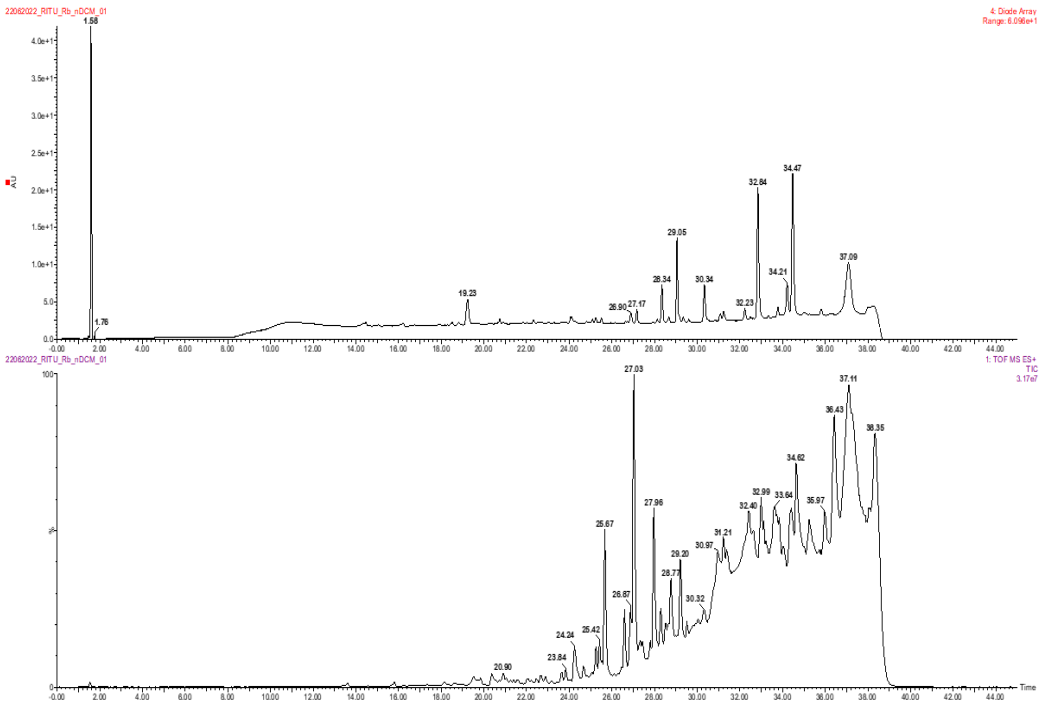
Appendix A21: UHPLC-QTOF-MS chromatogram of DCM extract of little millet



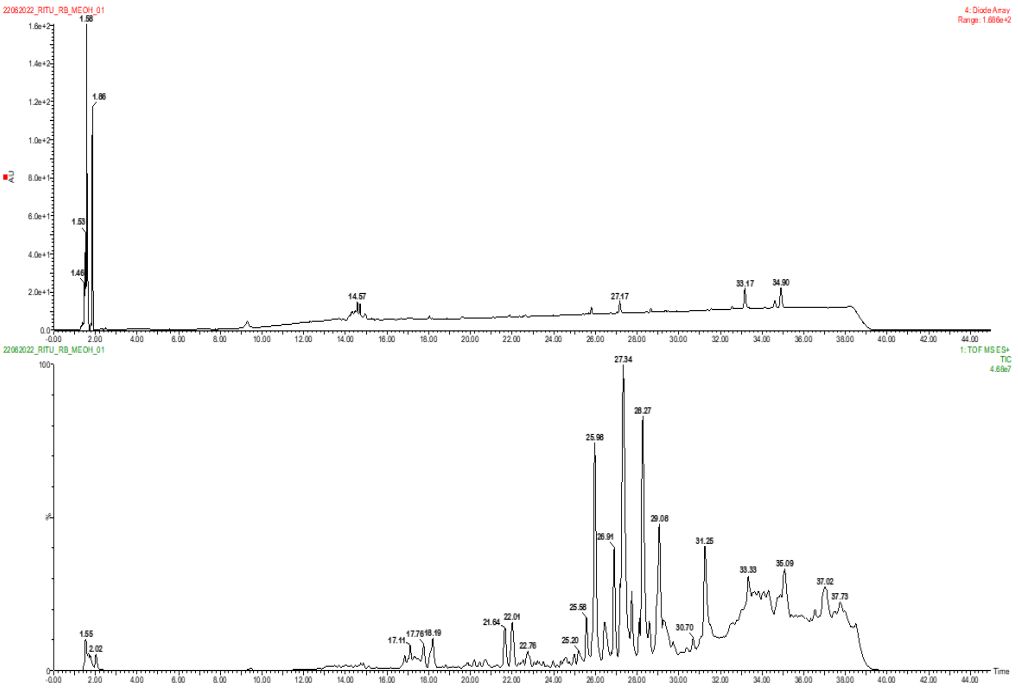
Appendix A22: UHPLC-QTOF-MS chromatogram of methanol extract of little millet



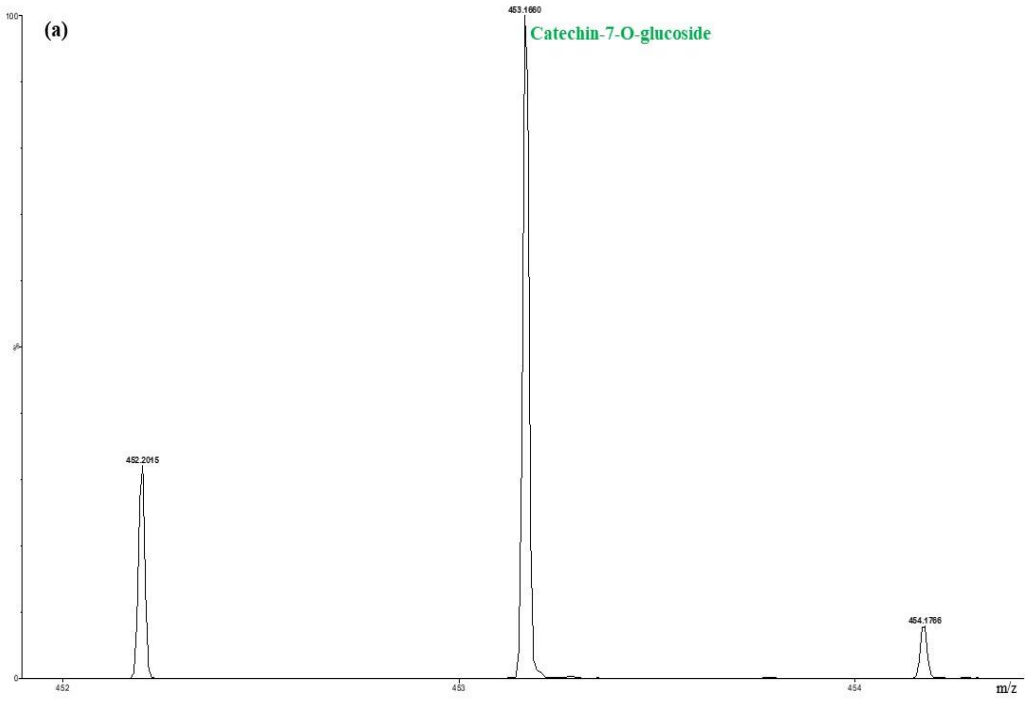
Appendix A23: UHPLC-QTOF-MS chromatogram of n-hexane extract of rice beans



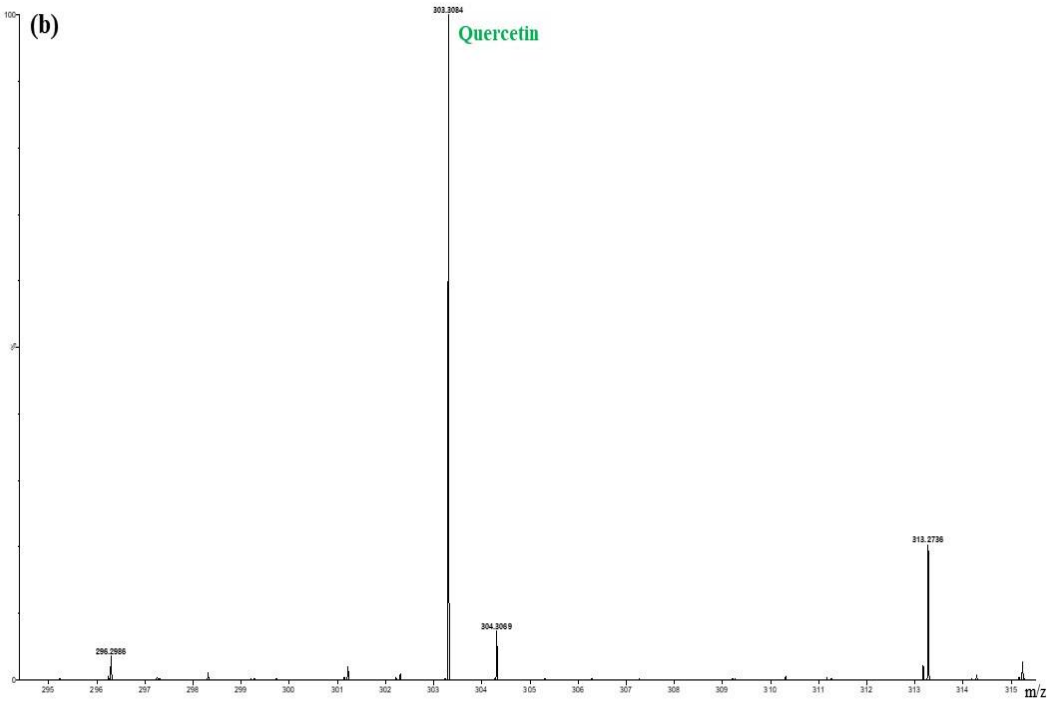
Appendix A24: UHPLC-QTOF-MS chromatogram of DCM extract of rice beans



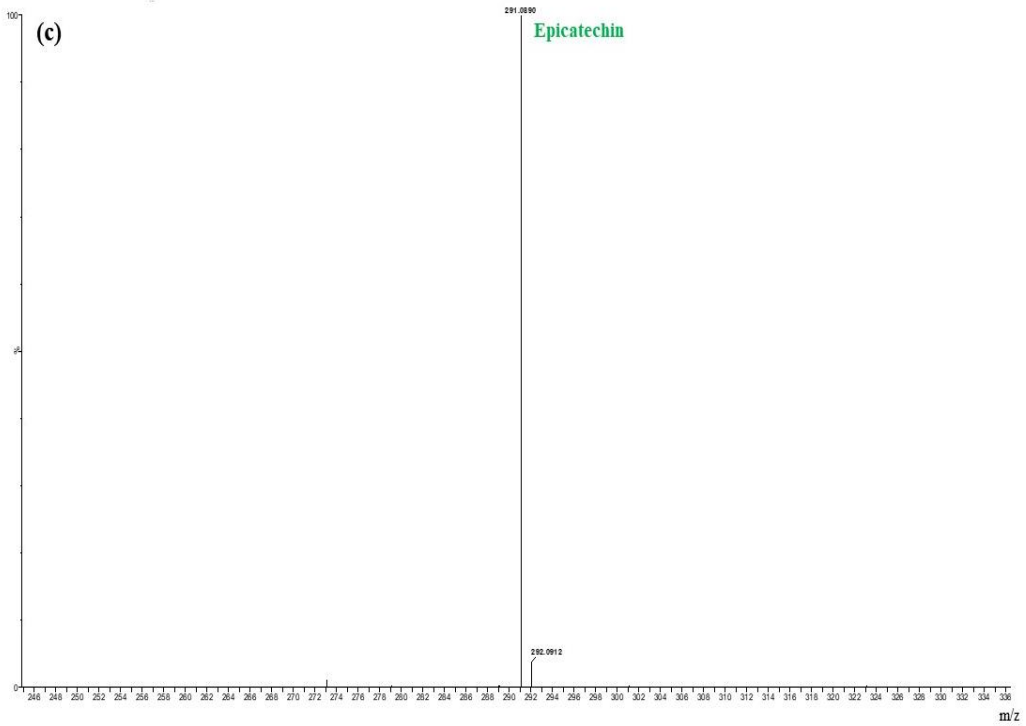
Appendix A25: UHPLC-QTOF-MS chromatogram of methanol extract of rice beans



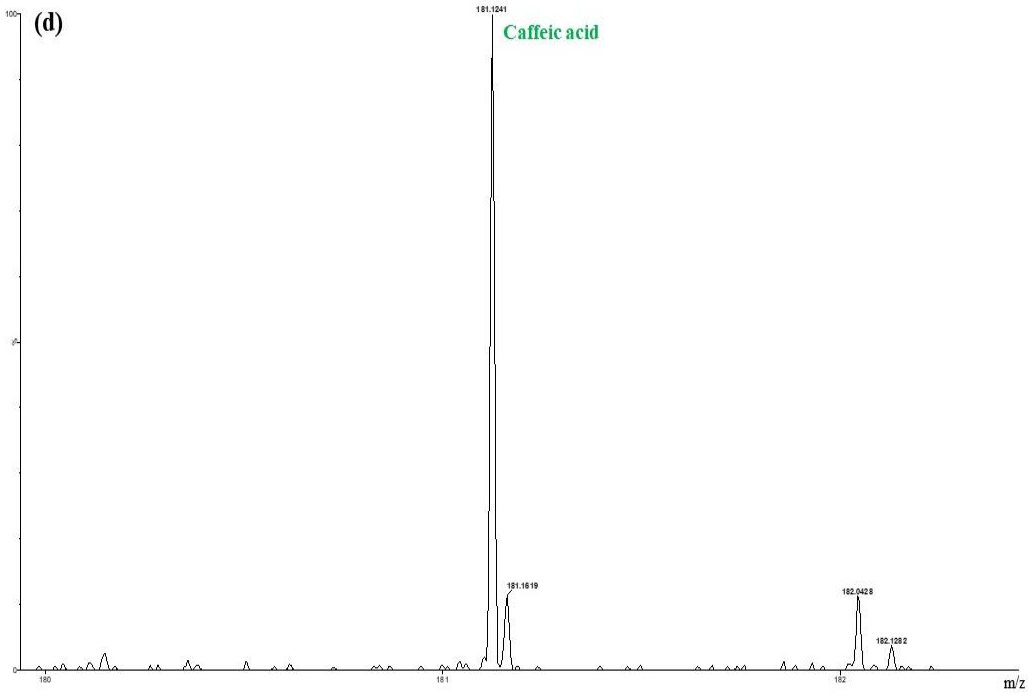
Appendix A26: UHPLC-QTOF-MS chromatograms of targeted metabolite - Catechin-7-O-glucoside



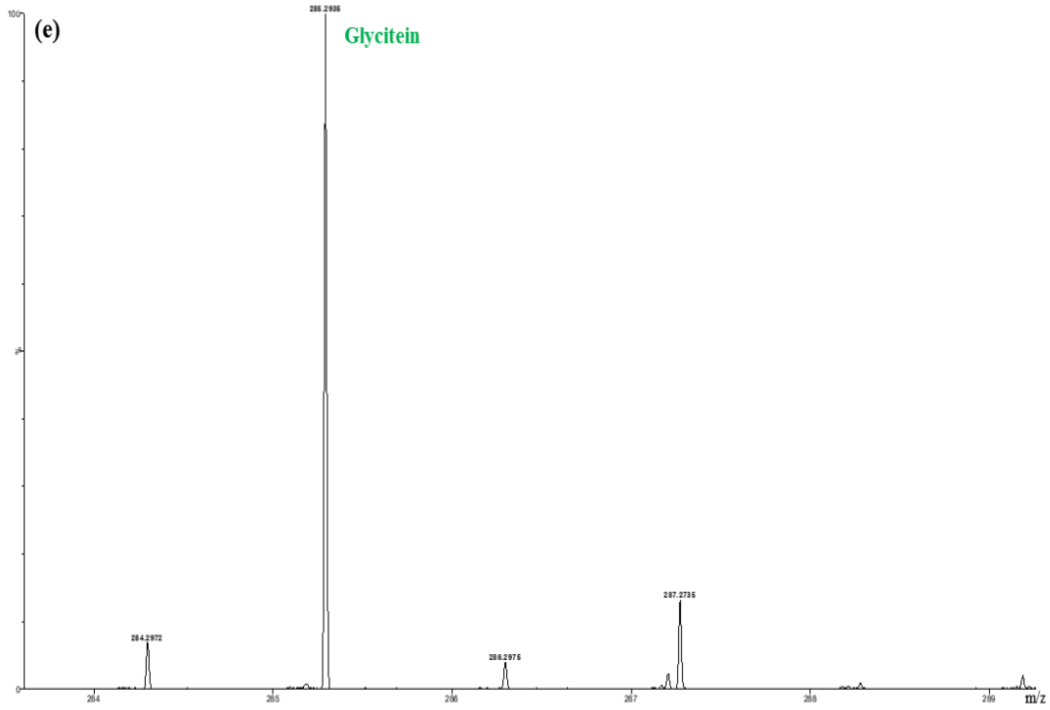
Appendix A27: UHPLC-QTOF-MS chromatograms of targeted metabolite - Quercetin



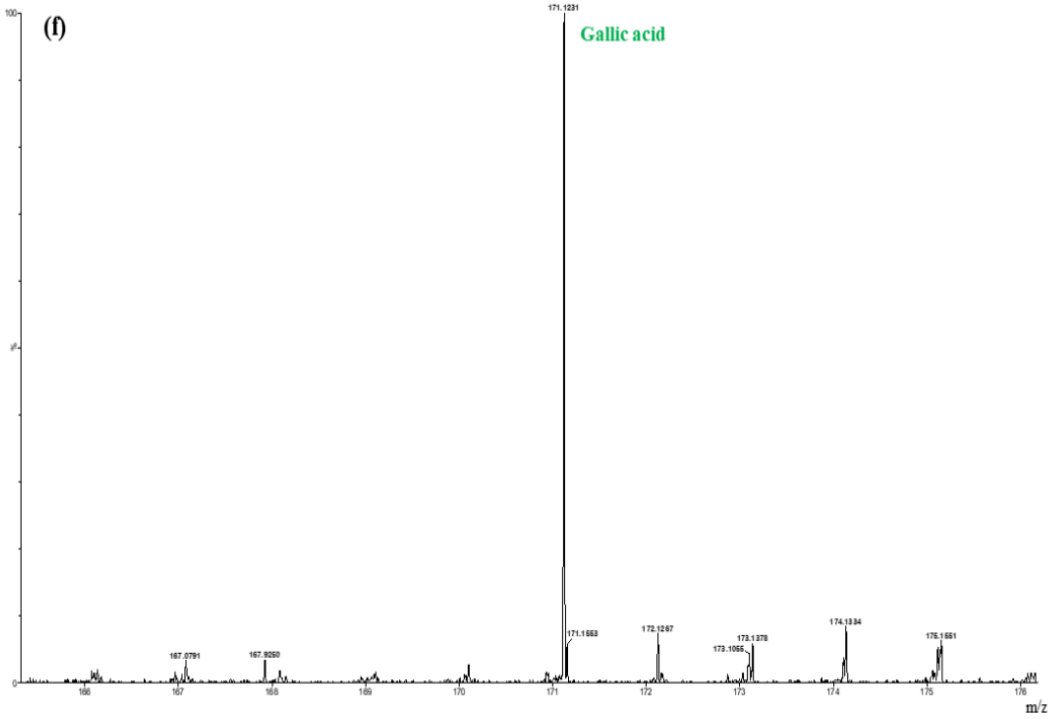
Appendix A28: UHPLC-QTOF-MS chromatograms of targeted metabolite - Epicatechin



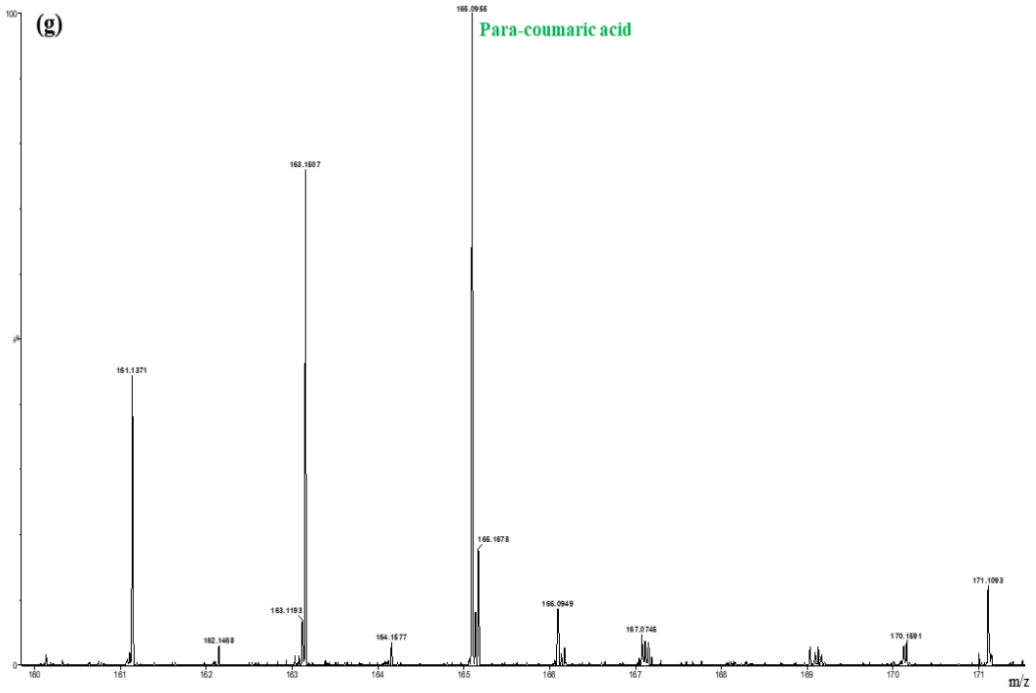
Appendix A29: UHPLC-QTOF-MS chromatograms of targeted metabolite – Caffeic acid



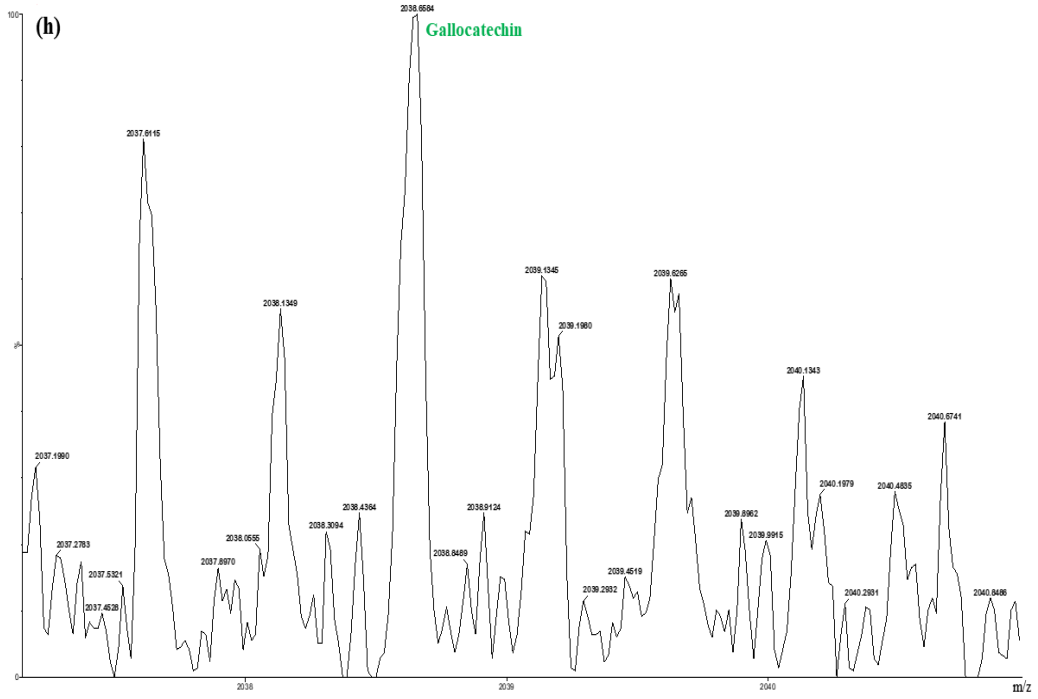
Appendix A30: UHPLC-QTOF-MS chromatograms of targeted metabolite – Glycitein



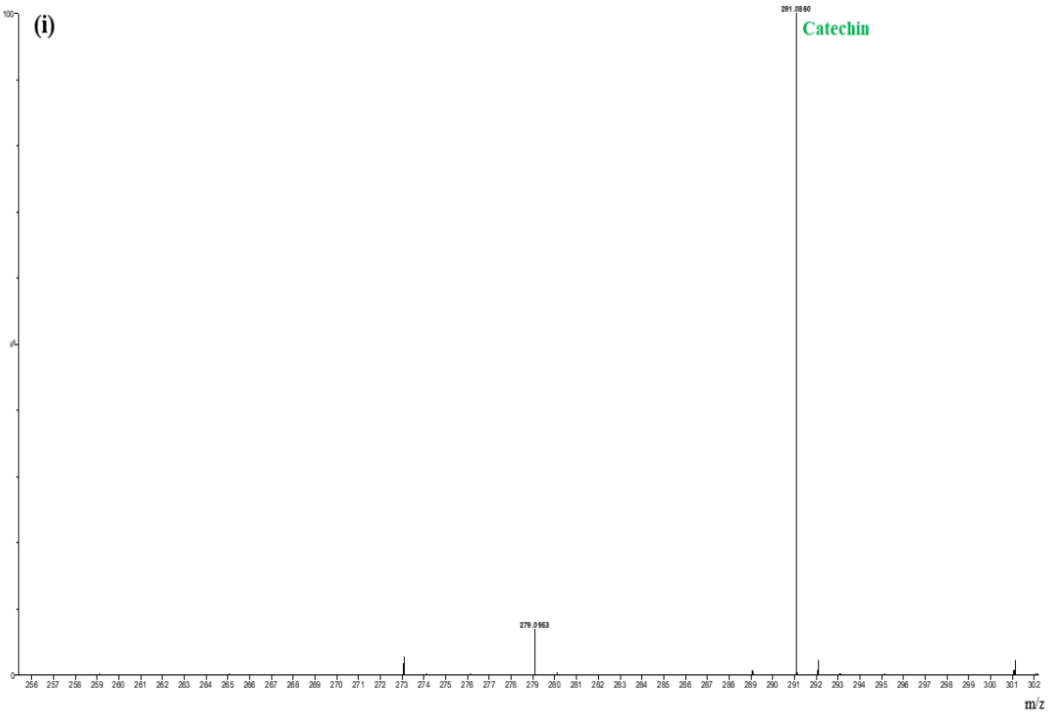
Appendix A31: UHPLC-QTOF-MS chromatograms of targeted metabolite – Gallic acid



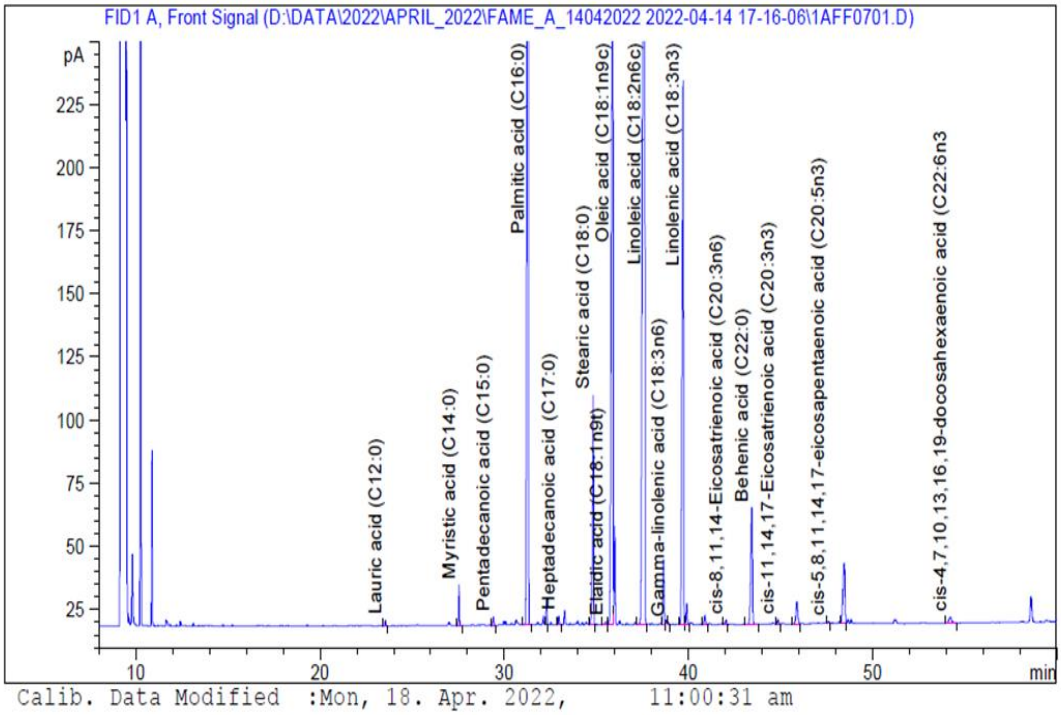
Appendix A32: UHPLC-QTOF-MS chromatograms of targeted metabolite – Para coumaric acid



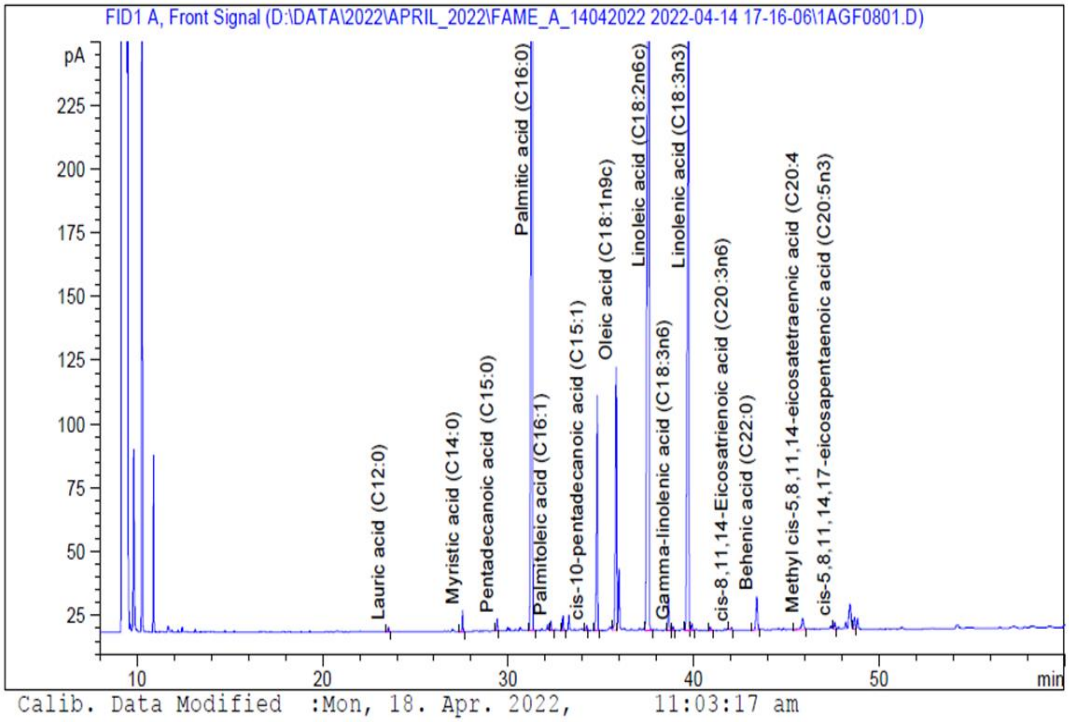
Appendix A33: UHPLC-QTOF-MS chromatograms of targeted metabolite – Gallocatechin



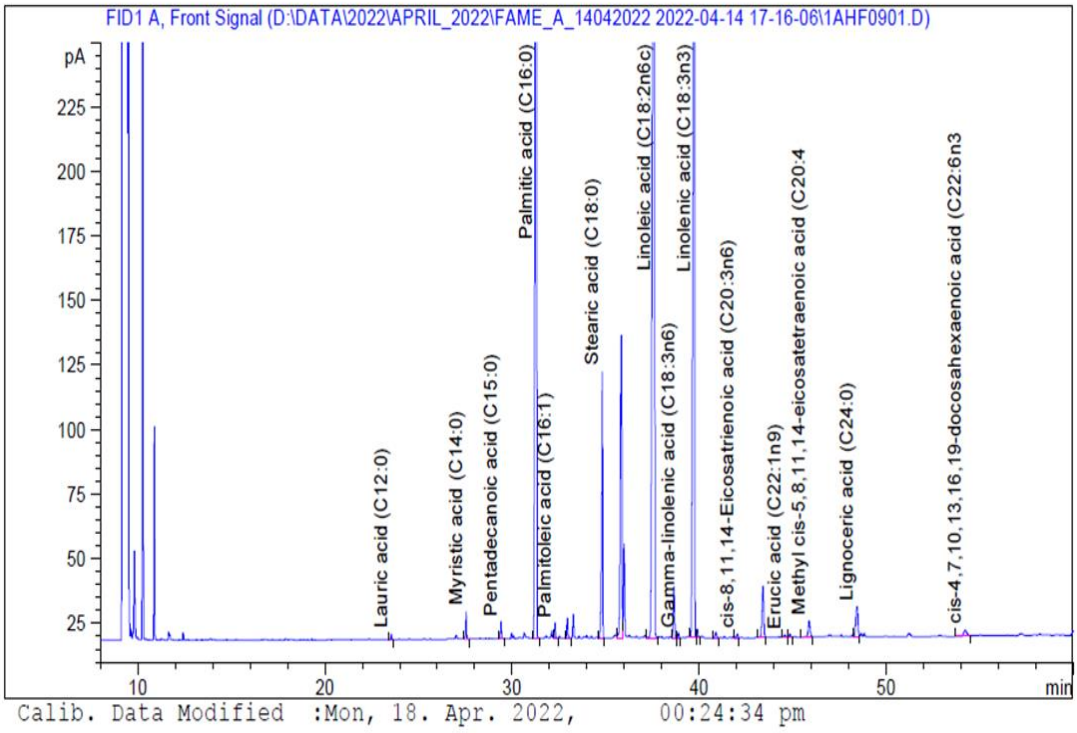
Appendix A34: UHPLC-QTOF-MS chromatograms of targeted metabolite - Catechin



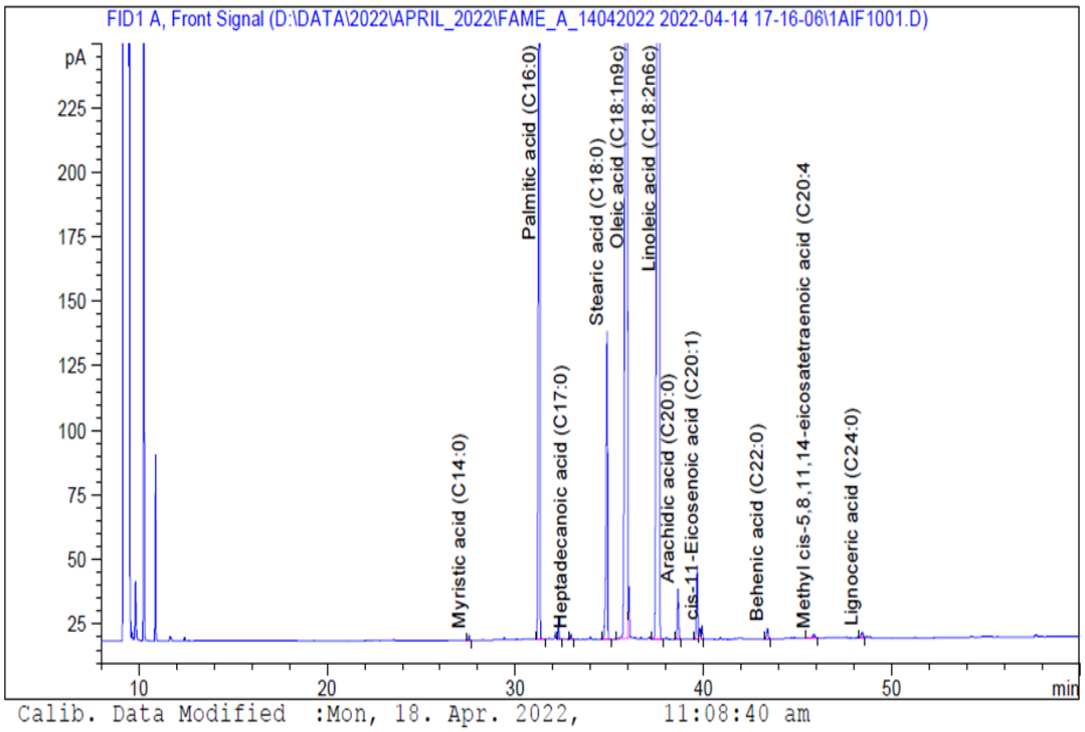
Appendix A35: FAME analysis GC-MS chromatogram of horse gram



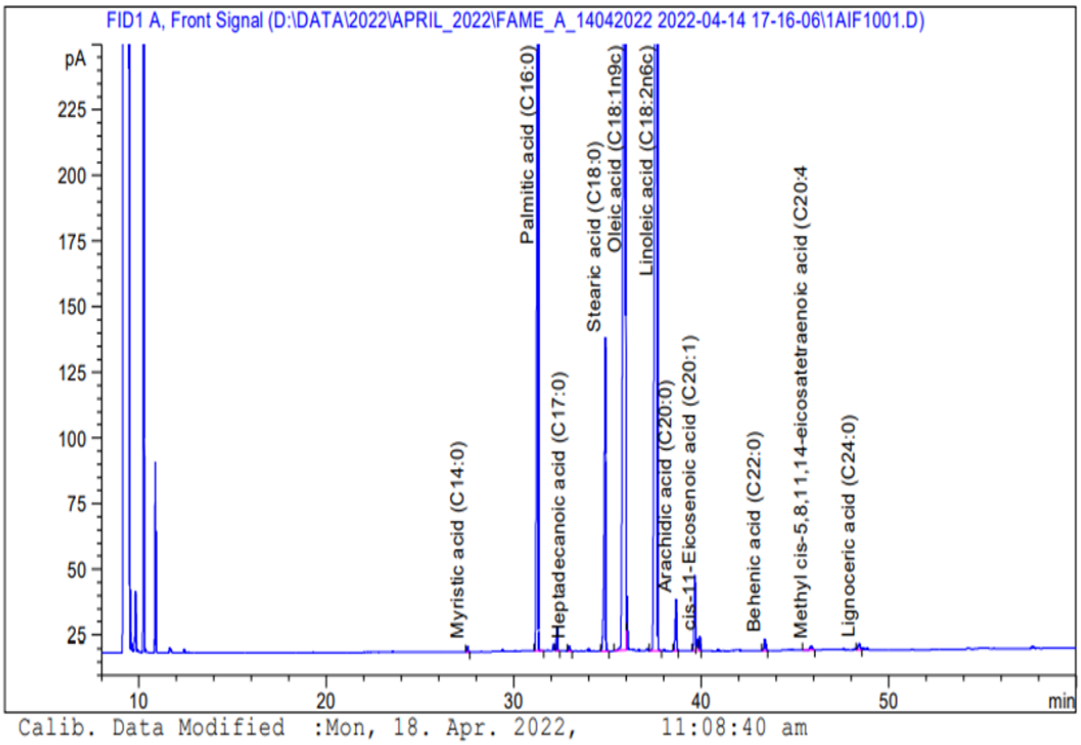
Appendix A36: FAME analysis GC-MS chromatogram of adzuki beans



Appendix A37: FAME analysis GC-MS chromatogram of rice beans



Appendix A38: FAME analysis GC-MS chromatogram of little millet



Appendix A39: FAME analysis GC-MS chromatogram of mung beans

LIST OF THESIS PUBLICATIONS

1. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2024) Bioactive profiling of two varieties of Indian legumes: Adzuki and mung beans, *International Journal of Food Science and Technology*, *59*(9), 6218-6230. doi:10.1111/ijfs.17358.

2. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2024) Phytochemical profiling of little millet (*Panicum sumatrense* Roth.), *Indian Journal of Natural Products and Resources*, *15*(4), 555-564. https://doi.org/10.56042/ijnpr.v15i4.9518.

3. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2025) Profiling of *Naurangi* and Kulthi dal used in traditional Indian cuisines and medicines, *Indian Journal of Traditional Knowledge*, *24*(7), 698-707. 10.56042/ijtk.v24i7.12511.

4. **Ritu Sharma**, Rajinder K. Gupta & Archna Rani (2023) Hydrogels based on mucilage of underutilized cereals: Synthesis and characterization, *Indian Journal of Chemical Technology*, *30*(4), 524-533. https://doi.org/10.56042/ijct.v30i4.70238%20.

5. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2025) Novel cereals and cookies to combat obesity using adzuki and mung beans, *ACS- Nutrition Science.* (Under review).

6. **Ritu Sharma**, Devendra Kumar & Rajinder K. Gupta (2025) Underutilized food crops of India as potential nutraceuticals: A review on nutritional profile, health benefits, and their applications in food, pharmaceutical, and cosmetics sectors. (To be submitted).

International Journal of Food Science and Technology 2024

Original article

Bioactive profiling of two varieties of Indian legumes: adzuki and mung beans

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(Received 5 March 2024; Accepted in revised form 4 July 2024)

Summary Nutritional benefits of legumes make them an important part of sustainable diets. They are rich not only in dietary fibres, proteins, and micronutrients but also in the bioactive compounds that can exhibit various biological activities. In the present study, we aimed to investigate the nutritional analysis, phytochemical composition, and *in vitro* anti-obesity activity of two varieties of legumes indigenous to India namely, adzuki beans (*Vigna angularis*) and mung beans (*Vigna radiata*). GC–MS, UHPLC-QTOF-MS, ICP-MS analyses were done for bioactive compounds, fatty acids, macro-elements, micro-elements, protein, and non-proteinogenic amino acids. GC-MS analysis showed that the major constituents identified were hydrocarbons, fatty acids, and terpenes, while the presence of various secondary metabolites, macro- and micro-nutrients were detected using UHPLC-QTOF-MS and ICP-MS, respectively. FAME analysis revealed a higher concentration of linoleic acid in adzuki beans, which is nutritionally beneficial for human consumption. Amino acid profiling revealed the presence of essential, non-essential, and non-proteinogenic amino acids. Further, adzuki beans and mung beans were screened for cytotoxicity on 3T3-cell line and it was observed that adzuki beans were less cytotoxic as compared to mung beans. Adzuki beans showed higher HMG-Co-A reductase enzyme inhibition activity (60%) as compared to mung beans (2.79%). The results indicate that these two legumes are a very good source of bioactive metabolites and can be developed into novel cholesterol-lowering and anti-obesity functional foods.

Keywords Adzuki bean, amino acids (protein and non-protein), bioactive compounds, HMG-Co-A reductase, mung bean, obesity.

Introduction

Obesity is a metabolic disorder caused by an abnormal build-up of body fat. It can cause diabetes, cardiovascular disease, metabolic syndrome, and other comorbidities, affecting global health (Nagao & Yanagita, 2008). When an adult's body mass index (BMI) is greater than 30 kg m^{-2}, they are classified as obese or overweight. According to the reports, obesity will affect approximately 20% of the adult population by 2030 (Smith & Smith, 2016). Obesity aetiology is highly intricate and it includes economic, social, genetic, and environmental factors that interact to avoid varied degrees to induce obesity development. Until now, researchers have actively explored effective techniques to treat obesity. The anti-obesity drugs like Setmelanotide, Metreleptin, Orlistat, Phentermine/Topiramate, Naltrexone/Bupropion, Liraglutide, and Semaglutide control body weight by reducing energy absorption, resulting in weight loss (Müller *et al.*, 2022; Chakhtoura *et al.*, 2023). According to WHO, some of these anti-obesity drugs are banned due to adverse negative side effects like diarrhoea, abdominal pain, flatulence, bloating, and a decrease in the absorption of fat-soluble vitamins. Therefore, it is necessary to develop novel anti-obesity drugs with minimal side effects in a lucrative manner that would benefit from natural sources.

In the present work, we studied two varieties of beans/legumes of India, i.e., adzuki beans (*Vigna angularis*) and mung beans (*Vigna radiata*). Adzuki beans (Family: Fabaceae), an underutilised legume, are grown in over 30 countries, and in India, the cultivation is limited to areas like the north-eastern and northern hill zones. It is widely consumed in China and Japan as a dessert (sekihan) (Hirota & Takahama, 2017). In recent years, adzuki beans have received a lot of attention due to their high nutritional content and distinctive phytochemicals, which can aid in treating various metabolic disorders like diabetes, obesity, fatty liver diseases, and hyperlipidaemia. According to Lee *et al.* (2019), the Chinese variant of adzuki bean water extract can aid in

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doi:10.1111/ijfs.17358

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Indian Journal of Natural Products and Resources
Vol. 15(4), December 2024, pp. 555-564
DOI: 10.56042/ijnpr.v15i4.9518

Phytochemical profiling of little millet (*Panicum sumatrense* Roth.)

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Received 12 March 2024; revised received 22 August 2024; accepted 27 August 2024

The little millet (*Panicum sumatrense* Roth.) has great potential to develop as functional food and source of nutraceuticals to prevent metabolic disorders. The aim of the current study aim is to identify the bioactive compounds present in little millet (*Panicum sumatrense*). The bioactives were analysed using GC-MS and UHPLC-QTOF-MS. GC-MS analysis majorly showed the presence of hydrocarbons, fatty acids, and terpenes, while UHPLC-QTOF-MS analysis showed 22 secondary metabolites, including quercetin, palmitic acid, β-stigmasterol, luteolin, and kaempferol. ICP-MS detected 21 macro- and micro-elements, with potassium (K), magnesium (Mg), and molybdenum (Mo) as the major elements. FAME analysis revealed the presence of linoleic acid (42%), oleic acid (34.1%), and palmitic acid (15.7%)as the major fatty acids. Amino acid profiling indicated the presence of essential, non-essential, and non-proteinogenic amino acids. These findings suggest that *P. Sumatrense* could be a valuable natural source of bioactive metabolites and can be utilised to develop value-added functional foods.

Keywords: Amino acids, GC-MS, ICP-MS, Little millet, *Panicum sumatrense* Roth., Phytochemicals, UHPLC-QTOF-MS

IPC code; Int. cl. (2021.01)– A61K 36/00, A61P 31/00, A61P 39/00

Introduction

Malnutrition and food security are major public health issues in India, affecting millions of people of all ages and socio-economic backgrounds. In light of the growing population, it becomes increasingly important to address these issues to maintain the food balance among everyone. India, being the highest producer of millets, holds great potential for addressing both malnutrition and food security issues through the use of millets[1].

Millets are small-seeded edible grass of the Poaceae family that can be found growing in marginal dry lands in tropical and subtropical regions of the world[2]. These millets help increase the genetic diversity of the food basket and food and nutritional security. Millets, like foxtail millet, pearl millet, sorghum, and finger millet, are a great option for those with celiac disease and diabetes because of their high nutritional content and low glycemic index[3]. Millets have been a staple food in traditional Indian diets for centuries, especially in rural areas. However, millet consumption has fallen drastically due to modernisation and the promotion of rice and wheat as the main grains.

Panicum sumatrense, commonly known as little millet or kutki, is an underutilised minor millet widely grown in countries like India, Africa, and China. It is a yearly crop that has resistant starch, phytates, phenolics, sterols, lignans, and gamma-aminobutyric acid as prominent phytochemicals[4]. It is regarded as a "cool food" because of its cooling effect on the body when consumed in summer[5]. The nutritional potential of little millet is entrenched with a good proportion of vitamins, minerals, and bioactive compounds. According to some previous studies, its high fibre content contributes to reducing fat deposits in the human body[6].

Severe chronic diseases in humans, such as cardiovascular disease, diabetes, cancer, cognitive dysfunction, and a variety of other normal activities, have been related to the oxidation of cellular molecules by reactive dietary antioxidants to protect against oxidative damage and maintain a healthy metabolic balance. Recently, plant bioactive compounds have gained noticeable attention from researchers for their numerous health benefits in reducing the risk of cancer, neurodegenerative, and cardiovascular diseases.

In view of the above, it is necessary to evaluate the phytochemicals and their bioactivity in little millet to utilise this millet as a food ingredient for the

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Indian Journal of Traditional Knowledge
Vol 24(7), July 2025, pp 698-707
DOI: 10.56042/ijtk.v24i7.12511

Profiling of *Naurangi* and *Kulthi Dal* used in traditional Indian system of cuisines and medicine

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Received 11 September 2024; revised 05 May 2025; accepted 25 June 2025

The development of fortified foods and nutraceuticals based on legumes used in our traditional Indian system of cuisines & medicine has gained much appeal in recent times due to their exemplary biological activities. Specifically, the use of traditional and underutilized legumes holds much scope for exploration. This study demonstrates the biological profiling and phytochemical screening of *Naurangi dal* (rice beans) and *Kulthi dal* (horse gram/*Kulthi* beans) extracts. The bioactive compounds were identified using UHPLC-QTOF-MS and GC-MS. Fatty acid profiling, proximate, amino acid, and elemental analyses were carried out to evaluate the nutritional profile of the legumes. Using GC-MS, it was found that the legumes had high concentrations of terpenes, hydrocarbons, and fatty acids. Various secondary metabolites (quercetin, catechin-7-O-glucoside, epicatechin, and catechin) were found using UHPLC-QTOF-MS. The legumes demonstrated rich concentrations of essential, non-essential, and non-proteinogenic amino acids, as well as linoleic, oleic, and palmitic acid. Elemental analysis showed the presence of 21 elements with magnesium, potassium, and molybdenum being the most prevalent. In biological profiling, anti-microbial and anti-oxidant activities were performed on the selected legume extracts. The anti-oxidant activity of *Kulthi beans* extracts was greater than *rice beans* extracts. Additionally, methanolic extract of the legumes also showed promising anti-microbial activity. These results suggest that these underutilized legumes could be an excellent source of bioactive compounds, anti-microbial and anti-oxidantagents that could be utilized in food, pharmaceutical and cosmeticsindustries.

Keywords: Amino acids, Anti-microbial agent, ICP-MS, Phytochemicals, Secondary metabolites, UHPLC-QTOF-MS

IPC Code: Int Cl.[25]: A23L 11/00, A01G 22/40

India is well-known for its agricultural diversity, with a vast array of legume species that have been grown for centuries. Legumes, classified under Fabaceae family, are considered as inexpensive and valuable sources of protein, and are ranked second most important food crop following cereals. Leguminous crops are not only rich in proteins, carbohydrates, minerals, vitamins, amino acids, and dietary fibre content, but they are also gluten-free and possess low fat and glycemic index.

Naurangi dal or rice beans (Family: Fabaceae), scientifically known as *Vigna umbellata,* are nutritionally rich grain legume which is cultivated primarily in hilly areas and utilized by traditional Indian system of medicine[1]. Owing to its immense nutritional benefits, it has been a staple food source in various cultures in form of stews, soups and curries[2]. This legume stands out for its dietary supremacy than other common traditional legumes in the *Vigna* family. Still, its potential to improve the well-being of humans is yet to be completely tapped. The beans are believed to have various health benefits, including aiding digestion and acting as a diuretic. It is a versatile crop traditionally utilized for its nutritional, agricultural, and cultural benefits across different regions. Besides, different parts of the rice bean plant are used in Chinese medicinal systems due to their nutraceutical potential[2]. According to recent research on rice bean, its protein hydrolysates may prevent breast and cervical cancer, while nutritional analysis of Himalayan accessions suggested it might improve food security[3,4]. While some Asian areas have a long history of its traditional use, it has yet to be adopted worldwide.

Similarly, *Kulthi* beans or horse gram (Fabaceae family), scientifically recognized as *Macrotyloma uniflorum,* are primarily grown in India, Africa, Australia, Malaysia, and Mauritius. It contains a substantial amount of proteins, amino acids, minerals, and vitamins. It also contains a plethora of natural

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Indian Journal of Chemical Technology
Vol. 30, July 2023, pp. 524-533
DOI: 10.56042/ijct.v30i4.70238

Hydrogels based on mucilage of underutilized cereals: Synthesis and characterization

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Received 9 January 2023; accepted 6 April 2023

Mucilage is a natural polysaccharide with a variety of physicochemical and structural properties. Plant-derived mucilage has a wide range of applications, such as binding agent, stabilizer, emulsifying agent, thickening agent, and gelling agent. This study investigated the potential of underutilized cereals' mucilage and further explored their application by synthesizing mucilage-based hydrogels. For this purpose, we have explored four new sources of mucilage, namely adzuki beans (A_b), amaranth (A_m), proso millet (P_r), and little millet (L_m). The underutilized cereals' mucilage application has been examined by developing hydrogels through the free radical co-polymerization technique. Mucilages are confirmed to be a natural thickening and a substitute for synthetic polymers after being evaluated physically and phytochemically. Structural analysis of mucilages and their hydrogels (A_bH, A_mH, P_rH & L_mH) were characterized by using FTIR-ATR, XRD, ^1H & ^{13}C NMR techniques. It confirms that all four mucilages are rich in polysaccharide residues and grafting of sodium acrylate has been successfully done on mucilages. Thermal gravimetric analyses represent the better thermal stability of the synthesized hydrogels than their respective mucilages. SEM confirms the porous structure of the mucilages and their hydrogels. All of these studies demonstrated that the underutilized mucilage from cereals might be a good feedstock for a hydrogel-forming agent, which can be explored in the food, cosmetics, and pharmaceutical industries.

Keywords: Co-polymerization, Hydrogel, Mucilage, Natural polysaccharide, Structural characterization

In recent years, there is a lot of interest in plant-derived mucilage owing to their non-toxicity, eco-friendliness, cost-effectiveness, and biodegradable nature. To meet the growing need, new sources are being investigated on a regular basis. Chemically, mucilage is a natural polysaccharide composed of highly branched structures of carbohydrates such as L-arabinose, D-xylose, and D-galactose monomer units[1]. They can be obtained from different parts of the plant like seeds, leaves, roots, and stems. The process of producing mucilage from the plant part is known as Myxospermy. Mucilage is partially soluble when it comes in contact with water[2]. These polysaccharides are composed of ten or more monosaccharide units. Mucilages obtained from different sources exhibit varied functional properties due to differences in the monosaccharide units, type of glycosidic bond, and conformation of the chains[3]. Due to their hydrophilic nature, these polysaccharides swell in water and form a gel-like solution, which has excellent and diverse use as a binding agent, stabilizing agent, emulsifying agent, thickening agent, etc.

Mucilage isolated from different plant materials has been extensively used in pharmaceutical and food industries. Recently, the isolation and characterization of new sources of plant-derived mucilage (chia mucilage, okra seed mucilage, marshmallow mucilage, and Chinese yam mucilage) and their applications have been investigated. Herein, we report four newer sources of mucilage, these are underutilized cereals of India i.e., Adzuki beans (A_b), Amaranth (A_m), Proso millet (P_r), and Little millet (L_m).

Adzuki bean (*Vigna angularis*) is a legume belonging to Fabaceae (Leguminosae) family. It is widely cultivated in countries like China, Japan, and Korea. It is widely utilized as an ingredient in desserts[4]. Amaranth (*Amaranthus*) belongs to the family Amaranthaceae. It is commonly known as Ramdana or Rajgira in India and is a nutritious pseudo-cereal. It is widely cultivated in different countries as a cereal, vegetable, weed, or crop. Proso millet (*Panicum miliaceum*) belongs to the Poaceae family. It is cultivated in India, China, Nepal, Africa, Turkey, Romania, and Russia. It is gluten-free and rich in proteins, vitamins, and minerals[5]. Little millet (*Panicum sumatrense*) belongs to the family Poaceae. It is widely cultivated across India, China, and Africa.

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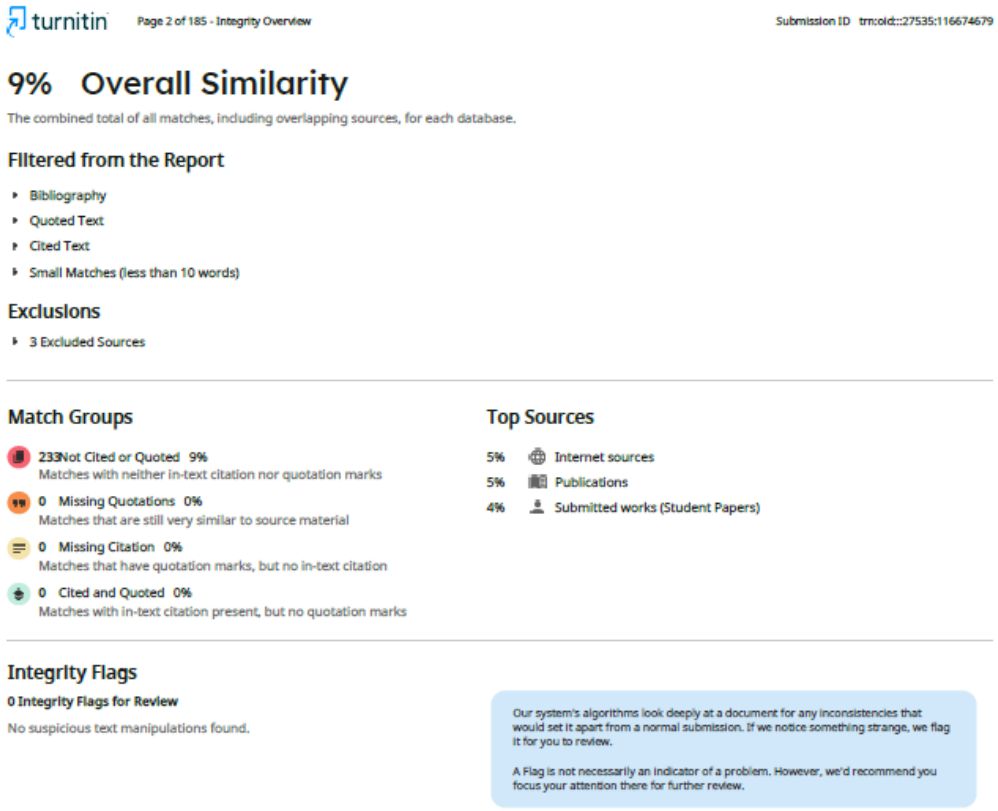

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