UTILIZATION OF *MORINGA OLEIFERA* FLOWER EXTRACT FOR ITS THERAPEUTIC PROPERTIES IN THE DEVELOPMENT OF STIRRED YOGURT

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

In

Food Science and Technology

By

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Supervised By

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DECLARATION

We hereby declare that the work done and presented in the Project entitled **"Utilization of *Moringa oleifera* flower extract for its therapeutic properties in the development of stirred yogurt"** is our own and original. The work has been carried out by us at the School of Agriculture, Lovely Professional University, Phagwara, and Punjab, India under the guidance of Dr. Mukul Kumar, Assistant Professor (Food Technology and Nutrition) of the School of Agriculture, Lovely Professional University, for the award of the degree Ph.D. Food Science and Technology.

Date: 03/02/2024

Poonam Jaglan (11919129)

Place: Phagwara, Punjab (India)

I certify that the above statement made by the student is correct to the best of my knowledge and belief.

Date: 03/02/2024 (**Supervisor- Dr. Mukul Kumar)**

Place: Phagwara, Punjab (India) Food Technology and Nutrition

Signature of External Examiner

CERTIFICATE

This is to certify that work embodied in the Ph. D. report entitled **"Utilization of *Moringa oleifera* flower extract for its therapeutic properties in the development of stirred yogurt"** has been carried out by **Poonam Jaglan** under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigations and study. No part of the project has ever been submitted for any other degree. The work has been carried out by her at the School of Agriculture, Lovely Professional University, Phagwara, and Punjab, India. She fulfilled the requirement for the award of the degree Ph.D. Food Science and Technology.

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Place: Jalandhar

Date: 03/02/2024 **Poonam Jaglan**

Abbreviations

ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
ATCC	American-type culture collection
ALT	Alanine transaminase
AST	Aspartate aminotransferase
CFU	Colony-forming unit
DES	Deep eutectic solvents
DMSO	Dimethyl sulfoxide
DPPH	2, 2-diphenyl-1-picrylhydrazyl
E. coli	*Escherichia coli*
EDS	Energy-dispersive X-ray spectroscopy
FESEM	Field emission electron scanning microscopy
FRSA	Free radical scavenging activity
FTIR	Fourier transform infrared Spectroscopy
GIS	Green Investigative Science
GAE	Gallic acid equivalent
GC-MS	Gas Chromatography-Mass Spectrometry
GRAS	Generally recognized as safe
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HPLC	High-Performance Liquid Chromatography
Ic_{50}	Inhibitory concentration
IL	Ionic liquids
MIC	Minimum inhibitory concentration

MOFE	*Moringa oleifera* flower extract
MRS	de Man, Rogosa and Sharpe agar
NA	Nutrient agar
pH	Potential of hydrogen
QE	Quercetin equivalent
RSM	Response surface methodology
S. aureus	*Staphylococcus aureus*
SPSS	Statistical package for the social science
TFC	Total flavonoid content
Tg	Glass transition temperature
TGA	Thermogravimetric analysis
TPC	Total phenolic content
XRD	X-ray diffraction analysis

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ABSTRACT

Phytomedicine is gaining researchers' interest over conventional drugs. *Moringa Oleifera* is a medicinal plant used for centuries for various medicinal purposes. *Moringa oleifera* is a plant known as the miracle tree, ben oil tree, and drumstick tree which belongs to the family Moringaceae and the Latin name of *Moringa oleifera* is *Moringa oleifera* Lam. The plant has been used for centuries for medical purposes as it has various health benefits and medicinal properties. It is abundant in macro and micronutrients as well as other bioactive substances that are crucial for the body's regular functioning and the prevention of specific disorders. The leaves, seeds, flowers, and nearly all other parts of the plant are edible and have a wide range of medicinal benefits. *Moringa oleifera* is a plant found in northern India and other tropical places such as Africa and Asia. Traditional medicine has used plant leaves, seeds, flowers, and roots to improve health and Moringa provides the remedy for diabetes, bacterial, viral, and fungal infections, inflammation, heart, cancer, and joint pain. Almost all the parts of the Moringa plant can be used for various purposes. Many researchers have argued the health benefits of different parts of *Moringa oleifera* for therapeutic purposes. This research is based on the utilization of *Moringa oleifera* flower extract for its therapeutic properties in the development of stirred yogurt. Characterization of the physiochemical and techno-functional properties of the *Moringa oleifera* flower powder was done and various techniques were used in this regard such as HPLC, GC-MS, TGA, FESEM, and EDS. Nutrient agar media was used for checking the antibacterial activity of the flower extract and Streptomycin was used as an antibiotic to check the activity of *Escherichia coli* and *Streptococcus aureus*. The water absorption capacity of *Moringa oleifera* flower powder increased from 10.60 to 11.40 and the oil absorption capacity increased from 6.10 to 7.80 whereas bulk density was found to be 0.44 to 0.38, with true density 0.30 to 0.23, foaming capacity 15.13 to 15.20, and foaming stability was found to be 56.23 to 56.34 for the *Moringa oleifera* flower powder. The phenolic content of *Moringa oleifera* flower extract for ethanolic and methanolic content is 15.97 and 13.82 mg GAE/g whereas flavonoid content was found to be 12.38 and 10.95 mg QE/gm. The antioxidant activity of the flower extracts was found to be 59.65 and 54.97%. The flower extract showed 11 mm and 8 mm of the zone of inhibition against *E. coli* and *S. aureus* respectively whereas in terms of antibiotic, streptomycin was used, and the zones of inhibition were found to be 20 mm and 18 mm respectively. Based on the finding of the research *Moringa oleifera* flower powder and extract can be used as food fortification, antioxidant, and antibacterial agents. In this study, results revealed the presence of various

phytochemicals: alkaloids, phenolic and flavonoid compounds, tannins, saponins, and terpenoids. HPLC analysis revealed the presence of various phytochemicals such as Gallic acid, Quercetin, and Rutin. A study was done to analyse the phytochemicals and to assess the antibacterial effects of *Moringa oleifera* flower extract (MOFE) against two common microorganisms: *E. coli* ATCC25922 and *S. aureus* ATCC33591. *Moringa oleifera* flower extract exhibited antibacterial effects against the two tested bacteria *E. coli* and *S. aureus* with the diameter of the zone of inhibition 11 and 9 mm, respectively. Five different concentrations (1000, 500, 250, 125, 62.5mg/ml) of *Moringa oleifera* flower extract were prepared in 5% DMSO (Dimethyl sulfoxide) and tested for the antibacterial effect by using the agar disc diffusion method. Minimum inhibitory concentrations were found to be 250 mg/ml. *Moringa oleifera* has high antibacterial action against bacterial pathogens that are pathogenic and virulent, and it may be a source of yet another efficient and practical antibacterial antibiotic. Deep Eutectic Solvents (DESs) based solvents have a vast majority of benefits, such as they are non-toxic, green solvents, with a highly efficient method of extraction of phenolic, antioxidant, and flavonoid compounds. DES solvents are made by combining two solvents, out of which one must be a hydrogen bond donor (HBD) and another must be a hydrogen bond acceptor (HBA) whereas, combining these two types reduces the melting point of the solvents. Six different types of DESs combinations were used in different ratios, for the optimization of the extraction process. The six combinations used were ChCl-Glycerol, ChCl-MA, ChCl-LA, ChCl-Glucose, L-Pro-Glycerol, and L-Pro-LA. Total phenolic content, flavonoid content, antioxidant activity, lipase activity, amylase activity, and glucose uptake assay of *Moringa oleifera* flower extracts were calculated *in vitro* at different temperatures ranging from 50-80 ℃. IBM SPSS statistics 23 software, Post Hoc Tests (Duncan) is used to optimize the extraction yield of the *Moringa oleifera* flower extract, and the homogeneous subsets are used to check the level of significance of the solvents, solvents ratio, and the temperature ranges and their effects. Maximum yield of the extract and the phytochemicals were found at 70℃ with the DES combination L-Pro-Glycerol in a 1:2 molar ratio where phenolic content was 32.40±0.02, flavonoid content 44.11±0.002, and antioxidant activity was found to be 74.83±0.002. Lipase inhibition activity was monitored to be 67.10% with an Ic 50 value of 62 and amylase inhibition activity was found to be 35.1% and the Ic50 value for the same is 46.72. *Moringa oleifera* is known for its high nutritive value as every part of this tree is a potential source of vitamins, minerals, and amino acids whereas yogurt is known for its potential health benefits because of its probiotics effects in maintaining good gut microbiota. The present study can be helpful to the dairy industry in

developing new probiotic products and may provide a rationale for selecting a combination of probiotic strains. *Lactobacillus bulgaricus* and *Streptococcus thermophillus* ferment the lactose in milk to produce yogurt, a coagulated milk product. Raw milk must have a low bacterial count, and be devoid of antibiotics, sanitizing agents, colostrum, and contamination by bacteriophages to produce a product of high quality. In comparison to milk, nutrients in yogurt can be absorbed into the body more readily since the fermentation process makes it easier to digest. The study's goal was to look into the physical and chemical makeup of fresh yogurt along with its stability and shelf life. This research was carried out in the Department of Food Technology and Nutrition in the food science laboratory of Lovely Professional University, Phagwara, and Punjab, India. Statistical design RSM was used for the optimization of yogurt where two independent variables incubation time, and *Moringa oleifera* flower extract at various concentrations (1-5%) were taken, and various responses such as titratable acidity, pH, protein, antioxidant activity, total phenolic content, and total flavonoid content were determined. Results obtained from RSM software showed that yogurt enriched with 5% flower extract has the maximum number of polyphenols and protein content, whereas the optimized time for the yogurt was found to be 5 hrs. That means *Moringa oleifera* flower extract is responsible for accelerating the growth of the culture bacteria and minimizing the incubation time for the preparation of the yogurt. According to sensory analysis, there was no discernible difference between flower extract-enriched yogurt and control in terms of texture, and appearance, but in terms of flavor or general acceptance flower extract-enriched yogurt was better than that of the control yogurt. The results of this study proved that *Moringa oleifera* flower extract-enriched yogurt was found to be more nutritious and self-stable when compared with the control yogurt. Various parameters such as titratable acidity, pH, viscosity, and syneresis were considered during the storage study of the yogurt and the results showed that flower extract-enriched yogurt is more stable than the control yogurt. Cost analysis of the product showed a remarkable difference in the cost of the products available in the market with this product.

Keywords: *Moringa oleifera*, traditional medicine, techno-functional, characterization, phenols, flavonoids, antioxidants, antimicrobial, anti-inflammatory, antidepressant, deep eutectic solvents, hydrogen bond donor, hydrogen bond acceptor, lipase activity, amylase activity, glucose assay, bioavailable, coagulation, probiotics, titratable acidity

Chapter-1

INTRODUCTION

Plethora numbers of herbal plants had been in use for the treatment of life-threatening ailments from ancient times. In the present times, herbal/medicinal plants are still receiving substantial attention as indicated in a report by annual growth of the flavoring plants that is based mostly on trade in the developing countries which are growing at the rate of 7 to15 percent annually (Sandeep et al., 2019). Within developing countries, a massive section of the population (about 80%) uses herbal medicines for natural action. The explanation behind their widely unfold use is that their effectiveness, safe to consume, reliability, non-toxic nature, ease of access, and cheap as in comparison to allopathy. According to Khan and Ahmad (2019), herbal medicine is a combination of indigenous and several other therapeutic experiences since generations which is plant-based and usually helps in the treatment of various diseases. Developing countries like India, China, and Egypt are still using herbal remedies for the treatment of ailments and even a WHO report says 80% of the population in developing countries totally depends on plant-based remedies for the treatment of various life-threatening disorders (Abuhajar et al., 2023).

MORINGA OLEIFERA

Moringa (*Moringa oleifera)* is one out of thousands medicinal Indian herb with great health benefits. The other terms used for the Moringa tree are Drumstick tree, Marango, Horseradish tree, Sajna, and Benzolive. *Moringa oleifera* belongs to the family Moringaceae, and the Genus Moringa. It was first reported in the sub-Himalayan sections of North-West India. Besides being used as a vegetable for the local population, it is also known for its wide range of health benefits. It is also known as a miracle tree among the general population due to its amazing healing properties for multiple kinds of ailments (Mahmood et al., 2010). Moringa production requires growing trees under suitable agro-climatic conditions. Moringa is well adapted to different soil types and can therefore grow in a variety of environments, including arid and semi-arid regions (Devkota & Bhusal, 2020). It is a plant that can withstand harsh conditions, making it a hardy and easy-care crop. *M. oleifera* is known for its high productivity and rapid growth (Daba, 2016). This tree begins to leaf out just a few months after being planted and continues to produce yields throughout the year. The leaves are rich in essential nutrients such as vitamins, minerals, and protein, making them a valuable source of

nutrients. Moringa is also known for its fast growth, which allows farmers to harvest it several times a year to provide a steady supply of a variety of products. Production of Moringa involves few key factors that contribute to the successful cultivation and harvesting of this versatile plant (Horn et al., 2022).

Agricultural requirements

Site selection: A well-drained site with appropriate sunlight is considered best to produce Moringa. Areas prone to frost should be avoided as Moringa is a cold sensitive plant and grows well in temperatures ranging between 25-35°C.

Climate: *M. oleifera* has relatively low agricultural requirements, making it suitable for cultivation in a wide range of areas. Moringa can grow in both tropical and subtropical climates. It grows best in areas with temperatures between 25 and 35 degrees (Rai et al., 2023).

Soil: Moringa is adaptable to a variety of soil types but prefers well-drained soil. Waterlogged conditions can lead to root rot and other diseases. It can grow in poor and degraded soils, making it a potential crop for land reclamation and agroforestry projects (Dhakad et al., 2023). Moringa is drought tolerant but benefits from regular watering, especially during the early stages of growth. However, it survives better in times of water scarcity than many other crops (Wong et al., 2024). Moringa is somewhat tolerant of saline soils, but excessive salinity can adversely affect its growth. Proper irrigation management and drainage can help mitigate salinity issues. Incorporating organic matter into the soil not only improves fertility but also helps in moisture retention. This is especially beneficial in arid and semi-arid regions. Moringa can tolerate a wide range of soil pH, from slightly acidic to slightly alkaline. The ideal pH range is around 6.3 to 7.0. Soil testing can help determine the pH of the soil and whether any amendments are needed (Dhakad et al., 2023).

Sunlight: Moringa requires plenty of sunlight to grow optimally. It is important to plant it in a location that receives plenty of sunlight throughout the day. Adaptability to different climates and soils, and versatility make it an attractive crop for sustainable agriculture and food security efforts (Kumar et al., 2024).

Pruning: Regular pruning helps promote bushier growth and enhances leaf production. Pruning can be done to maintain the desired shape and size of the tree along with enhancement of overall productivity. Pruning can be done once the plant has reached about 1 to 1.5 metres or 3-5 feet in height. Pruning encourages the growth of lateral branching and helps creating a bushier plant. Removing dead ends is one of the important steps in pruning

that helps in the growth of new branches. During pruning it needs to be made sure that avoid removing leaves from a single branch to maintain the plants growth (Rai et al., 2023). Pruning should be done during the growing season when the plant is actively producing new branches, this is typically done in spring or early summer depending on the local climate.

Pest and disease control: Moringa is generally resistant to pests and diseases, making it relatively low maintenance crop. However, occasional monitoring and appropriate measures may be required to manage any issues that arise.

Herbal plants are still reliable in phytomedicine and are widely used as one of the cost-effective and alternate way of the medicinal system due to its affordable price (Abuhajar et al., 2023). All around the world *Moringa oleifera* is in use for treating multiple complications such as anxiety, chest congestion, skin infections, blackheads, urine problems, anaemia, blood impurities, and many more (Mahmood et al., 2010). It is also used as a diuretic, anti-ulcerative, anti-diabetic, hepatoprotective and anti-pyretic (Huang et al., 2012). These days Moringa has been used in cosmetic products due to its amazing range of benefits such as miniaturisation of skin and conditioning of hairs. Moringa oil is also used as an ointment for the skin since Egyptian times (Mahmood et al., 2010).

TRADITIONAL USES OF *MORINGA OLEIFERA*

Moringa was traditionally used extensively for its health benefits by kings and queens to improve attentiveness and to keep skin healthy Indian warriors were given *Moringa oleifera* leaves as energy booster and pain and stress relief during the war (Mahmood et al., 2010). Other traditional uses of this genus include recovery from skin infection, healing of wounds, anxiety relief, asthma cure, fevers reduction, diarrhoea cure, and sore throats relief. Leaves of the plant are used as animal forage, biogas production, green manure, and biopesticide whereas seed oil is used as biofuel, cosmetic oil, and cooking oil. Wood of the tree is used for preparing blue dye, and pulp. Seeds are used as fertilizer, honey- and sugar cane juice-clarifier, water purification (Liu et al., 2022).

NUTRITIONAL CONSTITUENTS OF *MORINGA OLEIFERA*

Moringa oleifera is a great source of nutrition with number of health benefits such as vitamin A present in *Moringa oleifera* leaves is essential for the good health of the eyes and hair. For a better tissue growth protein is the basic need and in the deficiency of proteins growth can be retarted for example Kwashiorkor. *Moringa oleifera* leaves contains about 22.99%-29.36%

protein (Sultana, 2020) whereas the flowers contain 17.87%-21% protein (Madane et al., 2019). There are multiple types of minerals present in the *Moringa oleifera* like potassium, calcium, zinc, copper, and iron. Calcium is necessary for the bones and teeth health (Gopalakrishnan et al., 2016). *Moringa oleifera* flowers and leaves are potential source of fibre and according to American dietetic association; an adult must consume about 25-30g of fibre per day for a healthy gut (Madane et al., 2019). Other phytochemicals are also found in *Moringa oleifera* flowers, seeds, and leaves *viz.* tannins, amino acids, phenolic compounds, flavonoids and antioxidants are also present in (Masurekar et al., 2015).

Moringa oleifera is a plant with incredible health benefits and are claimed by number of cultures and communities on the basis of their vitamins, minerals, carotenoid content and amino acids. Moringa is also found to contain omega-3 as well as omega-6 fatty acids (Kasolo et al., 2010). Nutritional composition of this plant plays an imperative role in the nutritional, medicinal as well as therapeutic properties. Moringa leaves contains high amount of Vitamin-C, β-carotene, protein, potassium, calcium, and vitamins. It is a rich source of antioxidants. Almost all parts of the *Moringa oleifera* tree *viz*. seeds, flowers, leaves, bark and roots contain ample number of healthy nutrients. It is out of those rare plants which contain multiple numbers of high-quality compounds. It was also mentioned in an article published by "Trees for Life" organisation that "each and every single part of the Moringa is shown to have some beneficial properties which serve for the mankind" (Zaku et al., 2015).

Moringa oleifera flowers are bisexual and their fragrance is so pleasant. The flowers are about 2.5cm wide and are white and cream coloured and there are yellow dots on the base of the flowers. These flowers are really good source of nectar for the honey production. They can be eaten as salad in raw form as well as after blanching, used to prepare tea whereas fried Moringa flowers taste like mushrooms. Flower powder is supposed to be more suitable as a food additive because of negligible effect on the color and appearance of the food. These flowers are of great importance for medical purpose because they are good source of antioxidants, anti-inflammatory substances (Alhakmani et al., 2013). For many of the herbal plants it is seen that they lose their nutrients on heat treatment and after cooking, bleaching but *Moringa oleifera* flowers and leaves are out of those one which maintains its properties even after heat treatment (Abdull et al., 2014). Boiling of leaves and flowers in hot water result in three times of the digestible iron than that of the raw form and these results can also be seen in dried, and powdered form of leaves and flowers. It also contains several unique compounds such as Rhamnose and sugar, which are not at all commonly found in other plants

(Amaglo et al., 2010). These components are also seen to have cancer preventive activity including apoptosis (Brunelli et al., 2010).

DEEP EUTECTIC SOLVENTS (DESs)

These days, worldwide supportability challenges are personally interconnected being human wellbeing and ecological effect the fundamental concerns. In this unique circumstance, it is not conceivable to manage explanatory science in disconnection (Valcarcel, 2012). Remembering that scientific science strategies are utilized to take care of issues, just all-inclusive and troublesome approaches coordinating exploration, improvement, and advancement in interdisciplinary furthermore, trans disciplinary studies will make progressive advances. During the last not many a long time, logical science network has been assembled on the advancement of green practices. Concerning the present-day objectives of explanatory scientists, the incredible test is to adjust the standards of Green Investigative Science (GAC) with the standards of Green Science (Valcarcel, 2012).

Despite unchallenged advances in systematic equipment, test preparation is the bottleneck of each systematic technique. The unfriendly ecological effect of expository methods can be defeated applying the accompanying techniques: scaling down the expository scale or potentially supplanting perilous solvents by more secure options. Although the perfect circumstance is the advancement of dissolvable free extraction plans (Vian, 2017), this idea is still rather utopic. In this manner, the quest for elective solvents is of most extreme significant (Pereira, 2015). In recent decades, ionic fluids (ILs) increased incredible consideration as green media. In any case, their greenness is regularly contended, because of their poor biodegradability, biocompatibility, and manageability (Espino, 2016). Another class of solvents, which depend on the partner's eutectic behavior, was then questioned as an alternative to ILs. High eutectic solvent (DES) was developed by Abbott et al. al., 2003 demonstrate the wide range of liquids available as soluble materials and their interesting properties. The eutectic framework comes from the Greek term “eu” means simple and "teksis” means liquefaction, which melts and freezes at a single temperature lower than the intended liquefaction of various substances. Means a mixture of substances that form a common super section component, and the DES has been used for spontaneous reactions at 60 °C, spontaneous extraction, electrochemistry, and protein reactions (Dai, 2015). Later DES was coined as NADES (natural deep eutectic solvents) and this was totally based on the principles of green chemistry.

NADES are obtained on mixing of hydrogen bond donors with hydrogen bond acceptors molecule, to lower its melting point. There are some molecules such as alcohol, aldehyde, amines, and ketone groups which react as both hydrogen donor along with hydrogen acceptors. On the basis of chemical nature of the NADES are divided into four parts; a) mixture of sugars, b) derivatives of organic acids, c) derivative of choline chloride, and d) other combinations (Espino, 2016). Due to major advantages of NADES like readily available components, reusability, environment friendly (biodegradability), and cost effectively it is appealing research to go for their analytical application.

STIRRED YOGURT

Moringa oleifera belongs to the family Moringaceae which is known for its soft wood, and deciduous trees. It is one of those plants that has numerous health benefits and still is not known to the general population. It is a drought-tolerant, fast-growing medicinal plant with numerous health benefits therefore it is also known as a "miracle tree" (Madane et al., 2019). Most of the parts of *Moringa oleifera* are known for their medicinal and nutritional properties such as anti-diabetic, anti-inflammatory, anti-spasmodic, hepatoprotective, and diuretic (Divya et al., 2019). Taking nutritional values into consideration leaves contains fiber, proteins, as well as minerals such as K, S, Mg, Zn, Fe, and Ca (Rockwood et al., 2013) whereas seeds consist of fatty acids *viz.* linolenic acid, linoleic acid, and behenic acid (Sutalangka et al., 2013), roots and bark contain alkaloids like moriginine, morphine, and minerals such as Ca, Na, and K (Adeyemi et al., 2014), flowers consist of minerals (Ca, K, Na, Fe), vitamins (A, B, B12, C) and amino acids (leucine, arginine, lysine, isoleucine, valine) (Ningsih et al., 2021). The dried flower of *Moringa oleifera* is known as *Murungi poo* and is used to cure inflammation and muscle diseases. In Siddha, the flower part of the plant is used to cure cough, chronic bronchitis, chest diseases, and urticaria which is caused by the beetle sting (Patel et al., 2019). Due to the proven medicinal/nutraceutical potential of the plant parts researchers have been more concerned about the leaves and not about the flowers of the plant so flowers only received a little bit of attraction from the researchers despite the significant traditional healing and nutritional properties. Thus, due to the extremely high nutritional value of the *Moringa oleifera* flower, it could be used for the enrichment of various food products such as bakery dough, herbal medicines, dairy products (yogurt, tofu, paneer) as well as meats (Kc et al., 2021).

In recent era, plant-based products had been the centre of attraction for consumers because of their potential nutraceutical values so they are designed to satisfy the increasing demands of the market for alternatives to animal-derived products. Dairy products had always been on the top list of consumers due to their significant health benefits and their availability in the market. Secondly, the increasing trend of vegetarianism is leading the maximum number of people towards plant-based products and yogurt is one of those products because even patients with lactose intolerance can consume yogurt (Montemurro et al., 2021). Dairy is one of India's biggest agribusinesses with 8.47 million people involved in the industry that is contributing a significant amount to the economy (4%) of the country. Yogurt is one of the fermented dairy products which is prepared with the action of lactic acid bacteria on the milk and due to that action coagulation and acidification in the milk occurs and milk gets converted into yogurt (Han et al., 2016). Yogurt is a probiotic with a great source of bio-available calcium and is reported to be beneficial in multiple health problems especially related to gastrointestinal diseases (Inflammatory bowel disease, Crohn's disease, indigestion) (Katke & Deshpande, 2022). Presently yogurts are enriched with fruits, and flavors such as raspberry, banana, strawberry, vanilla, and peach are easily available in the market but there are other fruits, vegetables, and plant sources available that have potential health benefits and can be used as alternatives to these flavors such as *Moringa oleifera* flowers. Because neither it is imparting any color nor affects the flavor and texture of the yogurt and providing great benefits with no side effects (Akajiaku et al., 2018).

Chapter-2

REVIEW OF LITERATURE

Since ancient times, plethora numbers of herbal plants have been employed to treat various ailments (diabetes, cancer, hypertension, etc.). A growing trend to explore the medicinal potential and beneficial usage of various plant species has been reported in recent years (Paarakh et al., 2010). Currently, herbal plants are still receiving substantial attention as a source of flavouring components in food industries based mostly on trade in industrialized countries (US, Canada, Australia) which is growing at a rate of 7 to 15% per annum. Within developing countries like India, Pakistan, and Africa, a massive section of the population (about 80%) uses herbal medicines for natural action, as they are considered effective, safe, reliable, non-toxic, and simply obtainable and cheap compared to synthetic medicines (Ganatra et al., 2012 & Tabassum et al., 2013). Poor diet patterns include vitamin and mineral-deficient diets, which are responsible for several life-threatening diseases. So, the human diet must include an adequate amount of every constituent required for the normal functioning of the body (Islam et al., 2021). Most of the population looks for cheap alternatives, especially in developing countries and those alternatives do not include good protein content, meat, or any dairy product. *Moringa oleifera* is out of those plants which are getting huge popularity in industrial as well as general population levels due to its multiple beneficial effects (Fig. 1). *Moringa oleifera* plant parts had been in use for decades especially leaves seeds and bark but there is still one more part of the plant which needs to be explored i.e., the Moringa flower.

Morphological parts like seed coat, stem, bark, root, and flowers of the *Moringa oleifera* are known for their antimicrobial activities. Several studies on flowers and leaves of *Moringa oleifera* have shown that they are highly antioxidants (Iqbal et al., 2006). Pakade et al., 2016 determined the phytochemical constituents of the Moringa and observed that the Moringa contains almost double amount of these phytochemicals as compared to other fruits and vegetables. Whereas the flavonoids present in the Moringa ware three times higher than the vegetables. So, it is therefore clear from the results that Moringa has antioxidant activity more than that of the selected vegetables. Kalappurayil et al., (2017) studied that *Moringa oleifera* flowers are an unexplored part of the Moringa and they have a storehouse of the valuable bioactive phytochemicals. Primary investigations have shown that they contain anti-

bacterial, anti-inflammatory, antioxidant, antiviral, antifungal, and anti-cancer properties. They also mentioned that there is a high scope for the studies of the *Moringa oleifera* flowers' other therapeutic properties as well as for isolating, purifying, and characterization of the bioactive phytochemicals. *Moringa oleifera* flowers are bisexual, have a pleasant fragrance produced throughout the year, and are generally unequal in size. They are considered a delicacy in many places, whereas the flowers' sepals are more significant than the petals. It contains five sepals and petals, is spathulate, and is reflexed (Fig. 2.1). The flowers are about 2.5cm wide, 10-25 cm long, and are whitish and cream-colored, with yellow dots present on the base of the flowers (Paikra et al., 2017). These flowers are potential source of nectar for honey production and are also eaten as a salad in raw form and, after blanching, used to prepare tea. Fried Moringa flowers taste like mushrooms, and their powder is used as a food additive due to their negligible effect on the color and appearance of the food. These flowers are of great importance for medical purposes because they are a good source of antioxidants, anti-inflammatory substances, hepatoprotective, anti-diabetic, and other properties (Alhakmani et al., 2013, Mahajan and Mehta 2010). For many herbal plants, it is seen that they lose their nutrients on heat treatment or after cooking and bleaching, but *Moringa oleifera* maintains its properties even after heat treatment or a higher temperature of about 50-80℃ (Abdull et al., 2014). On boiling treatment, iron's bioavailability in leaves increases three times more than in the raw form (Wickramasinghe et al., 2020). It also contains rhamnose and sugar, which are unfamiliar to other plants and showed chemo preventive activity, including apoptosis (Amaglo et al., 2010 and Brenelli et al., 2010). Morphological parts of *Moringa oleifera,* like seed coat, stem, bark, root, and flowers, are known for their antimicrobial activities, anti-bacterial, anti-inflammatory, antioxidant, antiviral, and antifungal properties (Auwal et al., 2013). The flower contains more phenolic and flavonoid content than other herbs such as *Carum carvi*, *Borago officinalis, Lavandula officinalis, Nigella sativa,* and *Arteminisa dracunculus* (Pakade et al., 2016 and Kalappurayil et al., 2017).

2.1 DEVELOPMENTAL STAGES OF *MORINGA OLEIFERA* FLOWER

Moringa oleifera flower inflorescence arises in axillary or terminal panicles with wide branches and the topmost flowers develop earlier than the lower branches. Before the initiation of petal primordium takes place, sepal initiation occurs sequentially in two to five phyllotaxis. The sepals expand quickly and cover the bud in a quincuncial aestivation. The three sepals are intermediate in size and emerge to the right or left of the first sepal in equal

amounts. Size transformations are maintained throughout flower development and the sepals develop a thick indumentum of long unicellular hairs when they are initiated. They are tall, petaloid, and recurved at maturity (Guzman et al., 2020).

Figure 2.1 Flowering on *Moringa oleifera* tree

The following phase starts a five-angled platform that has been flattened. In the corners of this platform, alternate sepals' protuberances appear more or less simultaneously. They develop quickly into triangular primordia for petals. The petal primordium between sepals three and five continues to be steady and quickly enlarges. Four additional petals experience skewed growth because of their apical growth patterns. The smallest and the first-formed buds are globular in shape and greenish white in color (Fig. 2.2).

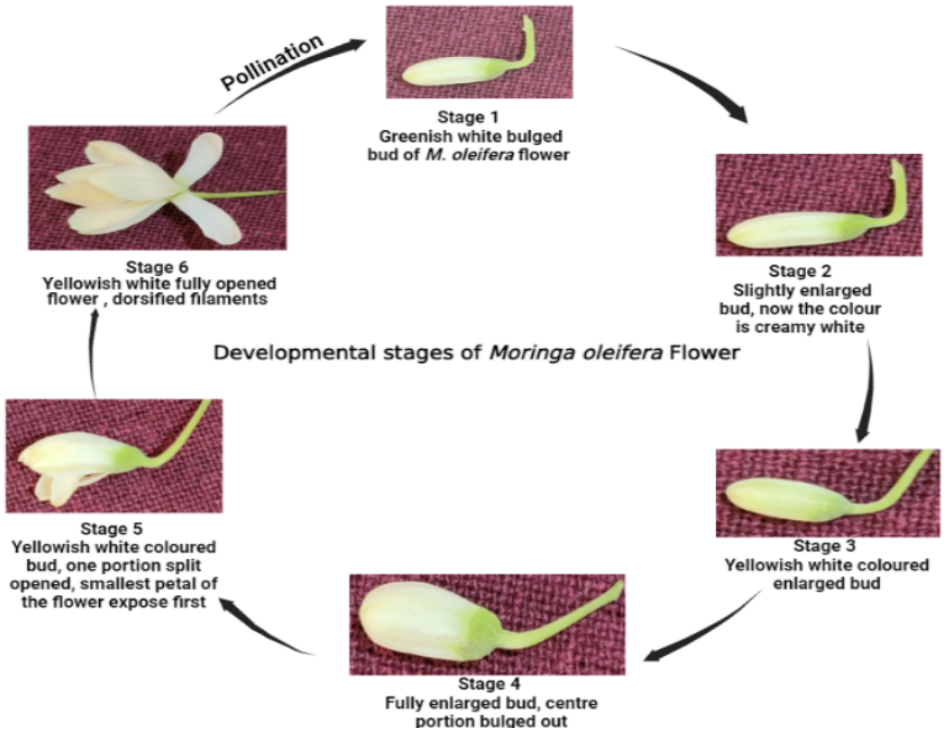


Fig. 2.2 Developmental stages of *Moringa oleifera* flower

Moringa oleifera flowers are yellowish white, bisexual, and about 1-2.5cm in size, with compound inflorescence. Sepals and petals are each five in number, unequal, free, and reflexed, five stamens which are all of the different sizes, filaments are dorsifixed, and the thalamus is set as a cup also named hypanthium (Kalappurayil et el., 2017). As it develops to the next stage, it slightly enlarges and becomes creamy white which enlarges more and becomes yellowish white in color in the later stage. As it develops more, it becomes a fully enlarged bud where the centre portion bulged out, and thereafter yellowish white bud split opens and the smallest petal of the flower exposes first, after that, it is a fully open flower where each sepal and petals are five in number and yellow-colored anthers can be seen with different lengths of dorsifixed filaments (Muhl et al., 2013).

2.2 CHEMICAL COMPOSITION AND PHYTOCHEMICAL POTENTIAL OF *MORINGA OLEIFERA*

Moringa oleifera contains a good amount of ascorbic acid which is a great source of antioxidants and plays an imperative role in free radical scavenging activity (Madane et al., 2019). It also contains vanillin, indole acetic acid, flavonoids, polyphenols, protein components, vitamins, and minerals (Divya et al., 2019). Various qualitative and quantitative

analyses of the *Moringa oleifera* flower extract confirmed that it contains saponins, tannins, terpenoids, and steroids (Sankhalkar & Vernekar; 2016). Additionally, *Moringa oleifera* flower extract also possesses hepatoprotective, diuretic, and anti-inflammatory properties and also helps in preventing various diseases such as asthma, pertussis, spleen, and muscle problems (Yang et al., 2018). Moringa flower contains nine amino acids sucrose, wax, nine amino acids, calcium, potassium, and iron (Ansari et al., 2020).

2.2.1 Nutritional constituents

Moringa oleifera flowers are potential source of multiple vitamins *viz.* A, B, C, D, and E, minerals (potassium, magnesium, iron, zinc, calcium, and copper) (Gopalakrishnan et al., 2016). It shows a higher amount of vitamin E (113 mg), B_2 (20.5 mg), C (17.3 mg), A (16.3 mg), and B_3 (8.2 mg) (Zaku et al., 2015). It also contains several trace minerals such as calcium (2003 mg), potassium (1324 mg), sodium (870 mg), magnesium (368 mg), phosphorous (204 mg), and iron (28.2 mg) (Gandij et al., 2018). Similarly, it contains a huge amount of different amino acids such as leucine (1.95 g 16^{-1} gN), arginine (1.33 g 16^{-1} gN), lysine (1.32 g 16^{-1} gN), threonine (1.19 g 16^{-1} gN), valine (1.06 g 16^{-1} gN), and isoleucine (0.83 g 16^{-1} gN) (Zaku et al., 2015).

2.2.2 Proximate composition

The proximate values of *Moringa oleifera* flower, dried leaves, seed, and barks are represented in Table 2.1 per 100gm. It shows significantly higher unstructured carbohydrates (57.88 % to 59.88 %) compared to *Moringa oleifera* leaves (26.60 % to 41.58 %), seeds (8.45 to 21.00 %), and bark (25.00 % to 52.00 %) (Tope et al., 2017 and Abdulkadir et al., 2016). *Moringa oleifera* is one of the potential sources of the protein, flower consist of 14.94% to 15.54%, leaves 16.40% to 18.34%, seeds 38.55% to 43.26%, whereas bark contains 5% to 12.70% of the protein content.

Table 2.1 Proximate Composition of *Moringa oleifera* Flower (Guzman-Maldonado et al., 2020, Tope et al., 2017, Abdulkadir et al., 2016, and Arise et al., 2014)

Proximate Composition	Values in %			
	Flower	**Dried Leaves**	**Seeds**	**Bark**
Ash	7.56-8.86	8.00-13.38	4.48-7.00	7.10-11.00
Carbohydrate	57.88-59.88	26.60-41.58	8.45-21.00	25.00-52.00
Fat	1.57-2.57	2.43-4.50	21.36-43.00	0.75-1.00%
Fiber	4.34-11.00	16.31-37	9.00-22.00	21.01-35.00
Moisture	5.30-5.80	9.00-11.00	6.43-8.00	6.40-9.00
Protein	14.94-15.54	16.40-18.34	38.55-43.26	5.00-12.70

2.2.3 Bioactive compounds

Moringa oleifera also contains several bioactive compounds such as alkaloids, terpenoids, ketones, alkenes, alkanes, alcohol, sterols, tocopherols, aldehydes, fatty acids, flavonoids, carotenoids, quercetin, kaempferol, β-Carotene, ascorbic acid, polysaccharides, D-mannose, D-glucose, protein, niazirinin, glycoside niazirin, and niaziminin A & B, (Chhikara et al., 2020, Vats and Gupta, 2017, Ganatra et al., 2012, Tabassum et al., 2013 and Mishra et al., 2011). Fig. 2.3 represents the chemical structure of the bioactive compounds of *Moringa oleifera* flower**.**

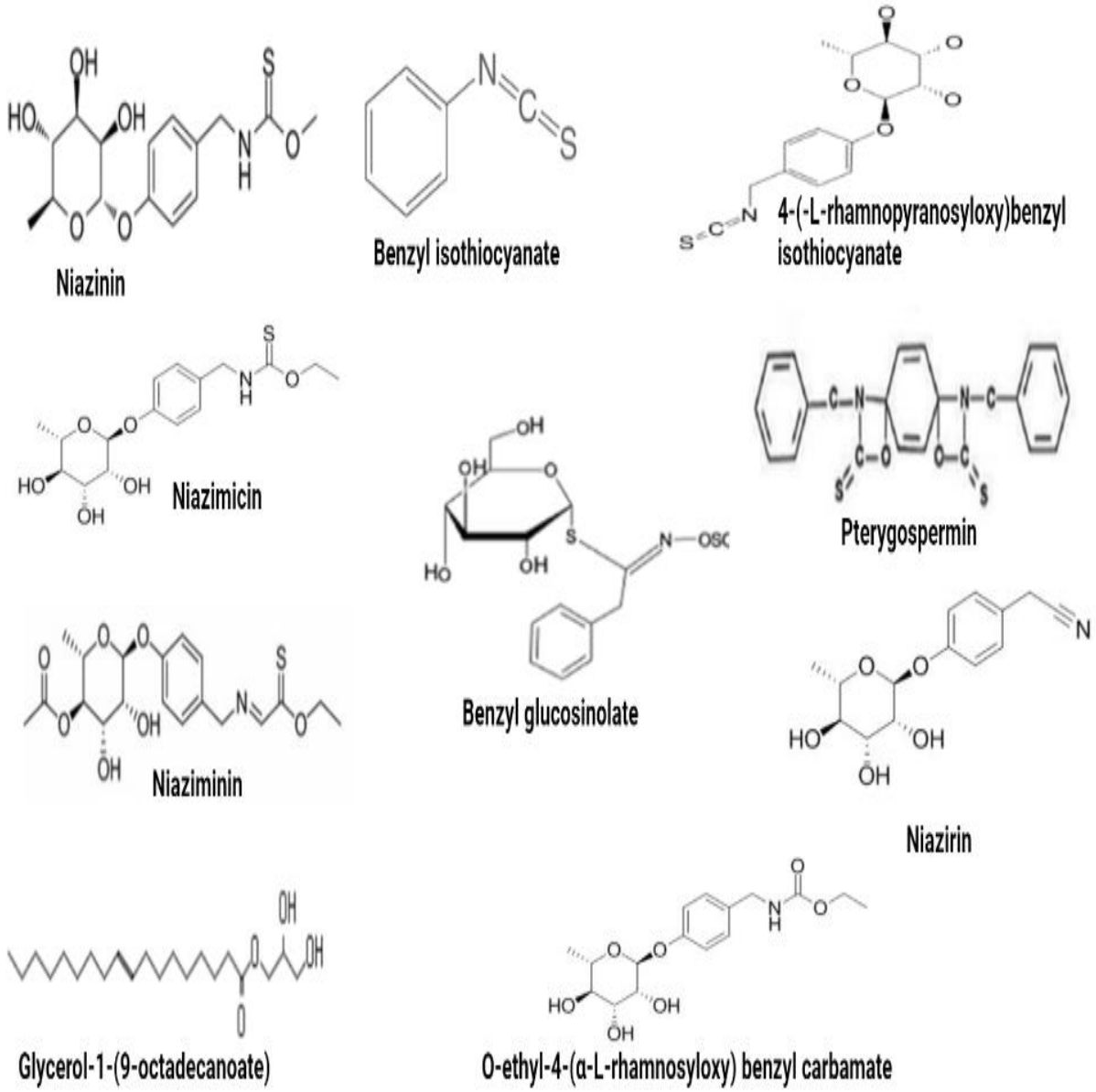


Fig. 2.3 Chemical Structures of bioactive compounds of *Moringa oleifera* flower (Anwar et al., 2007 and Sharma et al., 2011, PubChem)

2.3 CYTOTOXICITY OF *MORINGA OLEIFERA*

Cytotoxicity may be defined differently, depending on purpose of the study as well as the factors the cells were killed or else their uptake was altered. Testing a compound for its cytotoxic potential has two purposes- first is this compound is believed to be an anticancer agent, in this case, cytotoxicity may be important for its efficacy. Alternatively, number of compounds are used in the cosmetic and pharmaceutical industries where their side effects

need to be eradicated (Guevara et al., 1999). Alkaloids, flavonoids, and phenolics, among other bioactive substances, have all been identified in *Moringa oleifera* as having anticancer and antioxidant action. Antioxidants appear to prevent the growth of cancer cells. The cytostatic effect reduces cell multiplication, whereas the cytotoxic effect reduces cell survival. The following paragraphs cover several pertinent research to show the cytotoxic effects of *Moringa oleifera* on various cell types.

Moringa oleifera's bioactive substances have been researched since 1999. From everything stated above, Niazimicin appears as a powerful antitumor agent whereas 4 (alpha-L-rhamnosyloxy)-benzyl isothiocyanate also presented a substantial action (Pop et al., 2022). Niazimicin and benzyl isothiocyanate inhibit the growth of phorbol esters in the lymphoblastoid cells. Parvathy and Umamaheshwari (2007) looked into how *Moringa oleifera* extract affected human multiple myeloma (cancer) cell lines. Cytotoxicity of *Moringa oleifera* extract with several extracting solvents, including ethanol, chloroform, methanol, as well as ethyl acetate was evaluated using neutral red dye absorption method. According to findings, the methanolic extract of *Moringa oleifera* leaves showed the highest cytotoxic effect on the cells. Mulvihill et al., (2016) summarized in their review the recent developments in the underlying effects of flavonoids in modulating lipid metabolism, atherosclerosis, and other metabolic syndromes. Flavonoids, like nobiletin, hesperidin, tangerine, and naringenin have emerged as treatments for metabolic disorders. Various studies revealed an association between the intake of citrus flavonoids–containing foods and a reduced prevalence of cardiovascular diseases. Flavonoids diminish the inflammatory response in metabolically essential tissues including adipose, the kidney, the liver, and the aorta. Although the mechanisms which work behind the flavonoid-induced metabolic regulation are not been completely well-known yet, still several potential targets have been identified. In the animal models, flavonoids showed marked destruction of the atherogenesis through enhanced metabolic parameters along with the direct impact on the vessel walls. A few recent studies support the probable role of flavonoids in the treatment of obesity, raised lipids levels, insulin resistance, fatty liver, and atherosclerosis. The efficacy of bioactive ingredient release can be affected by various variables including solvent type, temperature of the extraction, extraction time, the extraction method, pH, and concentration of the extraction solvent. For example, the optimal extraction conditions for the bioactive components of *Moringa oleifera* was ethanol extraction at 80 °C, for 45 minutes (Chen et al., 2017). Bioactive ingredients are protected using encapsulation approaches with a variety of carriers

or coatings like biopolymers, animal or plant proteins, polysaccharides, as well as gums (Mulat et al., 2020).

2.4 PHARMACOLOGICAL ACTION

Medicinal plants have always been a topic of interest for researchers because of their medicinal properties, *viz.* anti-diabetic, anti-inflammatory, anti-cancer, cardiovascular, etc., due to presence of higher phytochemical constituents, as shown in figure 2.4. Primary phytochemical screenings showed that various secondary metabolites in the *Moringa oleifera* flower extract have anti-inflammatory and antioxidant activity. Secondary metabolites, like alkaloids, tannins, steroids, etc., adds another characteristic to it (Paikra et al., 2017). Out of them, a few are great sources of antioxidants, such as quercetin and chlorogenic acid, which mitigate the risk and progression of diseases like cancer and heart diseases along with strokes due to the presence of free antioxidant radical scavengers. Aqueous, ethanolic, .and methanolic extracts of *Moringa oleifera* leaves show great antioxidant activity, and *in-vitro* studies of these extracts showed to prevent oxidative damage, which is majorly caused by high-fat diets (Sharma et al., 2011). *In-vivo* studies of ethanolic extracts of *Moringa oleifera* leaves showed antiepileptic activity in Swiss albino mice (Joy et al., 2013 and Amrutia et al., 2011). *Moringa oleifera* root and bark extract reduced the stone weight produced by ethylene glycol in urolithiasis. It showed anti-urolithic and curative properties (Fahad et al., 2010).

Plants have played a substantial role in the treatment of sickness since ancient times and throughout history. All across the world, people continue to use plants for various purposes and according to estimates from the World Health Organization (WHO), 80% of the population in some poor nations uses herbal medicine for part of basic healthcare (Sharma *et al.,* 2022). For the vast majority of the impoverished, there are no other treatment options outside indigenous cures, whereas only 11% of the population, according to estimates, has access to formal health care. *Moringa oleifera* is one out of the 14 species in the Moringaceae family. According to the literature, the Moringa tree was brought from India to Africa at the beginning of the 20th century to be utilized as a dietary supplement (Kashyap *et al.,* 2022). The tree's name in the Nile Valley (Sudan) is Shagara al Rauwaq, that stands for tree for cleansing (Kaur *et al.,* 2023). Due to *Moringa oleifera's* antibacterial activity, it has been widely utilized to treat various ailments. It is abundant in substances that include the simple sugar rhamnose as well as a special class of substances known as glucosinolates and isothiocyanates. It is known for its antipyretic, antibacterial, anti-ulcerative, hepatoprotective,

and anti-inflammatory properties along with antioxidant and cholesterol-lowering effects (Maisto *et al.,* 2022). *Moringa oleifera* flower had been tested for various health benefits but the bioactive compounds may vary according to the various geographical sites (Penalver *et al.,* 2022). It is a potential source of phytoconstituents and alkaloids. A diet high in antioxidant fruits and vegetables, according to epidemiological research, dramatically lowers the risk of multiple oxidative stress-related diseases, as well as cancer, diabetes, and cardiovascular. The majority of dietary antioxidants come from polyphenols, which are easily absorbed in the intestine (Sehrawat *et al.,* 2022). Alkaloids are hypothetically a new class of antibiotics with an extensive antibacterial range, with no side effects, and a very low tendency to induce the drug resistance; due to which, major research determinations are focused on them (Yan *et al.,* 2021). Nakajima et al. (2019) investigated the role of compounds in obesity and some general trends worth highlighting. *In vitro* studies have shown that polyphenols induce adipocyte differentiation, adipocyte apoptosis and lower cellular lipid levels, and thus may be useful in the treatment of obesity. Biological tests are not completely consistent, moreover, most of them showed a significant reduction in adipose tissue. Increased gene expression indicates stimulation of glycaemia, better lipid profile and β-oxidation. There is also some evidence of improvement in inflammatory conditions. Several clinical studies have shown beneficial effects of flavonoids in reducing pro-inflammatory cytokines in humans and mitigating complications of obesity and obesity-related diseases. However, several clinical studies have been developed to investigate its role in reducing obesity.

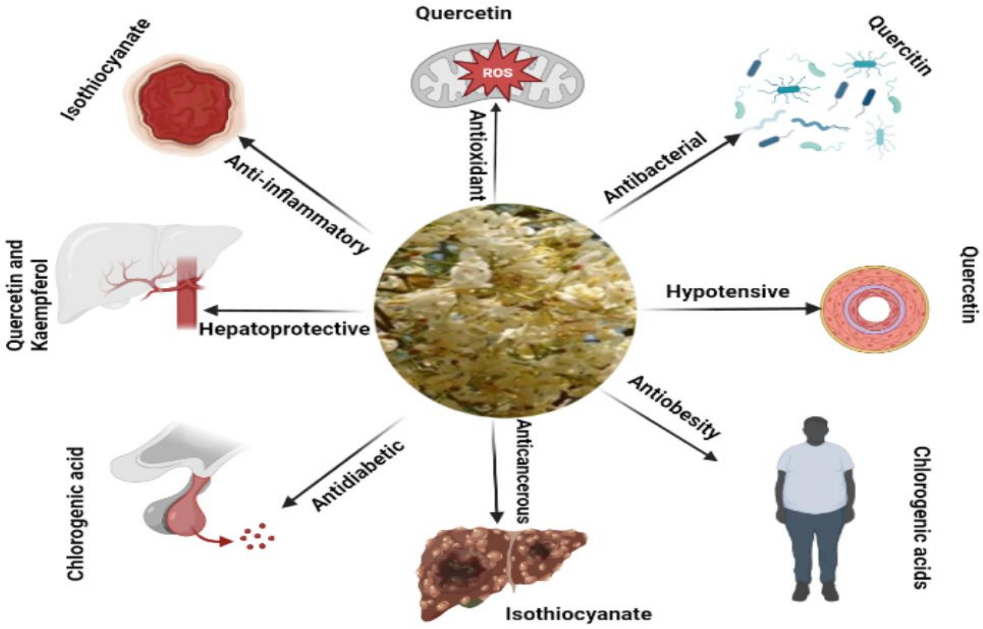


Figure 2.4 Pharmacological properties of *Moringa oleifera* flower

2.4.1 Anti-diabetic

Diabetes is a lifestyle conditions that is considered by high postprandial and fasting blood sugar levels. In normal conditions, food gets broken down into small pieces and generates energy in the form of glucose which is used by the body for regular functioning (Villarruel-sLópez et al., 2018). When blood sugar levels get increased, it signals the pancreas to release insulin, but in this condition, the pancreas releases little or no insulin, due to which blood glucose levels increase (Wang et al., 2022). *Moringa oleifera* extract contains compounds like phenyl glycoside, carbamates, and thiocarbamate that stimulate insulin secretion from the pancreatic beta cells and help the body to manage the blood glucose levels. Secondly, it also contains terpenoids that stimulate the beta cells of the pancreas and subsequently secret insulin (Razis et al., 2014). The hypoglycaemic property of *Moringa oleifera* extract has been studied in both standard and Streptozotocin-induced (STZ) diabetic rats and it was reported that administration of the *Moringa oleifera* extract orally reduces sugar levels by about 26.7% (Tang et al., 2017). Aqueous *Moringa oleifera* extract also exhibited a great hypoglycaemic effect in alloxan-induced rats by reducing the rate of gluconeogenesis and regenerating the damaged hepatic cells as well as the pancreatic β-cells (Figure 2.5) (Abd El Latif et al., 2014). In a randomized study Anthanont et al., 2016 revealed that consuming 7gm *Moringa oleifera* powder continuously for three months resulted in a 13.5% of reduction in postprandial as well as fasting blood glucose levels. Intake of *Moringa oleifera* leaves extract

exhibited hepatoprotective activity in response to the liver fibrosis in animal models by reducing serum activities of aspartate transaminase (AST) and alanine transaminase (ALT) (Hamza 2010). Figure 2 represents the Hypoglycemic property of *Moringa oleifera* flower extract.

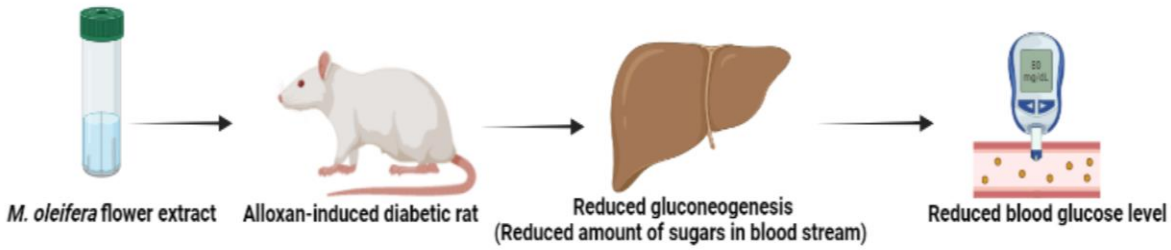


Figure 2.5 Hypoglycaemic property of *Moringa oleifera* flower extract

Moringa oleifera extract has shown the properties to cure both kinds of diabetes, type I and type II. In type I type of diabetes patients are unable to produce insulin on their own and they are dependent on insulin injections. Insulin is a hormone that upholds the levels of plasma glucose at normally essential levels. In type 2 diabetes patients' bodies produce insulin but not in the required amount but less than that so they are dependent on a kind of medication for the release of insulin hormone (Cerf, 2013). Studies are available which showed that Moringa has anti-diabetic properties where aqueous extract of the *Moringa oleifera* can treat streptozotocin-induced diabetes along with insulin-resistant in animal models (Divi et al., 2012). Another study done by researchers showed that when STZ-induced diabetic rats were fed with the Moringa powder, the fasting glucose of the rats dropped to a significant amount (Malki et al., 2015). Ethanolic extracts of the flowers of the *Moringa oleifera* plant contain thiamine, ascorbic acid, nicotinic acid, riboflavin, folic acid, rhamnetin, pyridoxine, and kaempferol whereas the aqueous extracts of Moringa contain D-glucose, D-mannose, unidentified monosaccharides, along with ascorbic acid (Jadhav et al., 2000). The findings showed that the MOFE (*Moringa oleifera* flower extract) supresses the development of the proinflammatory cytokines. *Moringa oleifera* flower extract is also responsible for the alteration of cytokines (Mahajan et al., 2009).

2.4.2 Anti-cancerous

Cancer is a life-threatening disease where abnormal cells divide irrepressibly and can abolish normal body cells. Anti-cancerous compounds, also known as antineoplastic drugs, are more effective in treating malignant and cancerous cells. *Moringa oleifera* has an ample number of therapeutic properties and contains a large amount of vitamins A, B, C, and E, potassium,

phosphorus, zinc, sodium, calcium, arginine, lysine, proline, and valine, which makes it a potential candidate for preventing and treating the cancer (Dixit et al., 2016). The plant extract has shown multiple benefits, such as chemo preventive properties, inhibits cancer cell growth, and reduces colony formation and cell motility (Karim et al., 2016). Modi et al., 2016 revealed that *Moringa oleifera* extract encourages apoptosis in the A549 cell line via depolarisation of the mitochondrial membrane, which results in a significant reduction in the ATP (Adenosine triphosphate), due to which reactive oxygen species levels (ROS) get increased which are accountable for the high level of depolarisation in the mitochondrial membrane. ROS level keeps increasing; this diminishes the level of antioxidant GSH (Glutathione) and causes cancer cells to undergo apoptosis. In a recent study on Wister rats, the ethanolic extract of the *Moringa oleifera* displayed great anti-cancerous activity against hepatocellular carcinoma by improving antioxidant activity (Sadek et al., 2017). Krishnamurthy et al., 2015 revealed the use of the Hep2 human cancer cell line for the determination of the anti-cancerous effect of the *Moringa oleifera*, and results were monitored after 24 and 72 hours of incubation. To determine the viability of the cells, sulforhodamine B was used as a stain in cells due to its effective binding properties with cellular proteins. *In vivo* studies observed that a 2000 mg/kg dose of *Moringa oleifera* extract resulted in comparatively less toxicity in the fed Swiss albino mice model due to no abnormalities found in internal organs and zero death rates. *Moringa oleifera* has ample number of therapeutic properties and other characteristics which makes it a good choice to a cancer inhibition and management plan. It contains large number of nutritional constituents such as vitamins, minerals, amino acids and other substances which makes it a potential candidate for the treatment of cancer and good health (Dixit et al., 2016). It is isolated with calcium, iron, phosphorus, and zinc along with many more other properties which promote the health which is a good tool against the cancer.

2.4.3 Anti-hypertensive

Hypertension is a condition when blood pressure gets extensively high (140/90 mmHg) due to poor dietary patterns and lifestyle. However, diabetes and obesity can also cause hypertension due to increased catecholamine sensitivity and reabsorption of insulin-induced sodium. *Moringa oleifera* extract help in reducing high levels of blood pressure by improving the vascular disfunction as well as by decreasing the oxidative stress. Niazirin and niaziminin are the bioactive compounds that are found in the *Moringa oleifera* flower, which are known as hypotensive agents and help to relieve high blood pressure (Aekthammarat et al., 2018).

Niaziridin acts as a hypocholesterolemic, anti-hyperlipidaemic, and hepatoprotective agent (Lee et al., 2013). ACE (Angiotensin converting enzyme) is an enzyme inhibitor that relaxes the arteries and veins to lower blood pressure. ACE prevents the production of angiotensin, a substance that contracts the blood vessels and this contraction of the blood vessels can cause high blood pressure, resulting in the heart working harder (Bakris, 2022). Preliminary results of Acuram et al., 2019 revealed that *Moringa oleifera* extract showed the highest ACE inhibitory results at 64.23 %, whereas standard Captopril showed the same results at 87.57 %. The study showed that nitrile and thiocarbamate glycosides from the *Moringa oleifera* flower are responsible for lowering blood pressure, and these compounds are scarce (Toma and Dayno 2014). Chan et al., 2012 reported that artificially induced pulmonary hypertension in rats with monocrotaline (injectable for hypertension) caused high blood pressure in pulmonary arteries. After three weeks of induction (4.5 mg/kg), *Moringa oleifera* extract was given via intraperitoneal injections, resulting in a significant decrease in pulmonary arterial blood pressure. Even continuous administration of the extract reversed the effect of the infusion (Monocrotaline).

Hypertension is an important risk factor for various cardiac diseases (Ungler, 2002). The development of such kind of diseases is influenced by many factors like obesity, genetic disposition, lifestyle, aging and nutrition (*Groziak & Miller, 2000*). According to WHO (World Health Organization) reporting of August 2013 the Ischaemic heart disease (IHD) is considered as to be the top major killer all over the world during the past ten years. This risk increases with the factors like smoking, hyper cholesterolaemia, age, diabetes, and high blood pressure. Hypertension has affected almost 15-20% of the adults. The people suffering from diabetes are always on the high risk for hypertension and cardiac diseases. ACE (Angiotensin converting enzyme) is an enzyme inhibitor which relaxes the arteries and veins to lower down the blood pressure. ACE prevents the production of angiotensin- a substance which contracts the blood vessels. This narrowing/ contraction of the blood vessels can ground high blood pressure and it results for the heart to work harder (Hansen et al., 1995).

2.4.4 Anti-inflammatory

Inflammation is the immune system's biological response, triggered by various factors such as toxins and pathogens. In this situation, the body's white blood cells help to fight against antigens, diseases, and toxins, which could be in the form of pain, swelling, heat, or redness (Razis et al., 2014). *Moringa oleifera* inhibit swelling and inflammation due to the presence

of polyphenols and isothiocyanates because they suppress the inflammatory enzymes and proteins (Paikra and Gidwani 2017). *In vivo* and *in vitro* studies by Mbikay (2012) on plant extract showed significant effectiveness in treating inflammation, hyperglycaemia, and hyperlipidemia. Almost thirty-six anti-inflammatory compounds which are present in the *Moringa oleifera* plant's aqueous and methanolic extract were shown to act as an anti-inflammatory agent by inhibiting the edema in rat models compared to anti-inflammatory drug indomethacin. Crude extract of the plant reduced the inflammation to 85% against carrageenan-induced edema in mice models at a concentration of 3mg/kg body weight (Padayachee and Baijnath 2020). Lectins present in *Moringa oleifera* function as an anti-inflammatory by using LPS-stimulated murine peritoneal macrophages due to the acquaintance of macrophages to the bacterial Lipopolysaccharide (LPS) starts a signal transduction cascade which leads to the higher amount of production of proinflammatory cytokines, nitrites, and reduction in bacterial activity (Araujo et al., 2013). In a study, scientists investigated anti-inflammatory activity by protein denaturation method where Diclofenac sodium (an anti-inflammatory drug) was used as standard, and the flower extract of 100-500 µg/ml was taken for the resistance against the heat-induced protein denaturation in the fresh egg albumin (Alhakmani et al., 2013).

2.4.5 Cardioprotective

According to WHO report of December 2020, ischemic heart disease (IHD) has been considered the top cause of death worldwide for the past ten years. IHD is described as a reduced blood supply in the heart muscles because of coronary artery disease (CAD) (Ford et al., 2019). This risk increases with the factors like smoking, hypercholesterolemia, age, diabetes, and high blood pressure and has affected almost 15-20% of adults. People with diabetes are always at substantial risk for hypertension and cardiac diseases (Hossain et al., 2020). *Moringa oleifera* preparations in metabolites and extracts improve cardiovascular diseases by inducing non-shivering thrombogenesis (Alia et al., 2022). Quercitin in *Moringa oleifera* flower extract improves cardiac contractability and prevents heart diseases. It also protects the heart by reducing cell oxidative stress (Aekthammarat et al., 2019). Chronic treatment by using *Moringa oleifera* flower extract demonstrated favourable changes in the biochemical enzymes like glutathione, creatine kinase-MB, as well as superoxide dismutase. In that case, the extract (400 mg/kg/day) prevented the rise of lipid peroxidation, which is the major reason behind cardiac diseases (Biswas et al., 2012). A study by Madkhali and coworkers, (2019) reported that obesity also triggers oxidative stress in conditions when an

imbalance of pro-oxides and antioxidants occurs in the body. In obesity, there is extreme formation of fat from glucose (lipogenesis) and inhibition of the lipid breakdown, in the intuitive areas where metabolic activities occur at a higher level. This situation leads to dyslipidemia and inflammation, which can lead to endothelial dysfunction.

Fuglie et al., (2005) studied the hypocholesterolemic and anti-arthritic potentials of the *Moringa oleifera* along with urinary tract curing properties. Sutalangka et al., 2013 showed that *Moringa oleifera* contains amino acids, calcium, and potassium. They contain nectar which is a viable source for honey production used by beekeepers. Velaga et al., 2017 studied the evidence of promising therapeutic properties of the *Moringa oleifera* for the treatment of inflammation. In case of edema, it is sensitive for both extracts as well as Indomethacin mainly in the second phase. *Moringa oleifera* has shown its properties to use clinically as efficient as medical drugs against edema. The results obtained with respect to phytochemicals indicate that *Moringa oleifera* flowers may be useful in managing and treating the effects of inflammation, for example as an antipyretic and analgesic. Other studies showed that plant-drug interaction have demonstrated teratogenic as well as mutagenic effect of *Moringa oleifera* in human studies, as well as time- and dose-dependent side effects, but promoters believe that in long-term it advocates reviewing its use as well as its efficacy in the chronic diseases. The phytochemicals are regarded one of the potential applicants for the drug delivery, discovery and also play important roles in drug development and drug research programs.

2.4.6 Hepatoprotective

The liver is one of the vital organs which plays a substantial role in the detoxification as well as protein synthesis. Hepatoprotective are compounds that decrease or prevent the liver from damage. *Moringa oleifera* flower contains caffeic acid, phenolic compounds, and flavonoids which contain a good number of antioxidants and help to protect the liver from free radicals generated during infection. When flower extract of *Moringa oleifera* is administered to mice, it reduces the oxidative stress caused by DNA damage *via* tumor necrosis factor-alpha (TNF-α), which reduces hepatic enzyme activity and prevents liver damage. *Moringa oleifera* leaves extract was given to two animal models of liver cirrhosis, and assessed the models for anti-cirrhotic activity. The animal models seemed to be in control from moderate to severe at dose concentration of 200, 400mg/kg (Biswas et al., 2012). *Moringa oleifera* extract showed a hepatoprotective effect in hepatic damage in histopathology. Wistar rats injected with

500mg/kg of extract had less necrotic effect than the negative control group. They demonstrated lower creatinine and alkaline phosphatase (ALP) levels caused by the high doses of medications (Buraimoh et al., 2013).

2.4.7 Antioxidant

Antioxidants are the effective components that show a significant role in oxidation processes in different sources such as food and body functions of humans and animals (Speisky et al., 2021). These compounds protect us from various diseases, such as inflammation, stroke, diabetes, and cancer (Shahidi et al., 2015). The antioxidants in *Moringa oleifera* flower are quercetin, kaempferol, cis-1-chloro-9-octadecene, ascorbic acid, gallic acid, Niazirin, Benzyl isothiocyanate, Benzyl glucosinolate, 4-hydroxy benzyl glucosinolate, Quercetin-3-O-eutinoside, and Isorhamnetin-3-O-(6-malonyl glucoside) and Kalappurayil et al., 2017). The human body generates free radicals during physiological activities, which causes oxidative stress on cells. These compounds act like the free radical scavengers to relieve the cells' oxidative stress, which helps to cure life-threatening diseases (Hossain et al., 2020). Figure 2.6 represents the antioxidant activity of *Moringa oleifera* flowers. Pakade et al., 2016 determined the phytochemical constituents of *Moringa oleifera* flower and revealed that it contains almost double and triple the amount of total phenolic 287.1 mg gallic acid equivalents/g and flavonoid content 254.3 mg quercetin equivalents/g (Nwidu et al., 2016) than fruits and vegetables such as spinach (2.69mg GAE/g & 1.33 mg QE/g), red pepper (180.3 mg GAE/100gm & 10.4 mg QE/100g), beetroot (2.57 mg GAE/gm & 0.62 mg QE/g), strawberry (3.63 mg GAE/gm & 14.6 mg QE/g). Administration of *Moringa oleifera* extract in rats at a concentration of 50 and 100 mg/day for two weeks showed a significant ($p<0.05$) decrease in lipid peroxides and an increase in glutathione concentration in liver and kidneys, which occurred due to the presence of high antioxidant activity (Ma et al., 2018). Shady et al., (2022) revealed the peroxide scavenging effect of *Moringa oleifera* extract observed in the mice model for four weeks of studies, resulting in great wound healing in mice at the rate of 49.26% with 1000µg/ml dose.

2.4.8 Anti-bacterial

Its primary purpose is to slow down the growth rate of the bacteria or completely kill the bacteria that cause life-threatening diseases. *Moringa oleifera* flower extract contains quercetin and glucosinolates, which are anti-bacterial and antifungal agents (Abdull Razis et al., 2015). Kalappurayil et al., 2015 studied that *Moringa oleifera* flowers are an unexplored

part of the Moringa and have a storehouse of valuable bioactive phytochemicals. Primary investigations have shown that they contain anti-bacterial, anti-inflammatory, antioxidant, antiviral, antifungal, and anti-cancer properties. According to current research by Yang et al., 2020, the anti-bacterial action of quercetin includes the destruction of the bacterial cell wall, change in permeability of the cell, reduction in enzymatic activities, and inhibiting nucleic acid synthesis. Razis et al., (2014) revealed an aqueous and ethanolic extract of *Moringa oleifera* showed significant results for gram-negative bacteria (*Enterococcus faecalis* and *Streptococcus aureus*) and non-significant effect against gram-positive species (Salmonella, *Aeromonas caviae,* and *Escherichia coli*) and the same results were seen in the other study by Peixoto et al., (2011). *Moringa oleifera* oil treats ringworms and *Aspergillus flavus* (fungus causing aspergillosis in mammals). These findings prove the ability of *Moringa oleifera* to treat some strains of fungi and bacteria (Shikwambana and Mahlo, 2020).

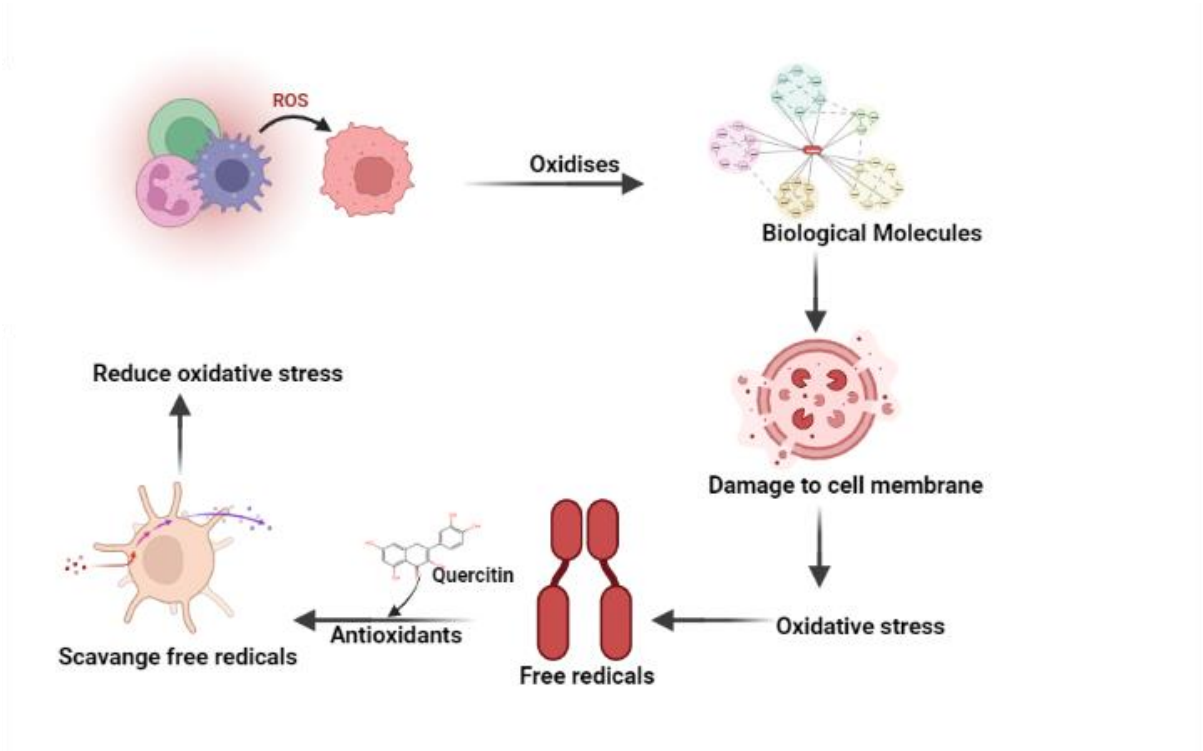


Figure 2.6 Antioxidant activity of *Moringa oleifera* flower extract

Establishing the antibacterial activity of *Moringa oleifera* flowers requires scientific proof of their antimicrobial potential because the majority of earlier attempts to pinpoint the antibacterial properties of *Moringa oleifera* utilized tests against human clinical bacterial isolates rather than approved standard bacterial species (Mohammed *et al.,* 2022). Given the worldwide difficulty in treating infections brought on by superbugs, the multidrug resistance of the bacteria utilized in some of these assays was not clearly established. Additionally,

numerous researches looked at the effectiveness of *Moringa oleifera* against bacteria originating from plants and animals as well as those from other environmental sources including water and sewage, perhaps because some of these bacteria can be acquired by humans and may cause diseases (Adji *et al.,* 2022). Although these investigations suggested that *Moringa oleifera* has antibacterial capabilities, it would be helpful to know how it performs in the presence of pathogenic, virulent, and multi-drug resistant bacteria, for which effective therapeutic alternatives are urgently needed. The aim of the study was to assess phytochemical properties, and minimal inhibitory concentration of *Moringa oleifera* flowers.

2.4.9 SYNERGISTIC EFFECTS

The synergistic effects of *Moringa oleifera* are also done to understand its health benefits. In traditional medicine, an extract from a combination of herbs is used for chronic ailments. As per the researchers, when the herbs are syndicated, the resultant effect of the herbs collectively provides the patients more benefits than a single use of herbs. Many researchers have also found improved anti-diabetic usefulness for diabetic patients when there is a combination of *Vernonia amygdalina Del*. and *Azadirachta indicia*. Aghara (2014) has reported an animal model's antagonistic effect of combining the Moringa leaf extracts and Telfairia on erythropoietin activities. The study observed that the combined effects of both extracts accounted for the increase in HB and PCV (packed-cell volume). Furthermore, the extracts of both plants contain natural hematinic agents like vitamin B, folate, iron, and copper. Table 2.2 represents several *in-vivo* studies on the *Moringa oleifera* flower. Alcoholic and aqueous both types of extracts of flowers and leaves exhibits potential antioxidant activity due to presence of the antioxidants such as beta carotene, vitamins (A, C, E) and minerals (copper, zinc, selenium) (Anwar et al., 2007). Along with antioxidant activity, these antioxidants are anti-cancerous, anti-inflammatory, and immune boosters.

Table 2.2 *In-vivo* study of *Moringa oleifera* flower

Diseases	Plant part	Compounds	Animal model	Mechanism of action	Reference
Antioxidant activity	Flower	Quercetin	Mice	Scavenge the peroxide activity and reduces the oxidative stress	Shady et al., 2022
		Ascorbic acid	Rats	Free radical scavenging activity	Hossain et al., 2020
		Methionine	Mice	Reduction in arsenic-induced oxidative Stress (arsenic induce hepatotoxicity and increase the oxidative stress)	Singh et al., 2009
Anti-inflammatory		Isothiocyanate	Rats	By inhibiting of heat-induced protein denaturation	Alhakmani et al., 2013

			Rats	Inhibits the activation of the NF-kb signaling pathway	He et al., 2022
Antibacterial		Quercetin	Mice	The destruction of bacterial cell wall, changes in the permeability of cell, reduction in enzymatic activities, and inhibiting the nucleic acid synthesis	Yang et al., 2020
Antiobesity		Chlorogenic acid	Mice	Lead to less cravings for carbohydrates and lower appetite levels	Abd El Latif et al., 2014
Antidiabetic		Chlorogenic acid	Rats	Reducing the rate of gluconeogenesis and regenerating the damaged hepatic cells	Abd El Latif et al., 2014
Hepatoprotective		Quercetin & Kaempferol	Rats	By lowering creatinine and alkaline phosphatase (ALP) levels	Buraimoh et al., 2013
Anti-hypertensive		Quercetin	Rats	ACE inhibitory action	Chen et al., 2012

Anticancerous		Isothiocyanate	Swiss albino mice	Effective binding properties with cellular proteins	Krishnamurthy et al., 2015
Cardioprotective		Quercetin	Rats	By preventing the rise of lipid peroxidation	Biswas 2012
			Rats	Improves cardiac contractability and prevents heart diseases	Aekthammarat et al., 2019
		Vitamin C	Rats	By converting cholesterol into the bile acids	Leone et al., 2015
Anti-fibrotic		Quercetin	Rats	Decrease acid-pepsin secretion and blocks the increase of the serum ALT (alanine aminotransferase), AST (aminotransferase)	Verma et al., 2012

2.5 TRADITIONAL USES OF *MORINGA OLEIFERA*

According to the Vedas, *Moringa oleifera* is used 5000 years ago to cure different life-threatening diseases. Folk medicines treat paralysis; wound healing, fever, diarrhea, sore throats, energy level, anxiety, asthma, skin problems, pain relief, alertness, scabies, and stress (Rani et al., 2018, Mahmood et al., 2010). In Ayurveda, *Moringa oleifera* flowers treat intestinal worms and help to maintain the balance in Pitta and Kapha. Table 2.3 shows the traditional uses of the various plant parts of the *Moringa oleifera.*

2.6 OTHER USES OF *MORINGA OLEIFERA*

Moringa oleifera is a multipurpose plant used for various purposes such as fertilizer, water purifier, honey, and sugarcane juice clarifier (Reddy et al., 2015). Oil extracted from different plant parts, such as bark, seed, and flowers, is used for cosmetics, cooking, biofuel, and scabies treatment. The plant's bark is used for making ropes, gum, and disinfectants (Mahmood et al., 2010, Tahany et al., 2010, and Al-Dhaheri 2016). Table 2.4 represent the other uses of *Moringa oleifera* parts.

Table 2.3 Traditional uses of various parts of *Moringa oleifera*

Plant Part	Phytochemicals	Therapeutic properties	Traditional method of preparation	References
Leaves	Niazirin, niazirinin, niaziminin A & B,	Anti-bacterial, HIV-AIDS, ulcer, hepatic, prostate, antitumor, diuretic, thyroid, antioxidant, diabetes, headache, lactation enhancer	Cooked as food, dries leaves powder, or as spinach in soups	(Ganatra et al., 2012 and Tabassum et al., 2013)
Roots	4-benzylglucosinolate	Common cold, renal pain, dental caries, diarrhea & scurvy	Prepared as decoctions	(Ganatra et al., 2016 and Dalei et al., 2016)
Pods	Nitriles, thiocarbamates, isothiocyanate, β-sitosterol	Skin cancer, anti-hypertensive, diabetes, and joint pain	Cooked as peas, fried in oil, or fresh	(Ganatra et al., 2016)
Flowers	Polysaccharides, ascorbic acid, and protein	Throat infection, abortion, antitumor and diuretic	As extract for improving the taste	(Mishra et al., 2011)
Seeds	Carbohydrate, fat, protein, moringyne, alkaloids, phenolic acids, flavonoids, glycosides	Antispasmodic, antitumor, ulcer, goitrogen, vitamin/mineral deficiency, warts	Roasted, powdered or extracts in the form of oil	(Ganatra et al., 2016 & Meireles et al., 2020)

Table 2.4 General uses of *Moringa oleifera* plant parts

Plant part	Uses	References
Seeds	Fertilizer, honey and sugar cane juice clarifier, water purification, treatment of diabetes in the Sultanate of Oman.	(Reddy et al., 2015)
Bark	Gum, rope, disinfectant, treatment of fever and headache	(Mahmood et al., 2010 and Tahany et al., 2010)
Leaves	Animal forage, biogas production, green manure, biopesticide	(Nawash and Al-Horani 2011)
Flower	Yellow flowers for color in food items	(Nawash and Al-Horani 2011)
Oil	Biofuel, Cosmetic oil, cooking oil, scabies treatment	(Al-Dhaheri 2016)
Wood	Blue dye, pulp	(Mahmood et al., 2010)
Leaves	Animal forage, biogas production, green manure, biopesticide	(Mahmood et al., 2010)

2.7 FOOD INDUSTRIAL APPLICATIONS OF *MORINGA OLEIFERA* Flower

Moringa oleifera has benefits in the food industry due to its many nutrients. There are functional foods that provide health benefits to people because they have more than basic nutrition and have the power to reduce the possibility of diseases as well as other health issues. Functional foods contain beta carotenes that are known for their role against free radicals; potassium lowers the blood pressure risk; flavonoids and calcium reduce osteoporosis; dietary fiber provides gastro intestine health and fatty acids for heart diseases. These kinds of nutrients provided by plants are more needed to cure diseases. However, the functional food industry is a high-growth market globally in which one natural food source has made its functionality to other foods. The plant (*Moringa oleifera*) is considered to have the potential to improve health issues and nutrition, encourage rural development and provide food security (Martín et al., 2013). This tree is a small, fast-growing, attractive tree with a low nutrient of soil, and the low demand for water makes its production easy. All parts of Moringa trees are used for industrial, potential food, and agricultural purposes. According to Torres-Castillo et al., (2011), Moringa is beneficial for multiple purposes such as medicine, functional food, water cleansing properties, as well as nutraceutical and is considered the most useful plant. In the food industry, its applications include the consumption of increasing milk production and also treating women having anaemia.

Moringa oleifera flower powder enhances the nutritional value of weaning foods and, is used in soups, salads, and tastes like mushrooms (Babayeju et al., 2014 and Arise et al., 2014). It is also used in muscle foods due to its potential source of protein. Even after being delicious and nutrient-dense, meat is highly deficient in dietary fibers and polysaccharides, which are an essential component of the diet and are present in plant sources. They contain a high number of micronutrients *viz.* iron, vitamins, sodium, magnesium, and potassium (Lorenzo and Pateiro, 2013). Studies proved that adding *Moringa oleifera* leaf extract by 2% reduces the chicken nuggets' redness and improves the nuggets' texture. *Moringa oleifera* flower extract also improves oxidative stability by reducing the process of lipid oxidation during storage. It has been noticed that it also increases the shelf life of cooked chicken nuggets by up to 15 days (Madane et al., 2019). *Moringa oleifera* extract improves the salinity resistance in wheat by increasing enzymatic and non-enzymatic antioxidants, high levels of potassium content, and increased grain weight and yield on comparison with other growth stimulators (Yasmeen et al., 2013). Late sowing of quinoa significantly reduces the growth and yield of the crop; however, *Moringa oleifera* extracts show improvement in physiological,

biochemical, and yield parameters. It was found to be the most promising stimulant for better growth and high quinoa yield (Rashid et al., 2021). *Moringa oleifera* powder supplementation in porridge improved the nutritional value by increasing vitamin A by 15% (Gomez and Angulo 2014). *Moringa oleifera* flower powder is used in the fortifying cake as it does not affect the texture and color and also improves the cake's nutritional value (Karim et al., 2013).

Leaves of Moringa are full of nutritious properties which are one hundred percent edible and they are a potential source of nutrients *viz.* beta carotene, minerals, vitamins, fiber, carbohydrates, and minerals like potassium, calcium, magnesium, iron, and phosphorous. Falowo et al., (2008) showed that the Moringa plant has many nutrients that provide health benefits in the food industry. Furthermore, the plant is also known for the purpose of fixing nitrogenous compounds in soils, and the wood provides fuel for the cooking purpose. The leaf juice of the plant is also a good source of hormone growth. The Moringa contains the compounds like nitrile, mustard oil glycosides, and thiocarbamate glycosides that can lower blood pressure. All parts of the tree used in food consumption also have diuretic activity, and its fruit reduces the heat and artery in Hypercholesterolemic rabbits and the lipid profile of the liver. Sahay et al., (2017) studied Moringa's properties in an animal model by treating rats with different sources of iron. The study showed that rats fed with Moringa leaf extract had higher hemoglobin than other rats.

2.8 AVAILABILITY OF *MORINGA OLEIFERA* PRODUCTS IN THE MARKET

The demand for *Moringa oleifera* flower increased due to its higher therapeutic value, which diverted the mind of humans towards it, and the export market also increased by 30%. It is used as a food and nutraceutical product due to its higher bioactive compounds. Flowers are demanded, and other fragments of the *Moringa oleifera* plant, like leaves, seeds, and barks, are also in higher demand for different purposes in the international market. Research shows that 80% of the total production of Moringa is from India, which makes crores of foreign exchange for our country. In India, different states, *viz.* Tamil Nādu, Karnataka, Andhra Pradesh, as well as Odisha, are big exporters of Moringa. China, Germany, Canada, South Korea, and the US also export *Moringa oleifera* products to countries like Ghana, Cambodia, Nigeria, Haiti, and Kenya (Thomas 2016). The various big brands available in the market are Miracle Garden Texas Moringa, Sun Potion Moringa, Organic Veda, Kiva, and Organic India, which export and import the Moringa leaves, leaves powder, dry fruit, turmeric Moringa latte,

liquid green Moringa drops, and Moringa leaves tablet. The leading exporter and importers in the Global market of Moringa are Kuli Kuli, Genera Organics, Moringa Farms, and Moringa Initiative Ltd.

2.9 DEEP EUTECTIC SOLVENTS (DESs)

These days, worldwide supportability challenges are personally interconnected being human wellbeing as well as ecological effect on the fundamental concerns. In this unique circumstance, it is not conceivable to manage explanatory science in disconnection (Valcarcel, 2012). Remembering that scientific science strategies are utilized to take care of issues, just all-inclusive and troublesome approaches coordinating exploration, improvement, and advancement in interdisciplinary furthermore, trans disciplinary studies will make progressive advances. During the last not many a long time, logical science network has been assembled for the advancement of green practices. Concerning the modern-day objectives of the explanatory scientists, the incredible test is to adjust the standards of Green Investigative Science, with that of the twelve standards of the Green Science (Valcarcel, 2012, Anastas, 1999 & Armenta, 2015). Despite unchallenged advances in the systematic equipment, test prep is the bottleneck of each systematic technique. The unfriendly ecological effect of expository methods can be defeated applying the accompanying techniques: scaling down the expository scale or potentially supplanting perilous solvents by more secure options. Although the perfect circumstance is the advancement of "dissolvable free" extraction plans (Vian, 2017), this idea is still rather utopic. In this manner, the quest for elective solvents is of most extreme significant (Pereira, 2015). In recent decades, ionic fluids (ILs) increased incredible consideration as green media. In any case, their greenness is regularly contended, because of their poor biodegradability, biocompatibility, and manageability (Espino, 2016). Another class of solvents, which depend on the partner's eutectic behavior, was then questioned as an alternative to ILs. High eutectic solvent (DES) was developed by Abbott et al., al., 2003 demonstrate the wide range of liquids available as soluble materials and their interesting properties. The eutectic framework comes from the Greek “eu” means simple and “teksis” liquefaction, which melts and freezes at a single temperature lower than the intended liquefaction of various substances. Means a mixture of substances that form a common super section component, and the DES has been used for spontaneous reactions at 60 °C, spontaneous extraction, electrochemistry, and protein reactions (Dai, 2013). Later DES was coined as NADES and these solvents were totally built on the principles of green chemistry.

NADES are obtained on mixing of hydrogen bond donors with hydrogen bond acceptors molecule, to lower its melting point. There are some molecules such as alcohol, aldehyde, amines, and ketone groups which react as both hydrogen donor along with hydrogen acceptors. On the basis of chemical nature of the NADES are divided into four parts; a) mixture of sugars, b) derivatives of organic acids, c) derivative of choline chloride, and d) other combinations (Espino, 2016). Major advantages of NADES like readily available components, reusability, environment friendly (biodegradability), and cost effectively it is appealing research to go for their analytical application. NADES can be used for the separation and extraction of both organic and inorganic compounds like phenols, flavonoids, amides, and aromatic compounds. They are low cost, less volatile, and easy to prepare for extraction and separation of bioactive compounds. The one and only fact for the best results is to figure out the proportion of hydrogen bond donors and hydrogen bond acceptors, so it needs to be adjusted according to the samples.

Three strategies are most ordinarily utilized for getting ready NADES:

a) **Heating and stirring**: the blend of the salts is put in a container with a mixing bar and then they are warmed with unsettling till a reasonable fluid is framed (around 30–90 min) (Dai, 2013 & Dai, 2015).

b) **Evaporating technique**: segments are broken down in the water and dissipated with the help of rotatory evaporator, then this fluid acquired is putted in desiccator with the silica gel till it reaches at consistent weight (Dai, 2013).

c) **Freeze drying technique**: fluid arrangements of each of the salts is freeze dried.

Chandran et al., (2019) studied Deep Eutectic Solvents, which were mixture of acids and bases with different arrangements of anions as well as cations and were responsible for making a new form of ionic liquids. For reducing the Sulphur content during fuel production desulphurization is employed, which is done by hydro desulphurization method. This method increases the cost of production of fuel, thus DESs can be a suitable replacement for ionic liquids. These solvents maintain their liquid form upto a temperature of 343K. Based on the combination of quaternary ammonium salts, metal halide, hydrated metal halide, and hydrogen bond donors, DESs are classified into type I, II, III, and IV. DESs preparation involved the selection of different hydrogen bond donors as well as the hydrogen bond acceptors in different molar ratios i.e., 1:2. 1:3, and 1:5. The extraction time was 10 min to attain equilibrium in solvent containing toluene. The desulphurization yield decreased with an

increase in temperature, a 20% decrease was reported at 333K than at 293K. DESs solvents are cost-effective as they can be used again for extraction upto five times, after that the DESs lose their extraction efficiency due to change in viscosity, solute-solvent interactions, saturation limits, impurities, and solvent degradation during extraction. Thus, the study concluded that DESs are a better choice for desulphurization than ionic liquids as DESs can be used multiple times with full extraction efficiency. Secondly DESs shows impressive properties such as they are easy to prepare, biodegradable as well as non-toxic (Unlu et al., 2019).

Achkar et al., (2021) studied the basic characteristics of DESs which could be alternatives of the conventional organic solvents. DESs are classified in the four types which is based on a formula ($Cat^+ X^- zY$) and Cat is ammonium, X is a base and Y is an acid and the number of molecules of Y is represented by z. DESs are prepared using the heating method in which the compounds are mixed and heated at temperatures between 50-100^o C. The other method of preparation involves grinding of compounds using mortar and pestle for making a clear ionic liquid. The eutectic solvent should have a melting point below the ideal DES to be considered good for deep extraction. DESs usually have high density than water but the density depends on multiple factors *viz.* temperature, with the increase in temperature the density decreases. The viscosity of DESs is extremely high at room temperature, sugar-based eutectic solvents are more viscous than choline and ethylene-based solvents. With an increase in temperature, the viscosity decreases, and the ionic conductivity of the solvents increases, which means DESs have poor ionic conductivity. Due to high viscosity, the surface tension of DESs is also high. Presence of water for making DESs is not recommended as it is considered an impurity, physiochemical properties are highly affected, and water interferes with the viscous network of the solvents. Due to above-mentioned properties DESs are the most studied green solvents nowadays.

Zainal-Abidin et al. (2017) worked on usage of the DESs for extracting different bioactive components. Bioactive compounds are particularly useful in providing many health benefits thus their extraction is particularly important which is growing in the pharmaceutical industry and more interest is toward emerging green solvents. The physicochemical properties of the DESs are mostly temperature dependent. DESs has a lower melting point than ideal eutectic solvent is considered best for the extraction of different compounds. Different DESs have been considered good for extracting phytochemicals such as ammonium based Deep Eutectic Solvents are optimum for extracting the phenolic content from *Lonicerae japonicae.* Malonic

acid and choline based DESs are optimum for the extraction of flavonoids, a higher yield of rutin i.e., 0.39g/g was extracted using DESs. Similarly, protein partition, tools, terpenoids, polysaccharides, keratin, and astaxanthin are extracted in more concentration with DESs. The viscosity of DESs sometimes hampers the extraction of compounds which can be reduced with the addition of water, the addition of excessive water may suppress the capability of extraction of the solvents. The study concluded that due to low toxicity and less harmful effects on the environment, DESs can be an alternative for extracting the phytochemical constituents.

Dai et al. (2015) studied a new kind of solvents called NADES. NADES are highly viscous which sometimes proves to be a disadvantage for the extraction of compounds. Different NADES were prepared using the heating method with different solvents mixed in varying molar ratios. The NADES were evaluated for physical properties by diluting them with deionized water. The molecular structure of the solvent was proved to be best in a 1:1 ratio of malic acid and proline but after dilution with water, the molecular structure compromised, and hydrogen bonds disappear. NADES viscosity decreases with an increase in water content whereas conductivity of the solvent first increases and then decreases after a peak value of 10-100 times. The density of the solvent decrease linearly with water and water activity increases with an increase in water content. Thus, NADES has a hydrogen bonding interaction between compounds which can be decreased by dilution with water. This will decrease the hydrogen bonding in the solvent above 50% due to which different combinations are required to maintain the properties of NADES.

Ivanovic et al. (2020) studied innovative methods for extracting and isolating the various phytochemicals by using DESs. People are making a shift toward the use of functional food which provides nutrition and health benefits. These foods are made with the help of bioactive compounds from diverse sources, which require efficient extraction methods. DESs and NADES are the solvents that are employed for efficient extraction of compounds. Identification of bioactive compounds uses sampling, pre-treatment, extraction of compounds, filtration of crude extracts, and identification of bioactive compounds. The DESs were first reported in 2003, and since then extensive studies are done on their use in replacing ionic liquids with green solvents which are less toxic. Ultrasound-assisted extraction makes use of DESs for extracting the bioactive compounds with action of contraction and expansion cycles of the material. Another method is microwave-assisted extraction which uses 0.3-300GHz of frequencies. It is done in a closed vessel where the temperature is increased

rapidly with less extraction time and higher yield. The study confirms the use of DESs and NADES for the extraction of bioactive compounds with different techniques.

Antony & Farid (2022) studied the effect of temperature for extraction of phytochemicals. Compounds with high free radical scavenging activity and other health benefits found in plants are called polyphenols, which include phenolics, flavonoids, lignans, and stilbenes. There are two types of polyphenol extraction through traditional or emerging technologies. Polyphenol extraction was highest at 60-80^{o}C with solvents ethanol, methanol, and acetone used for extraction. The polyphenolic extraction increases with an increase in temperature however thermal degradation of polyphenols occurs at exceedingly high temperatures. Anthocyanin extraction decreases rapidly with an increase in temperature above 45^{o}C. Different conditions for the extraction of polyphenols can affect the degradation of compounds such as catechins can be oxidized due to presence of oxygen. Presence of enzymes *viz.* glycosidases, polyphenol oxidases, and peroxidases causes the degradation of polyphenols. Anthocyanin can be broken down into anthocyanidins and unstable sugars by glycosidase which causes degradation of polyphenols. Solvent concentration and pH also play a significant role in preventing the degradation of compounds. Different compounds degrade at different temperatures and sometimes Maillard reaction products also promote degradation of compounds. The type of compound to be extracted from the plant and the form it is present in the plant and the overestimation of the compound also affect the extraction.

Abdelhamid et al., (2022) discussed the preparation of flavored yogurt incorporated with orange peel oil. It was examined for checking its antibacterial, antiviral, and antioxidant properties. The oil contains some biological activities such as flavonoids, limonoids, coumarins, sterols, organic acids, alkaloids, and phenolic compounds as well as dietary fibers, essential oils, and carotenoids. The orange peel oil was added in concentrations of 0.0, 0.5, and 1.0% into the yogurt. The fortified yogurt shows antibacterial activity higher in gram-positive than gram-negative. The oil peels do not show any effect on the growth of fungus and yeast (concentration 0.0mm) as compared to other concentrations. The antiviral activity was evaluated on the MA 104 cell line; the orange peel oil showed positive results against rotavirus and the orange peel oil has high radical scavenging activity of about 94%. By using this oil in preparing orange yogurt, a fermented milk product was obtained that was good for its sensory and health properties. Therefore, it could be recommended for using applications like these for their probiotic synergistic properties.

Smith et al., (2021) studied the applications of commonly used deep eutectic solvents. DESs are prepared through a eutectic mixture of compounds and can be used as an alternative for ionic liquids which are made of one type of anions or cations. Both DESs and ionic liquids have the same physical properties but differ in chemical properties. DESs are easily available and can be prepared from universally accessible compounds than ionic liquids. There is a linear relation between conductivity and fluidity of solvents. Firstly, ionic liquids were green solvents but later, their toxicity ruled them out and DESs proved to be green solvents with low toxicity. DESs are used in metal electrodeposition by chrome plating, aluminum, copper, zinc, nickel, and alloy coatings. Another application includes electropolishing which is done by immersing the metal surface into the solvent to reduce roughness, and corrosion and increase optical reflectance. DESs are used in the extraction of metals and chemical synthesis by iono thermal synthesis, biotransformation, gas adsorption, and purification of biodiesel. This study concludes the use of DESs as an alternative for ionic liquids in different applications.

2.10 STIRRED YOGURT

Yogurt is an effective food for the curing dysentery, constipation, diarrhea, alongwith carcinogenesis (Kamruzzaman et al., 2002). There are factors which are directly linked to the popularity of the yogurt, like sensory characteristics (Routray and Mishra, 2011). For increasing the sales of the yogurt continuous evaluations and modifications are required in the final product so that it can attract the consumer and fulfil the needs (Teshome et al., 2017). Dhavi et al.; 2020 and Weerathilaka et al.; 2014 studied effect of fortification on fermented foods along with their nutritional values. Buffalo milk is supposed to be the best for making dairy products because of high nutritional composition especially for the formation of curd, cheese and ice cream, it consists of almost twice as much as butter as of cow's milk along with higher amounts of solid particles and casein which makes it the first choice for consumers (Achi et al; 2019). There are ample number of fermented products available in the market which are enhanced with the medicinal plants or plant derived compounds (Iriondo et al; 2018). The formulation of the dairy products is done with the medicinal herbs to improve the medicinal potential of the yogurt (Jamshidi et al; 2018). *Moringa oleifera* or the drumstick plant is of great medicinal values like- antitumor, anti-inflammatory, anti-bacterial, hypocholestrolemia, and anti-diabetic. Moringa is of great importance because of its multifunctional medicinal values. Moringa can be added to the yogurt to fortify it and to see the health impacts of Moringa enriched yogurt. Then the effects

of the fortified yogurt is assessed by exploring the physiochemical and sensory properties of the yogurt (Van et al; 2011).

People have made a shift toward functional food for the increase in life expectancy. Das et al., (2019) reported the effect of yogurt manufacturing process on marketability of yogurt. Yogurt is a fermented milk product prepared using lactic acid-producing bacteria, Nomadic people discovered it in the Middle East. Yogurt is a complete package of nutrients such as proteins, minerals, and vitamins and it could be manufactured in stirred, drinkable, flavored, Swiss, and Greek styles. All these manufacturing styles affect different preferences of our society. Both starter cultures and the fermentation time are important in the manufacturing of yogurt. Other attributes like color, texture, and flavors highly affect the marketing of yogurt. Yogurt is sold as functional food which provides nutrition as well as protection from diseases. The introduction of probiotics, which increase the beneficial bacteria in the gastrointestinal tract is present in the yogurt and using a new concept of Made in Transit (MIT), increases the marketability of yogurt. MIT is a supply chain concept in which the product is made during its transport to the market location. Yogurt is made through fermentation which can be done during its transportation under a controlled environment. All these attributes and techniques help in increasing the marketability of yogurt.

Research was done by Wang et al., (2019) on the incorporation of apple pomace into stirred-type yogurt and drinkable yogurt. Fruit pieces along with non-concentrated yogurt are used for incorporation in stirred-type yogurt. Stirred-type yogurt is a semi-solid, pourable viscous product. Yogurt drinks are prepared using assorted flavors, sugars, and dispersion media for diluting the yogurt. Apple pomace is a product derived from apple cider. It has high fiber and phytochemical content. Apple pomace was added in concentrations of 1, 2, and 3% and the addition of apple pomace increases the viscosity of the yogurt more than the control yogurt. The pH of the yogurt decreased with the addition of apple pomace whereas titratable acidity increased with days of storage. This is due to the continuous fermentation by the lactic acid-producing bacteria. Apple pomace significantly reduces the accumulation of whey on the surface of the yogurt, whey off while stirring called syneresis from 23% in control to 9% in apple pomace enriched stirred yogurt. It increased the firmness, consistency, cohesiveness, and viscosity of the yogurt. The TPC of the yogurt increased from 41 to 74 mg GAE/g DW. These attributes show the positive effect of the addition of apple pomace in stirred yogurt and drinkable yogurt for higher nutrition while maintaining the yogurt matrix.

A group of researchers studied the use of *Moringa oleifera* extract in yogurt. Moringa is known for its medicinal use because it contains vitamins, minerals, and bioactive compounds that are useful in medicinal properties. Yogurt was incorporated with Moringa extract in 0-0.2% concentrations. The extract was prepared using Moringa leaf powder in water and heating it. The pH of the yogurt dropped, and the fermentation was significantly accelerated with *Moringa* extract. Yogurt incorporated with Moringa extract reduced the syneresis up to 21% and increase water holding capacity by 17%. Viscosity of the yogurt increased with the addition of extract, but it decreased along with storage. The antioxidant activity increased from 29% in control yogurt to 73% in Moringa extract yogurt. The extract increased the phenolic content two folds in the yogurt. Due to the bitter taste of Moringa extract, it increased the bitterness of the yogurt and the acceptability of yogurt decreased in comparison to control sample. The concentration of 0.05% does not change the overall acceptability of yogurt but the high concentration increased the bitter taste. Thus, the addition of Moringa extract increases the medicinal and nutritional properties of yogurt without sacrificing the overall acceptability of the yogurt (Zhang et al., 2019).

Bikheet et al., (2021) prepared a yogurt drink fortified with *Moringa oleifera* leaves. Functional foods are foods that provide nutrition as well as protection from various diseases. Yogurt is a fermented dairy product that can function as an excellent functional food due to its beneficial effects on human health. Moringa leaves are a reliable source of proteins, carbohydrates, iron, vitamins, beta-carotene, calcium, phosphorus, potassium, and other bioactive compounds. Moringa leaf extract prepared using ethanol and water, was used in the preparation of the yogurt drink. The yogurt drink prepared using the ethanolic leaf extracts showed more increase in the nutritional profile than the water leaf extract. Protein content was increased from 3.6 to 6.4% and the vitamin C content increased from 7 to 31 mg/100g. The viscosity and antioxidant activity of the drink also increased. Drink with 5% Moringa leaf ethanolic extract showed the highest nutritional content. Yogurt with 3 and 5% alcoholic extract gained the highest scores on the sensory evaluation. The study concluded that yogurt drinks fortified with ethanolic extract of Moringa leaf reported to be best for gaining substantial amounts of nutrients.

Effects of yogurt on infants and toddlers was studied by Donovan and Rao (2019). Yogurt is a nutrient-packed fermented product which is consumed all over the world and its health benefits have been studied extensively for adults but the effects on infants have not been studied properly. Consuming yogurt improves diet, metabolic activities, and many other

health benefits to the body. Different literature articles were assessed for the benefits of yogurt in infants. Studies supported that yogurt shows a positive effect against diarrhea. Consumption of yogurt daily helps in maintaining the good gut microbiota which maintains intestinal health and good digestion. Gut microbiota prevents several digestive tract diseases and prevents food sensitivity which occurs while eating some foods. Yogurt prevents many allergic diseases in infants. The study concluded that yogurt shows a positive effect in reducing the severity and duration of diarrhea, preventing allergy, and promoting gastrointestinal health which makes yogurt a good introductory food for infants.

The physicochemical, antioxidant, and sensory properties of yogurt fortified with flax seed powder was evaluated by Marand et al. (2020). Flax seeds are fiber and food crop that contains several vital fatty acids such as linolenic acid, eicosapentaenoic acid, as well as docosahexaenoic acid. Flax seed powder was added in concentrations of 0-5% in the yogurt. The pH of yogurt decreases because of the presence of the lactic acid-producing bacteria whereas titratable acidity increases. TPC of the yogurt reported to be increased with increase in powder concentration upto 19 mg GAE/g and the antioxidant activity also increased up to 55%. Although the nutritional profile of the yogurt was increased with flax seed, the sensory parameters scored highest for the control and lowest for the 5% powder-incorporated yogurt.

Different properties of yogurt incorporated with red propolis extract was studied by Santos et al. (2019). Yogurt is a product obtained after the fermentation of milk using different lactic acid-producing bacteria. The addition of thickeners, flavorings, and preservatives in yogurt has been practiced for ages, and the use of such ingredients in excess leads to allergies and digestive dysfunctions. There has been a shift toward the use of natural compounds such as red propolis for their therapeutic use which is mainly found in the mangrove region of Brazilian states. The yogurt was prepared with strawberry flavor and the addition of red propolis where yogurt with red propolis showed antibacterial, fungistatic, and fungicidal activity. Shelf life of the yogurt was checked up to 28 days, which showed the absence of harmful disease-causing bacteria. The apparent viscosity of the yogurt decreased with an increase in shear applied to the yogurt. Yogurt with red propolis was highly accepted in sensory evaluation however, the addition of excess red propolis may result in an unpleasant odor and color change. Overall, the yogurt was well accepted in taste, viscosity, and texture which are similar to traditional yogurt and showed its potential for commercial use.

A study done by Savaiano and Hutkins, (2020) revealed the health benefits of yogurt and fermented milk. Fermented dairy products such as yogurt have been the oldest food products consumed for good health. Consumption of fermented milk products improves gastrointestinal health. The intestine is home to many good bacteria that help boost our immunity, yogurt improves gut microbiota which performs critical health functions. Yogurt improves the digestion of lactose, decreases lactose intolerance, and shows positive outcomes against diarrhea and constipation. Studies showed the potential use of yogurt against irritable bowel syndrome and other digestive tract complications. Fortified yogurts showed positive effects against cardiovascular diseases and hypertension. It has been found to reduce LDL levels. Consumption of yogurt is known to have inversely associated with corectal, breast, and prostate cancer. Yogurt being a fermented product is already in simpler form which makes it easy to digest and prevent its accumulation as fat which results in weight loss. Yogurt helps in improving the action of insulin and insulin sensitivity which helps in reducing the risk of diabetes. Its consumption increases bone strength and reduces dental caries and risk of hip fractures. The study concluded that yogurt can be a healthy alternative for people with lactose intolerance to get all the health benefits of dairy products.

Li et al., (2020) revealed the effects of seasonal variations on the quality of different types of yogurts. Yogurt is a popular fermented product eaten by all age groups due to its unique texture and health benefits. Two types of yogurts set (regular) yogurt and Greek-style yogurt were prepared using lactic acid-producing bacteria. Variation in season did not have any effect on the fermentation time of both yogurts. The firmness of set yogurt had no effects for upto 7 days whereas the firmness of Greek-style yogurt increased in the first 7 days and then remained stable up to 28 days. There was also a difference in the firmness of yogurt with variation in seasons, as mid-season yogurts were not that firm compared to regular set of yogurts. Regular set yogurt showed the highest syneresis and lowest water-holding capacity during mid-season than Greek-style yogurt. The study showed that there is no effect of season on the fermentation time of both yogurts but early-season yogurt was firm as compared to mid-season yogurt.

The effect of fermentation temperature on yogurt was studied by Yang et al., (2021). Yogurt is a fermented product in which the proteins are been degraded into amino acids and peptides. Lactose-intolerant people can easily consume it as the lactose in yogurt is broken into simpler forms after fermentation. Yogurt contains 17 free amino acids and 8 essential amino acids which are affected by different fermentation temperatures. The amino acid concentration was

more at 42ºC than at 30ºC. Both mono-saturated and poly saturated fatty acid first reduced, and afterwards it was increased with the rising temperature, and was highest at 45ºC. The water retention ability of yogurt first increased and afterwards reduced with fermentation temperature. In the texture analysis the hardness increased with increase in temperature. Sourness of yogurt also increased on rising temperature till the maxima at 45ºC. The study showed the effect of different fermentation temperatures on nutrients and sensory properties of yogurt. The highest PUFA content was found at 45ºC, but the sensory analysis was not good.

According to Gilbert and Turgeon (2021) revealed a relationship between physical properties of yoghurt with the micro-structure. The microstructure of any food material is directly linked to rheological, sensory, and water-holding properties of the product. During fermentation, yoghurt undergoes milk gelation in which the low viscous milk is converted into a semi-solid or solid form. The qualities of stirred yoghurt are function of the microstructural elements like viscos nature, firmness, creamy attribute, or syneresis of microgels determine their sizes. It is currently included in most of the research on yoghurt since it is the easiest microstructural characteristic to detect. The qualities of stirred yoghurt have been claimed to be influenced by other features, such as microgel form or compactness, which can only be observed through imaging methods. The complexity of the structural organisation of microgels, their interactions, and aggregation can be dissected using techniques that involve varying degrees of sample destructuring, revealing phenomena like rebodying during storage or the influence of process and formulation on the properties of stir yoghurt. To evaluate the microstructure of the stirred yoghurt and look into molecular connections or water inclusions in the network, extra methods like tribology or studies of water mobility are useful tools. The intrinsic characteristics of microgels, such as network stiffness, porosity, entanglement with other microgels or EPS, and how to manipulate them to enhance stirred yoghurt qualities, are, however, only understood to a limited extent.

Mousavi et al., (2019) studied the effect of supplementation of flaxseed into yogurt. The rheology, colour indices, and sensory characteristics of flaxseed-fortified yoghurt samples were investigated using the response surface methodology. A good technique for assessing the hardness, stickiness, cohesion, chewiness, gumminess, and elasticity of yoghurts is the analysis of rheological characteristics. The findings demonstrated that all of the rheological parameters listed, with the exception of stickiness and sensory characteristics, significantly increased as a function of flaxseed content and storage duration in flaxseed-fortified yoghurt

samples. A* and b* values were higher in the flaxseed-containing samples than in the control yoghurt sample, however the L* value as a colour index was comparatively lower. In general, functional meals with good results may be produced by mixing 2.63% flaxseed with yoghurt samples. Adding 2.63% flaxseed to yoghurt samples often resulted in functional meals with acceptable texture and sensory qualities that persisted up to 17.17 days after being stored in the refrigerator. On an industrial scale, flaxseed-fortified yoghurt samples of acceptable quality may be produced using the optimisation quantities derived using RSM in this work. Therefore, this research might be used to the industrial manufacturing of yoghurt that has been enhanced with flaxseed as a functional food.

Research Gap- The utilization of *M. oleifera* flower extract in the development of stirred yogurt for its therapeutic properties presents a promising avenue for both the food and health industries. However, there are still notable research gaps that warrant further investigation and exploration. The existing literature highlights several areas where additional research could contribute valuable insights:

Optimization of Extraction Techniques: Existing studies may not comprehensively cover the diverse extraction techniques for obtaining *M. oleifera* flower extract. Further research could focus on optimizing extraction methods to enhance the yield of bioactive compounds with potential health benefits.

***Long-Term Health Effects*:** While short-term therapeutic effects may be studied, there is a lack of research on the long-term health implications of regular consumption of Moringa-fortified yogurt. Comprehensive studies on the sustained benefits and potential risks over an extended period are necessary for a thorough understanding of its impact on human health.

Addressing these research gaps will not only advance the scientific understanding of *M. oleifera* flower extract in yogurt but also contribute to the development of functional foods that promote both nutritional and therapeutic benefits. Future studies should strive to fill these knowledge voids, paving the way for the effective integration of *M. oleifera* flower extract into mainstream food products for improved health outcomes.

Chapter-3

HYPOTHESIS OF THE STUDY

Moringa oleifera flowers are one of the underutilized parts of the plant with potential health benefits. It could be utilized in a proper manner for developing functional foods or nutraceuticals. Other parts of the plant *viz.* leaf, fruit, and bark has been used for various purposes and are a great source of economy. *Moringa oleifera* flowers are an ironic source of antioxidants, flavonoids, and other nutritional components (vitamins, minerals) so they would be a potential source for the development of functional foods with added value.

Chapter-4

OBJECTIVES OF THE STUDY

The main objectives of the proposal are as follows:

1. To optimize the extraction process of *Moringa oleifera* flower by using DES (Deep Eutectic Solvents) for optimal therapeutic properties.
2. To develop and evaluate stirred yogurt enriched with *Moringa oleifera* flower extract.
3. To study the storage stability of the developed product.

Expected outcomes

On completion of the thesis work these outcomes are expected:

1. *Moringa oleifera* flower's nutritional, physiochemical, and textural properties will be in the form of a database.
2. *Moringa oleifera* flower extract-enriched yogurt will be developed.
3. The prepared product will be optimized, and its shelf life is more than that of normal yogurt.

Chapter-5

METHODOLOGY

5.1 Materials

Folin-Ciocalteu reagent (CDH, New Delhi), Sodium carbonate, gallic acid, sodium nitrite ($NaNO_2$), quercetin, aluminium chloride, methanol, 2,2-diphenyl-1-picryhydrazyl radical (DPPH), sodium hydroxide (NaOH), ChCl (choline chloride), glycerol, malic acid, lactic acid, glucose, *P*-nitro phenyl-butyrate (*p*-NPB), EDTA (LOBA Chemie Pvt Ltd., Mumbai), tris-buffer (Tris-HCl) (HIMEDIA, Mumbai, Maharashtra) ELISA reader (Biotek 800, Mumbai), α-glucosidase, phosphate buffer, L-proline (Avanscure lifescience Pvt. Ltd, Gurugram, India). *Moringa oleifera* flowers collection was done in Karnal, Haryana, in March and April 2021. The experiments were conducted in the Food Technology Laboratory, School of Agriculture, Lovely Professional University, Phagwara, and Punjab, India. Buffalo milk was procured from the Lovely Professional University farms and the starter culture (*Lactobacillus delbrueckii sp. Bulgaricus* and *Streptococcus thermophilus*) used was ordered from Biocelx lab by SRL, Surat, Gujarat.

5.2 Methods

5.2.1 Plant Material Collection

Collection of mature flowers of the *Moringa oleifera* plant was done in Karnal, Haryana in March and April 2021 (Fig. 5.1 A).

5.2.2 Cleaning

Collected flowers were then washed thrice with the tap water and once with distilled water for removing the impurities such as soil, dust, dirt, and insect waste (Matabura et al., 2022).

5.2.3 Drying and powder formation

Moringa oleifera flowers were then dried at 45°C in a tray dryer for 48 hours. Dried flowers were then powdered before use with the help of a Sujata Powermatic plus 900 watts grinder and sieved with a 100µm sieve with the help of an electric sieve shaker (SRI equipment, New Delhi) and stored in polypropylene bags (Ziploc®) and kept in a cabinet at room temperature to avoid moisture and contamination (Figure 5.1 C).

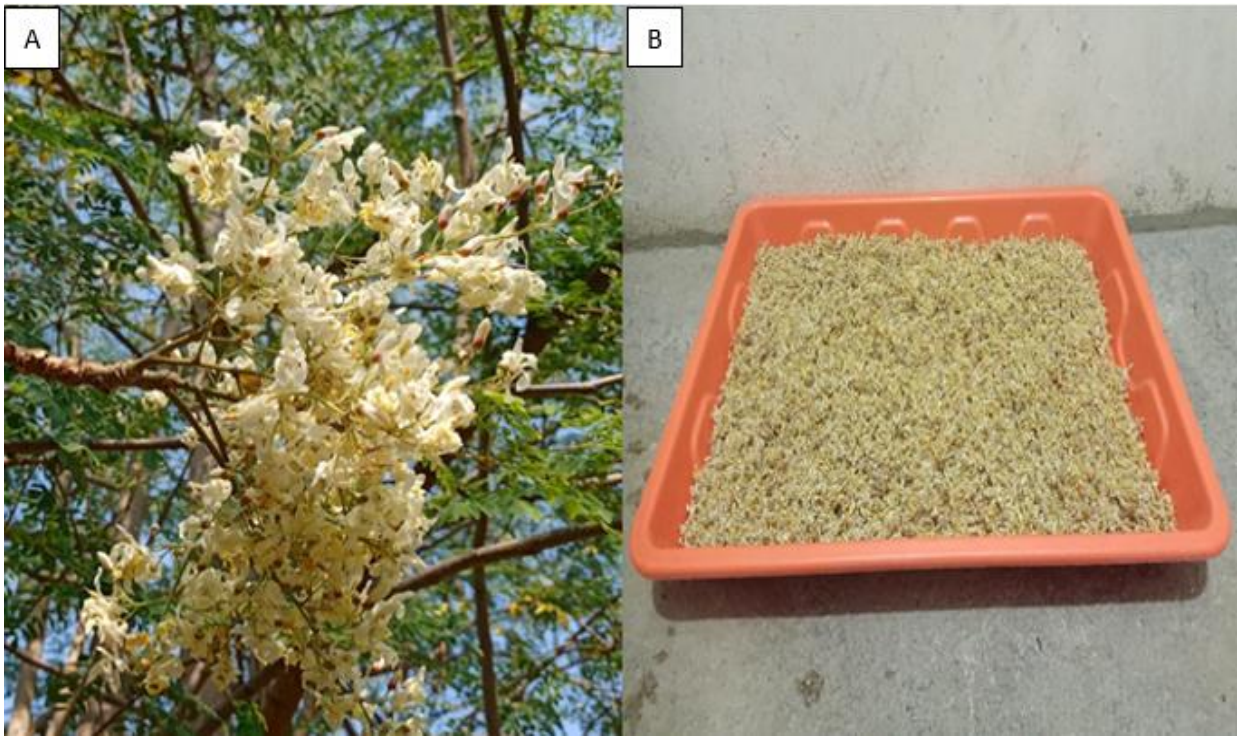


Figure 5.1-A, B: Pictorial representation of *Moringa oleifera* flowers

5.2.4 Sample preparation

The different sample was prepared by using *Moringa oleifera* flower powder. The first sample was used for fat estimation with the help of the soxhlet apparatus and after that fiber estimation was done to estimate the fiber content in the same defatted powder because the defatted powder is needed for the estimation of fiber content. Another sample was used for other tests such as proximate analysis (ash, fat, fiber, moisture content, and protein), physiochemical properties (water absorption index, water solubility index, bulk density, tapped density, Hausner ratio, porosity, oil absorption capacity, foaming capacity, as well as foam stability) and for making flower extract for the estimation of phenolic, flavonoid content, antioxidant efficacy as well as other parameters of the extract.

Figure 5.1-C: Pictorial representation of *Moringa oleifera* flower powder

5.3 Proximate analysis

The Chemical composition of the *Moringa oleifera* flower powder (moisture content, fat, fiber, protein, carbohydrates, and ash) was determined. To calculate the moisture content of flower powder oven dry method was used. The crude fat of the *Moringa oleifera* flower powder was determined by using the Soxhlet extractor method, where petroleum ether was used as the extractor, and ash content was determined by using AOAC, 2000. Kjeldahl's method of protein estimation was used for determining the protein content of the powder where nitrogen content was multiplied with the factor 6.25. Fiber content was determined by using method given by Madhu et al., 2017. Carbohydrate content was determined by the following formula-

Carbohydrates= 100- (Fat + Moisture + Ash + Fiber + Protein) (1)

5.3.1 Moisture content

To determine moisture content all the petri plates were washed and then dried properly by using an air dryer (Labfit India Pvt. Ltd. Mumbai, Maharashtra, India). After drying the empty Petri plates weight was recorded for further calculations. After that 5 gm of the sample powder was taken in the petri plate and the weight was recorded again. Then the petri plates were kept in the oven for an hour at 100℃. After an hour the weight was recorded, and same procedure was repeated until we get the constant readings. After removing from the oven, the crucible was transferred to the desiccator to cool down.

s

$$\textbf{Moisture content } \% = \textbf{W3} \times \frac{\textbf{W2}-\textbf{W3}}{\textbf{W1}} \times \textbf{100} \quad \textbf{(2)}$$

Where,

W1= Weight of the empty petri plate,

W2= Weight of the petri plate with sample before drying

W3= Weight of the petri plate with sample after drying

5.3.2 Fat Determination

The crude fat content of the *Moringa oleifera* flower was determined using the Soxhlet apparatus. 3 g of the powder were taken in a thimble and the extraction was done by using 150 ml n-acetone as a solvent at 60℃ for 6 hrs. After 6 hrs the evaporation of the solvent was done by using a heating mantle. The difference of the initial weight of the flask and the final weight after evaporation (using a heating mantle) was recorded and the difference was found in crude fat % (Sultana 2020).

Formula used-

$$\% \textbf{ Crude fat} = \frac{\textbf{W2}-\textbf{W1}}{\textbf{weight of the powder sample}} \times \textbf{100} \quad \textbf{(3)}$$

Where,

W2= Weight of crude fat+ flask

W1= Initial weight of the flask

5.3.3 Determination of crude fiber

Crude fiber of the *Moringa oleifera* flower powder was calculated by taking 2 gm of the defatted sample by using 200 ml sulphuric acid for 30 minutes. After that, it was filtered with the help of muslin cloth and washed with boiling water (100℃) until the acid was removed. After that, it was boiled with 200ml of the sodium hydroxide for 30 minutes and then again, it was then filtered using muslin cloth and washed with 1.25% of 25 ml of sodium hydroxide, then with water, and lastly with 25 ml of 90% alcohol. Afterward, the residue was kept in a pre-weighed crucible to dry it for two hours at 130℃. After that crucible was cooled at room

temperature and put in a desiccator. After that, it was ignited at 600°C in the muffle furnace for 30 minutes and the sample was then cooled and weighed (Madhu et al., 2017).

% Crude fiber=(W_2-W_1) (W_3-W_1)/ Weight of sample×100 **(4)**

Where,

W_1 = Initial weight of the crucible

W_2= Final weight of the crucible

W_3= Weight of crucible with sample

5.3.4 Determination of Ash

3 gm of the *Moringa oleifera* flower powder sample was taken in a pre-weighed crucible, and it was put in the muffle furnace at 600°C for 6 hours. After that, the crucible was cooled at room temperature and weighed (Sultana 2020).

Ash %=W_2/W_1×100 **(5)**

Where,

W_1= Weight of crucible and sample before ashing

W_2 = Weight of crucible and sample after ashing

5.3.5 Determination of protein content

Kjeldahl method of protein estimation was used for the determination of protein content. 1 gm of the sample was taken for the digestion process, and it was transferred to the digestion tube. A blank tube was kept without the sample and marked as blank test. 2 Kjeldahl tablets, along with 20ml sulphuric acid were added to each test tubes. Tubes were then placed in the digestion unit for 1 hr at 420±20°C. After 1hour tubes were removed from the unit and allowed to cool for 20 minutes. After cooling, 80ml distilled water added to each of test tubes. Distillation as well as titration were carried out by taking 25ml of boric acid in a conical flask. 50ml of NaOH was added and the distillation process was carried out. Titration of the conical flask content was carried out with the help of HCl as standard solution and a

few drops of the indicator were added to it. Total amount of titrant used was recorded, on appearance of pink colour (Nimyel & Lori, 2022).

% Nitrogen (N)= (V_S-V_b) × M(HCl) ×1×14.007/W×10 **(6)**

Where,

V_s=Volume of HCl used for sample

V_b= Volume of HCl used for blank

M(HCl)= Molarity of HCl

1= Acid factor

14.007= Molecular weight of Nitrogen

10= Conversion factor (mg/g to %)

W= Weight of the sample (gm)

$$\% \boldsymbol{Crude\ Protein} = \% \boldsymbol{N} \times \boldsymbol{F} \quad \textbf{(7)}$$

Where,

F= 6.25 (for all forages, mixed feeds, feeds, 5.70 for wheat grains, and 6.38 for milk and meat products)

% N= % Nitrogen

5.4 TECHNO-FUNCTIONAL PROPERTIES OF *MORINGA OLEIFERA* FLOWER

5.4.1 Water absorption index and water solubility index

2 grams powder sample was added to the 20ml of the water and mixed properly. The mixture was left at room temperature for 30 minutes and then centrifuged at 4000rpm for only 10 minutes. The supernatant was collected in a measuring cylinder and used to estimate the water absorption index as well as water solubility index of the flower powder **(**Awuchi et al., 2019 & Sharma et al., 2019).

WAI=Weight of residue in centrifuge tube/ Dry sample weight **(8)**

WSI= Weight of dissolved sample in supernatant/ Dry sample weight×100 (9)

5.4.2 Bulk Density

It is defined as the density of the powder which includes volume of voids and pores. To calculate bulk density of the powder sample 2 gm of the powder was filled in a measuring cylinder and the height of the sample in the measuring cylinder was noted (Bala et al., 2020). Bulk density was calculated by using the following formula

Bulk Density = M/ V **(10)**

Where,

M= Mass of the sample powder

V= Volume of the measuring cylinder

5.4.3 True/ Tapped Density

It is the actual density of the powder excluding the volume of the voids and pores of the powder sample. It was calculated when the powder in the measuring cylinder was tapped continuously until a constant volume was obtained. Tapped density of the sample is mass that is calculated after tapping the powder (Awuchi et al., 2019).

True density= Mass of compact powder on tapping/volume of the powder on tapping **(11)**

5.4.4 Angle of Repose

It is an indicator of the powder's clump-forming capacity. 5 gm of *Moringa oleifera* flower powder was taken and passed through a funnel to check its heap-forming capacity. The base was kept flat so that angle can be measured properly (Jha et al., 2021).

To calculate the angle of repose the following equation was used-

$\alpha = \tan^{-1}(r/h)$ **(12)**

Where,

r = radius of the heap in cm and

h= height of the heap in cm

5.4.5 Hausner ratio

It represents the cohesiveness and flowability of the powder samples and is defined as the ratio of the true density to the bulk density (Jha et al., 2021).

$H_r = T_d / B_d$ **(13)**

Where,

T_d= Tapped density and

B_d= Bulk density

5.4.6 Porosity

It is defined as the difference between the bulk densities to the true density **(**Awuchi et al., 2019).

Porosity=Bulk density - True density **(14)**

5.4.7 Foaming Capacity and foam stability

The foaming capacity of a powder was measured by using the method given by Awuchi et al., 2019. Protein is responsible for the foaming, so foam stability is the time required for the protein to lose its stability by 50%. Foaming capacity, foaming stability both depend on the interfacial spaces formed by the proteins and the foam or bubbles created by the suspension of proteins. 1 gm of powder was mixed in 50ml of water in a measuring cylinder and was thoroughly mixed for 5 minutes so that foam can be produced. The foam formed was measured instantly (Awuchi et al., 2019).

Foaming capacity and stability are measured by the following equation-

Foaming capacity (FC)%= $V_a - V_b / V_b \times 100$ **(15)**

Where,

Va = Volume of liquid with foam

Vb= Volume of the mixture before foaming (blending)

Foaming stability was determined by measuring the foam left in the measuring cylinder after 1 hr. It was calculated by using following formula-

Foam stability (FS)% $= \frac{T}{V} \times 100$ **(16)**

Where,

T= volume of foam after time

V= initial foam volume

5.4.8 Oil absorption capacity

1 gm flower powder was mixed in 10 ml oil in a centrifuge tube. After mixing well the mixture was then centrifuged at 2500rpm for 10 minutes. The oil remained as the supernatant was pipetted out and then the weight of the centrifuge tube was recorded. So, the amount of oil absorbed by the powder sample is the oil absorption capacity (Jha et al., 2021).

Oil absorption capacity (ml/g)=V_1-V_2/ W **(17)**

Where,

V1= Initial volume in centrifuge tube

V2= Final volume in centrifuge tube

W= Weight of the powder

5.5 CHARACTERIZATION OF THE *MORINGA OLEIFERA* FLOWER POWDER

5.5.1 Fourier transform infrared Spectroscopy (FTIR)

FTIR is used for identification of the functional groups of unknown compounds in the samples (4000-400cm^{-1} wavenumber). In FTIR wavenumbers are used because it allows the users to compare the IR spectrum conveniently (Chauhan et al., 2021). FTIR of *Moringa oleifera* flower powder sample was performed on the Perkin Elmer Spectrum IR Version 10.6.1 with ATR and Pallet accessories and the data was analyzed with the help of spectrum software (Systat). For analysis, 2mg of the sample was taken and put on the diamond ATR top plate because the diamond is the preferred choice for most applications because it is robust and durable. After putting the sample, the pressure arm of the ATR was positioned over the powder sample so that it can be locked in the precise position, and force is applied over it. FTIR software utilizes a preview so that the spectrum quality could be monitored in real-time, once it is clear then the final image was collected, and it only takes about 32 seconds to collect the spectrum (Anand et al., 2016).

5.5.2 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS is analytical method that gives results that are the combination of gas chromatography as well as mass spectrometry and is used to measure bioactive compounds with great accuracy even if they are present in trace amounts (Kandeepan et al., 2022). It was done by using Shimadzu TQ8040 at Q3 scan mode and was done with a start time of 4 minutes and an end time of 28 minutes, the initial temperature of the column was kept at 80 ℃ and then it was increased to a temperature of 280 ℃. The temperature of injection port was kept at 280 ℃ and the interface temperature was maintained at 290 ℃ and 2 ml of sample was introduced with a syringe in a split ratio of 5:1. In the end all the compounds present in *Moringa oleifera* flower extract were identified using the retention time, with mass spectra of NIST spectral library for GC-MS software. The carrier gas used in the gas chamber was helium at a flow rate of 2ml/min (Anand et al., 2016).

5.5.3 Thermogravimetric analyser (TGA)

A thermogravimetric analyzer is used to check the change in mass upon increasing the temperature of the system and it is used to obtain the decompositional temperatures and oil volatility of the substances (Escalante et al., 2022). Perkin Elmer TGA 4000 was used to check the thermal stability of the *Moringa oleifera* flower powder. 2.3 mg of the sample was taken and the initial temperature was set at 30℃. After that nitrogen gas was supplied at 20 ml/minute, and the temperature was raised up to 600℃ at 10℃/ min. Then the sample was kept for a minute at 600℃, and the weight changes were observed in the form of a graph and the values were noted as observed (Balogun et al., 2021).

5.5.4 Field Emission Scanning Electron Microscopy (FESEM)

FESEM was performed by using JEOL JSM-7610F Plus and it is the most versatile technique to observe the surface morphology of a sample. Conventional microscopy is not that advantageous when compared with electron microscopy because FESEM gives the advantage of high magnification power, great resolution, and easy sample preparation techniques (Gourai et al., 2023). FESEM provides topographical along with elemental information on the sample at various magnifications. In FESEM a field emission cathode was used which has an electron gun for the scanning electron microscope and it provides an extremely focused narrow beam for improved spatial resolution without damaging the sample. FESEM was performed in a high vacuum so that gas molecules could not affect the electron beam. A small

amount of powder (1mg) was taken on the glass slide and coated with gold film before morphological observation. After that the high magnification images were collected with various magnification powers (Subramanjam & Sathiparan, 2022).

5.5.5 Energy-dispersive X-ray Spectrometer (EDS)

OXFORD EDS was used for the EDS, and it is used for the elemental analysis at very low voltage. A few nanometres of the gold coating were done to provide electrical conductivity to the sample because it provides high-resolution images of the metals. Chemical characterization and elemental analysis of materials are made possible by the analytical technique EDS. Based on the atom from which they originate, X-rays have a distinctive spectrum. In this way, the composition of a specific sample volume that has been stimulated by an energy source may be determined. The element is identified by the position of the peak in the spectrum, and the concentration of the element is indicated by the intensity of the signal. In EDS sample material was irradiated with electron probe which resulted in the emission of the x-rays which characterizes the presence of the elements present (Gourai et al., 2023).

5.5.6 High-performance liquid chromatography (HPLC analysis)

Moringa oleifera flower extract was analyzed with two standards *ie.* Rutin (Tokyo chemical industry co. ltd.) and Quercetin hydrate (Loba Chemie Pvt. Ltd. Mumbai). The flower extract was quantified by using HPLC (Shimadzu Prominence i-Series LC-2030 Plus, Kyoto, Japan) consisting of an LC-20AD pump, an SPD-M20A ultraviolet-visible (UV-vis) detector along with an autosampler SIL-20AC, column oven CTO-10A. The pump was set at a low-pressure gradient so mixing was done before the pump and the sample was run at 1ml/minute at a pressure of 5400psi. The sample rack was 1.5ml with 105 vials and a rising volume of 500µl. The oven temperature was set at 30-85℃ whereas the wavelength was set at 190-800nm (Ntshambiwa *et al*., 2023).

5.6 PREPARATION OF DEEP EUTECTIC SOLVENTS (DESs)

6 types of DESs were prepared according to the procedure given by Bajkacz and Adamek, 2017. For preparing DESs Hydrogen bond donors and Hydrogen bond acceptors were mixed in various molar ratios (1:1, 1:2, and 1:3 respectively) and then they were allowed to mix properly by using a magnetic stirrer (REMI, Maharashtra) at 80℃ for 10 minutes, which resulted in a homogeneous and clear liquid, which is termed as DESs. All the chemicals used

in this study, along with their molar ratio are given in Table 5.1. For extraction, 1 g of *Moringa oleifera* flower powder was mixed in 10 ml of the DESs, where 30% of the water was used (v/v) to reduce the viscosity of the DESs. After that, all the mixtures were extracted at four temperatures ranging from 50, 60, 70, and 80℃ for 30 minutes. After this, all the mixtures were centrifuged (REMI, Arsh Enterprise, Ahmedabad) at 1000 rpm for 10 minutes. The supernatant was collected for further use in the experiments (Wu et al., 2020).

Table 5.1 Representation of Deep Eutectic Solvents with different molar ratios and temperatures

Solvents	Hydrogen bond acceptor	Hydrogen bond donor	Molar Ratio			Temperature (°C)			
ChCl-Gly	Choline chloride	Glycerol	1:1	1:2	1:3	50	60	70	80
ChCl-MA	Choline chloride	Malic acid	1:1	1:2	1:3	50	60	70	80
ChCl-LA	Choline chloride	Lactic acid	1:1	1:2	1:3	50	60	70	80
ChCl-Glu	Choline chloride	Glucose	1:1	1:2	1:3	50	60	70	80
LP-Gly	L-Proline	Glycerol	1:1	1:2	1:3	50	60	70	80
LP-LA	L-Proline	Lactic acid	1:1	1:2	1:3	50	60	70	80

5.7 EXTRACTION YIELD

After the collection of supernatants, (discussed under the preparation method) they were dried by using a rotary evaporator and then weighed to get the extraction yield. Extraction yield was calculated the following formula (Mahmoud et al., 2017).

Extraction Yield (%) = $W_2/W_1 \times 100$ **(18)**

Where,

W1= Weight of the raw material

W2= Weight of the extract

5.8 DETERMINATION OF TOTAL PHENOLIC CONTENT (TPC)

TPC of *Moringa oleifera* flower extract was estimated using method used by Wang et al., 2018. For that 50µL extract was mixed with 50µL of Folin-Ciocalteu reagent and incubated for 5 minutes at 30℃. After 5 minutes 100µL of Sodium carbonate (Na_2CO_3) was added to the test tube and incubated for another 20 minutes at 30℃. Then absorbance was recorded at 765 nm using UV spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer) and the calibration curve for the same was drawn with standard Gallic acid at concentrations (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml). The results were then expressed as mg GAE/g DW. For the working solution, 250µl of the solution was taken and it was raised to 1 ml by the addition of 750 µl of ethanol. 10 µl of the freshly prepared Folin-Ciocalteu reagent was added to the *Moringa oleifera* flower extract and was incubated for 10 minutes in the dark. After that 100 µl of aqueous Sodium carbonate (7.5%) solution was added and it was incubated for 30 minutes at room temperature (30℃). TPC was calculated by the regression equation and was denoted by mg (GAE) gallic acid equivalent/gm dry weight (Kumar et al., 2020).

5.9 DETERMINATION OF TOTAL FLAVONOID CONTENT (TFC)

TFC of *Moringa oleifera* flower extract was estimated by the method used by Wang et al., 2017. 100µL of the extract was taken in a test tube and the volume was raised to 500µL by adding methanol. Then 30µL (5%) sodium nitrite ($NaNO_2$) was added to the solution and kept for 5 minutes incubation period, after that 30µL of 10% aluminium chloride ($AlCl_3$) was added and incubated for another 6 minutes. Lastly, 200 µL 1M NaOH added and final volume was raised by adding methanol (1 mL). The mixture was kept for incubation for 30 minutes at 30°C and absorbance was taken at 510nm. The calibration curve was drawn between the standard Quercetin and various concentrations of the sample (100µg/ml, 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml). To prepare a stock solution, 1 mg of the *Moringa oleifera* flower extract was prepared in 10 mL ethanol and same procedure was followed as for the standard Quercetin.

5.10 DETERMINATION OF ANTIOXIDANT ACTIVITY ASSAY (DPPH RADICAL SCAVENGING ASSAY)

DPPH assay was performed by the procedure given by Muller et al., 2011. In this test, ascorbic acid was used as a standard for plotting the calibration curve. For checking the % antioxidant efficacy, 250µL of the flower extract was mixed with 2mL of 0.1mM DPPH solution and allowed to incubate for 30 minutes at 37°C. Absorbance was observed at 517nm by using UV spectrophotometer (Systronics, Ahmedabad) (Mehra et al., 2022). 1 mg of the *Moringa oleifera* flower extract was prepared using 10 mL ethanol. 250 µL of the sample was added from the stock solution, and 2 mL of 0.1mM DPPH solution was added and kept for incubation for 30 min at 37°C. Absorbance was measured at 517 nm using at a UV spectrophotometer (UV-1800 Shimadzu UV Spectrophotometer) for determination of the reduction of free radicals (Kumar et al., 2022).

The extracts antioxidant activity was calculated using the % inhibition formula:

$$\textbf{Inhibition (\%)} = (A\text{-}A_1)\ /A\times 100 \qquad \textbf{(19)}$$

Where,

A = absorbance of blank

A_1= absorbance of the extract

5.11 *IN-VITRO* ANALYSIS

5.11.1 LIPASE INHIBITION ASSAY

By dissolving 10 mg of lecithin, 5 mg of sodium cholate, and 80 mg of glycerol trioleate in 9 ml of 0.1 M TES buffer at pH 7.0, a substrate solution was made to test the lipase inhibition assay. Using 0.1 M TES buffer, several concentrations of the plant material (*Moringa oleifera* flower) extract were made (25, 50, 75, and 100 µgmL-1). A microplate well containing 20 µL of sample solution and 20 µL of the substrate solution and10µL of the lipase solution (20 g/ml) was added, mixed, and was incubated at 37 °C for 30 minutes. After 30 minutes of microplate reader (Patil et al., 2017).

Lipase inhibition (%) was checked by using the following formula-

Lipase inhibition (%) = [1-(OD_2-OD_1)/ (OD_4-OD_3) ×100] **(20)**

Where,

OD_1= Optical density of solution with extract, lipase, and substrate

OD_2 = Optical density of solution with plant material and substrate

OD_3 = Optical density of substrate & lipase

OD_4= Optical density of the substrate

5.11.2 AMYLASE INHIBITION ASSAY

Amylase inhibition activity of *Moringa oleifera* flower extract was determined by using method used by Karray et al., 2022. Starch solution was used as a substrate in this method.

The starch solution was prepared by using 0.1M tris-HCL buffer (pH 8), containing 20 mM NaCl and 0.1 mM $CaCl_2$. Amylase inhibitory activity was expressed in percentage for comparative study purposes. The inhibitory potency of *Moringa oleifera* flower extract was observed by calculating IC_{50} value.

Amylase inhibitory activity (%) = 1-[(OD_b-OD_a)/ (OD_d-OD_c) ×100 **(21)**

Where,

OD_a= Optical density of solution containing plant extract

OD_b= Optical density of solution containing plant extract and starch

OD_C= Optical density of solution containing starch and amylase

OD_d= Optical density of solution containing starch

5.11.3 GLUCOSE UPTAKE ASSAY

A 2000 KDa dialysis membrane was used for the determination of the effect of the glucose movement. The dialysis membrane was dipped in the distilled water so that it can expand, and then the dialysis membrane was sealed on one end and after that 1mg/mL of the flower extract was added, and then 15 mL of the 0.22mM glucose solution was mixed into it thoroughly. Afterward, the dialysis membrane was sealed from the other end and kept for incubation for 4 hours at 37℃ and it was allowed to centrifuge at 4800rpm for 20 minutes. Afterward, 45 mL 0.15 M NaCl was mixed into centrifuge tube and finally, glucose movement from dialysis membrane to the outer solution was calculated at the time intervals of 15, 30, 60, 120, 240, and 360 min using an Accu-Chek Active Glucose Monitor (Kumar et al., 2020).

5.12 ANTIBACTERIAL ACTIVITY ASSESSMENT

The antibacterial properties of *Moringa oleifera* flower extract were checked against one gram-positive bacteria (*Escherichia coli* ATCC25922) and one gram-negative bacteria (*Staphylococcus aureus* ATCC23235). The antibacterial activity of the *Moringa oleifera* flower extract was measured by using Nutrient agar media for the platting and disc diffusion method whereas Whatman filter paper discs were used to check antibacterial activity of *Moringa oleifera* flower extract (Kheir et al., 2014).

5.12.1 Preparation of stock solution

1g of *Moringa oleifera* extract powder was dissolved in 1 ml of 3% DMSO (Dimethyl Sulfoxide) to make a stock solution. *Moringa oleifera* flower extract stock solution was serially diluted in saline to make the working concentrations of 62.5mg/ml, 125mg/ml, 250mg/ml, 500mg/ml, and 1000mg/ml (Das *et al.,* 2020).

5.12.2 Antibacterial assay of Moringa oleifera flower extract

Antibacterial assay of *Moringa oleifera* flower extract was performed against the *Escherichia coli* and *Staphylococcus aureus* by using Agar disc diffusion method. The DMSO-soaked disc was used as a negative control, the Streptomycin-soaked disc as a standard antibiotic, and various concentrations of extract discs were used to check the antibacterial effect against the bacteria (Talath et al., 2022). Zone of the inhibition was measured by using a scale to check the antibacterial activity of the flower extract (Wali *et al.,* 2020).

5.12.3 Minimum inhibitory concentration (MIC) of Moringa oleifera flower extract

MIC is the minimum concentration of the *Moringa oleifera* flower extract which is required to inhibit the growth of the microbes. It was determined by using a 96-well microplate. 10 µl of nutrient broth (bacterial culture broth) was taken in each well and 200 µl of flower extracts (Concentrations ranging from 1000mg/ml to 62.5mg/ml) were added to the wells. The wells were then incubated at 37℃ for six hours and then 0.4mg/ml (40µl) of p-iodonitrotetrazolium violet (INT) was added in the wells and again was incubated for 12 hours at 37℃ temperature (Weli *et al*., 2019).

5.13 PREPARATION OF YOGURT

Buffalo milk was procured from the Lovely Professional University farms and the yogurt starter culture was taken from Biocelx lab by SRL, Surat, Gujarat, India. *Moringa oleifera* flower extract was extracted by using deep eutectic solvents L-proline and glycerol at a 1:2 ratio for the preparation of *Moringa oleifera* flower extract enriched yogurt.

5.13.1 Formulation of Moringa oleifera flower extract yogurt

Yogurt was prepared according to the standard parameters, 2% of the culture was inoculated in the homogenized milk (solid not fat was adjusted to 14% by adding skim milk powder) at a temperature of 60℃ and incubated for 6 hours at 40±2℃ temperature. The amount of skim milk required to adjust the solid not fat (SNF) content of buffalo milk to a particular level depends on the initial SNF content and the desired final SNF content.

Amount of skim milk = (Final SNF- Initial SNF)/ (SNF of skim milk) **(22)**

= (14-11)/ 9 = 0.33gm

After the incubation, 1-5% of *Moringa oleifera* flower extract was mixed according to the design given by Response Surface Methodology (RSM) software (Design-Expert.V8.0.6). Figure 5.2 represents the development of *Moringa oleifera* flower extract enriched yogurt.

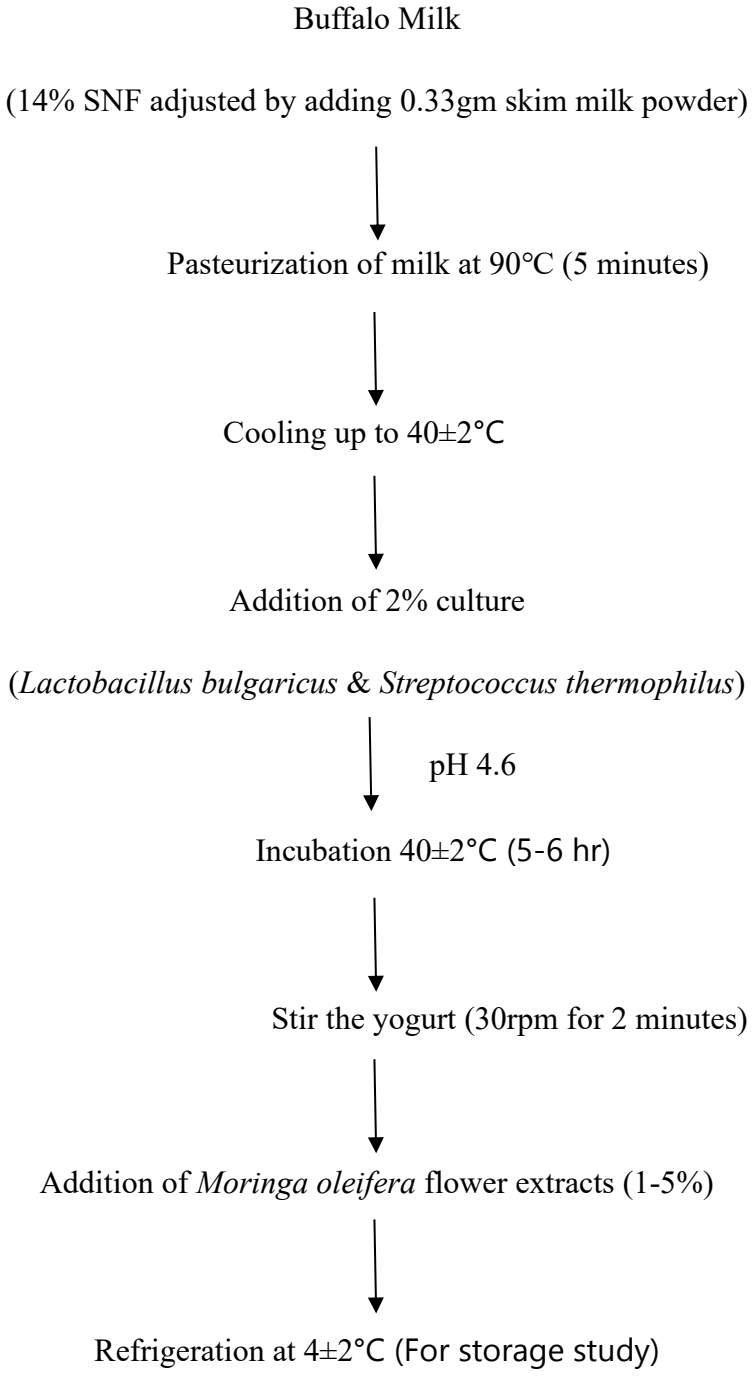


Fig. 5.2 Flowsheet of *Moringa oleifera* flower extract-enriched yogurt

5.13.2 Experimental design

Response Surface Methodology (RSM) is very frequently used software for the analysis of the data which results to standardization of the food products. In RSM central composite design (CCD) was used for analyzing the interaction between the variables. Response surface methodology (RSM) was used to know the optimum conditions of the *Moringa oleifera* flower extract-enriched yogurt using two parameters and three levels of central composite design (CCD), which depicted 13 runs for the experiment. Figure 5.2 shows the preparation method of *Moringa oleifera* flower extract-enriched yogurt.

Table 5.2 Indicating the independent variables and the levels of the factors used in the Central Composite Design (CCD)

Independent variables	**Levels**		
	Low (-1)	**Centre (0)**	**High (+ 1)**
A- Incubation Time (Hr)	5.0	5.50	6.0
B- Flower Extract (%)	1.0	3.0	5.0

In this analysis optimization of yogurt was done by using system Design Expert 8.0.6 software. Two independent variables were taken in this design which include Incubation time, and *Moringa oleifera* flower extract concentration. Three coded levels low (-α), center (0), and high (+α) were used in RSM (Table 5.2). Incubation time taken was between 5-6 hrs, and the flower extract concentration was taken between 1-5%. Multiple responses were considered for the experimental design which are Titratable acidity (Y_1), protein (Y_2), antioxidant activity (Y_3), total phenolic content (Y_4), and total flavonoid content (Y_5) of the yogurt. On using response surface methodology for *Moringa oleifera* flower extract enriched yogurt, a quadratic equation was achieved for the response variables which is shown in Table 5.3.

Table 5.3 Representation of levels of independent variables A. Incubation Time B. Flower extract concentrations (%) used in CCD design with all the responses

Run	Independent variables		Responses				
	A	B	Y_1	Y_2	Y_3	Y_4	Y_5
1	5.50	3.00	0.832	4.9	76.43	24.76	20.32
2	5.00	1.00	0.766	4.6	74.16	20.74	18.21
3	6.21	3.00	0.817	5.1	76.06	24.89	20.42
4	5.50	3.00	0.826	4.8	75.34	24.59	20.34
5	6.00	5.00	0.935	5.7	84.21	28.31	21.34
6	6.00	1.00	0.945	4.6	74.21	20.34	18.16
7	5.50	0.17	0.978	4.3	71.25	19.01	17.16
8	5.50	3.00	0.825	4.8	75.33	24.59	20.71
9	5.00	5.00	0.923	5.6	82.67	27.94	21.26
10	5.50	3.00	0.834	4.9	76.16	24.92	20.45
11	5.50	3.00	0.843	4.8	75.98	24.99	20.39
12	4.79	3.00	0.831	5.1	76.27	25.02	19.56
13	5.50	5.83	0.925	5.8	84.38	28.39	21.37

A Incubation time, B flower extract concentration, Y_1 titratable acidity, Y_2 Protein, Y_3 antioxidant activity, Y_4 total phenolic content, Y_5 total flavonoid content41

5.13.3 ANTIOXIDANT ACTIVITY OF YOGURT

The antioxidant activity of the *Moringa oleifera* flower extract-enriched yogurt was determined using the method given by Chipurura et al. (2021). 0.2 ml of yogurt was mixed in 0.3 ml of the DPPH solution (0.0012 gm/100ml) and the absorbance was measured at 517 nm (Chipurura et al., 2021).

The free radical scavenging activity was determined by the following calculations:

$$\textbf{\textit{Scavanging activity}}\ (\%) = \frac{\textbf{\textit{Absorbance of control} - \textbf{\textit{Absorbance of of sample}}}}{\textbf{\textit{Absorbance of control}}} \times \mathbf{100} \qquad \textbf{(23)}$$

5.13.4 TOTAL PHENOLIC CONTENT OF YOGURT (TPC)

The yogurt samples were defatted using hexane, for minimizing the oxidation process of the extracts. 50 ml of yogurt was added in 50 ml hexane and was defatted overnight. The next day it was filtered, and the residues were collected for estimation of phenolic content. 0.5 ml yogurt sample was dissolved to 9.5 ml of the distilled water and 500µl of the Folin-Ciocalteu (1N) reagent was mixed to it and 2% of 500µl NaOH was added. Then mixture was left for incubation for 40 minutes at 37°C and then absorbance was noted at 725nm whereas, 50% methanol was checked for absorbance as a blank. The TPC of the yogurt samples was then expressed as mg GAE /100g (Chipurura et al., 2021).

The formula used for determining the total phenolic content of the yogurt is-

$$C = \boldsymbol{c}\ \frac{\boldsymbol{V}}{\boldsymbol{m}} \qquad (24)$$

Where,

C= Total phenolic content mg GAE/g DW

c= Concentration of Gallic acid obtained from the calibration curve

V= Volume of flower extract in ml

m= Weight of extract in gm

5.13.5 TOTAL FLAVONOID CONTENT (TFC)

The extracted yogurt samples (0.5ml) were mixed with 2% methanolic aluminium chloride ($AlCl_3$) and was left for incubation for 15 minutes at 37°C. After 15 minutes absorbance of the sample was checked at 430nm against a blank (50% methanol) whereas Quercetin was used for the standard flavonoid and the total flavonoid content measured is expressed as mg QE/100gm (Chipurura et al., 2021).

Formula used for determining the total flavonoid content of yogurt-

$$\boldsymbol{T = c\ \frac{V}{m}} \qquad \textbf{(25)}$$

Where,

T=Total flavonoid content mg QE/g DW

c= Concentration of Quercetin obtained from the calibration curve

V= Volume of flower extract in ml

m= Weight of extract in gm

5.14 PHYSIOCHEMICAL ANALYSIS OF *MORINGA OLEIFERA* FLOWER EXTRACT-ENRICHED YOGURT

5.14.1 Titratable acidity

Titratable acidity of the *Moringa oleifera* flower extract enriched yogurt was determined by mixing 1 ml of the yogurt in 10 ml distilled water and then titrated with 0.1*N* NaOH and 0.5% phenolphthalein indicator which was used as an indicator of the endpoint for the light pink color (Izadi et al., 2015).

5.14.2 *pH*

A digital pH meter was used for the determination of the pH values (Labman 1mV Digital pH Meter). 50 ml of the sample was taken in a beaker, the knob of the pH meter was inserted into the yogurt to check its pH (Izadi et al., 2015).

5.14.3 Determination of Protein (%)

Determination of the yogurt protein was done using the Lacto plus milk analyzer. 20 ml of the yogurt sample was taken in beaker, the sample was loaded into the milk analyzer to check the protein content of the yogurt.

5.14.4 Viscosity (cp)

The viscosity of the *Moringa oleifera* flower extract-enriched yogurt was determined by using a rotational viscometer (Brookfield viscometer) that works on the principle of rotational viscometry. 150 ml yogurt was taken in a beaker and placed under the spindle to measure the viscosity of the *Moringa oleifera* flower extract enriched yogurt. Spindle number 4 was used at 300rpm Izadi et al., 2014).

5.14.5 Determination of syneresis (%)

Syneresis was checked by centrifugation method, where 10 ml of the yogurt was stirred properly and centrifuged at 1500rpm for 12 min and then supernatant was collected and syneresis was measured by using the following formula:

$$\boldsymbol{Syneresis}\ (\%) = \frac{\boldsymbol{Volume\ of\ Supernatent}}{\boldsymbol{Total\ volume\ of\ yogurt\ in\ centrifuge\ tube}} \times \mathbf{100} \qquad \mathbf{(26)}$$

5.15 MICROBIOLOGICAL ANALYSIS

Yogurt samples were analyzed for bacterial viability using method given by Bulut et al. (2021). Culture of both the yogurts (control and flower extract enriched yogurt) was made to grow on De Man Rogosa and Sharpe agar (MRS) media (Himedia Ltd., Bombay, India) at 45 ℃ for 24 hrs. After that, the viable cells were counted and expressed in terms of log CFU/ml (Naklong et al., 2023).

5.16 SENSORY EVALUATION

Sensory analysis of the control yogurt and *Moringa oleifera* flower extract-enriched yogurt was carried out at the School of Agriculture, Lovely Professional University, and Phagwara, Punjab, India. It was evaluated by using a descriptive scorecard, whereas, faculty members of the School of Agriculture, participated in the analysis. All the age groups were considered during this process because yogurt can be consumed by all the age group due to potential

source of calcium, protein, and fat. All the panel members were asked to analyze the yogurt on the basis of appearance, body and texture, taste, flavor, acidity, container, and closure. The yogurt samples were stored at 4±2°C during the period of sampling, evaluation, and 28 days of storage. The mean of all the attributes of the scorecard was calculated and the final score for a particular sample was obtained. IBM SPSS statistics 23 software, Post Hoc Tests (Tuckey) was used to optimize the level of significance of all the yogurt samples. The highest accepted sample of yogurt can be selected based on sensory analysis.

Table 5.4 Sensory analysis of *Moringa oleifera* flower extract-enriched yogurt

Samples	Storage time (days)	Flavor	Body & Texture	Acidity	Colour & Appearance	Container & Closures	Overall acceptability
Control	0	40	28	7	9	4.5	88.5
	7	39	27	7.5	9	4.7	87.2
	14	39	28	7.5	9.5	4.5	88.5
	21	40	27.5	8	9	4.8	87.3
	28	30	25	9	9	4.5	77.5
***Moringa oleifera* flower extract-enriched yogurt (1%)**	0	41	28	6	9	5	89
	7	39	27.5	7	9.5	4.8	87.8
	14	39.5	27	6.5	10	4.8	87.8
	21	38	28	6.5	9	4.9	86.4
	28	29	26	8	9	4.8	76.8
***Moringa oleifera* flower extract-**	0	40	28.5	6.5	9.5	4.8	89.3
	7	41	27	7	9	4.9	88.9

enriched yogurt (3%)	14	41	27	7.5	9	5	89.5
	21	40	27.5	8	9.5	5	90
	28	30	27	8.5	9	4.9	79.4
***Moringa oleifera* flower extract-enriched yogurt (4%)**	0	41	26	6.5	9	4.8	87.3
	7	41	27	6.5	9.5	5	89
	14	40	27	7	9.5	5	88.5
	21	42	28	8	9	4.9	91.9
	28	30	27	8	9	4.8	78.8
***Moringa oleifera* flower extract-enriched yogurt (5%)**	0	42	27	6	9	4.8	88.8
	7	42.5	26	6.5	9.5	5	89.5
	14	42	27	7	9.5	5	90.5
	21	42	28	7.5	9.5	4.8	91.8
	28	31	28	8	9	4.9	80.9

5.17 COST ECONOMICS OF *MORINGA OLEIFERA* FLOWER EXTRACT-ENRICHED YOGURT

For calculating cost of the product all the raw materials cost was taken into consideration such as procurement of milk, labour, packaging and labelling, extract extraction, *Moringa oleifera* flower cost, incubation, refrigeration, marketing, and taxation.

Chapter-6

RESULTS AND DISCUSSION

This study was carried out with the aim of "Utilization of *Moringa oleifera* flower extract for its therapeutic properties in the development of stirred yogurt". The results obtained from the study are discussed below.

6.1 Collection

Mature flowers of the *Moringa oleifera* plant were collected from Karnal, Haryana, India in March-April 2021. After collection the flowers were identified by the Department of Botany, School of Agriculture, Lovely Professional University, at Phagwara, and Punjab, India.

6.2 CLEANING, DRYING, PROCESSING

Collected flowers were then washed thrice with the tap water and once with distilled water for removing the impurities such as smoke, pollens, soil particles and dust. *Moringa oleifera* flowers were dried at 45℃ in a hot air oven for 48 hours. Dried flowers were then powdered with the help of a Sujata Powermatic plus 900 watts grinder and sieved with 100µm sieve with the help of an electric sieve shaker (SRI equipment, New Delhi). After that prepared *Moringa oleifera* flower powder was stored at room temperature in polypropylene bags (Ziploc®) to avoid moisture and contamination.

6.3 PROXIMATE ANALYSIS

Proximate analysis of *Moringa oleifera* flower powder was done to get to know the chemical composition viz. fat, carbohydrates, fiber, protein, and energy. Dried *Moringa oleifera* flower powder was analyzed, and observed that the fat content in 100gm of the flower powder was found to be 1.47%, fiber content 6.7%, protein 18.1%, ash 5.93%, carbohydrate 61.30%, and total energy count was 331kcal/100gm. Table 6.1 represents the proximate values of *Moringa oleifera* flower powder. The same results were shown by Guzman et al., 2020, the protein content of *Moringa oleifera* leaves was 18.34gm, fat content was 10.76gm, and carbohydrate content was 41.58gm. Similarly in another study it was reported that protein 25.16%, fat 1.57%, carbohydrate 53.67% of *Moringa oleifera* flower powder showed significant difference ($p<0.05$) with each other (Arise et al., 2014). The specific reason identified by Guzman et al. (2020) for the difference in proximate content was due to the climatic

differences, soil types, plant age and genetic factors. However, it was observed that the protein content of the sunflower (74%) and the soybean (66%) showed significantly ($p<0.05$) higher as compared with the legumes such as pigeon pea (18%), and cowpea (22%) showed significantly ($p>0.05$) lower protein content as compared with *Moringa oleifera* flower protein value (Moyo et al., 2011).

Ahmed et al., 2023 revealed that *Moringa oleifera* leaves consist of 7.94% of moisture, 13.55% of fats, and 11.65% ash whereas in terms of minerals, magnesium is 147.5ppm, Ca, 1110 ppm, potassium 559 ppm, and zinc 0.125 ppm which means *Moringa oleifera* is a potential source of multiple vitamins and minerals.

Table 6.1 Proximate composition of *Moringa oleifera* flower powder

Proximate composition (per 100gm)	Values (gm)
Fat	1.47 ±0.08
Fiber	6.70±0.08
Protein	18.10±0.32
Ash	5.93±0.09
Carbohydrates	61.30±1.12

Data are represented as mean ±SD (n=3)

6.4 TECHNO-FUNCTIONAL PROPERTIES

In this study techno functional properties of the *Moringa oleifera* flower powder were observed, that include water absorption capacity, foaming capacity, foaming stability, oil absorption capacity, bulk density, and true density. Techno-functional properties of both types of *Moringa oleifera* flower powder as well as defatted flower powder were calculated, and the results are shown in Table 6.2.

6.4.1 Water absorption and oil absorption Capacities

The water absorption capacity of the *Moringa oleifera* flower powder was 10.60% significantly ($p<0.05$) higher as compared to the defatted flower powder 11.40 %. Similarly, the oil absorption capacity was monitored to be 6.10% and 7.80% respectively and the oil absorption capacity of the defatted flower powder was significantly ($p<0.05$) higher than the defatted powder. The oil absorption capacity for corn silk was found to be 2.57 (g/m^3) which

was significantly ($p<0.05$) lower as compared to the *Moringa oleifera* flower powder. It was found that in *Moringa oleifera* flower powder water and oil absorption capacity both increased significantly ($p<0.05$) on defatting, because during fat content extraction all the fat was extracted, absorption capacity of the flower powder was increased due to presence of free space in the form of voids. However, oil absorption capacity also depends on the particle size because powder that absorbs maximum oil has a high oil absorption capacity (Lucas-González et al., 2017). In another report scientist revealed that the water absorption capacity of the banana flower powder was significantly ($p<0.05$) lower (7.76%) as compared with the *Moringa oleifera* flower powder (10.60) whereas the oil absorption capacity of banana flower powder was also significantly lower (1.4%) (Jha et al., 2021). The water absorption capacity of *Moringa oleifera* seed flour was reported 8.08% (Ljarotimi et al., 2013). However, as compared with the oil absorption capacity of oats leaves powder (39.85%), and wheat leaf powder (35.50%) showed significantly ($p<0.05$) higher value as compared with the *Moringa oleifera* flower powder (6.10%) (Getachew and Admassu, 2022).

6.4.2 Moisture content

The moisture content of the *Moringa oleifera* flower powder was significantly ($p<0.05$) higher 6.91% as compared to defatted flower powder 4.31%. The reason behind the low moisture content of defatted flower powder was that during the fat extraction, the sample was treated with hexane for 6 hours, during this process most of the moisture get evaporated and hence cause the low moisture content of the defatted flower powder. Mehmood et al., (2022) revealed that, the moisture content of *Moringa oleifera* leaves powder was reported at 6.37%, similarly in a study by Herlina and Sinaga moisture content was reported as 4.82%. Similarly, a study reported that *Moringa stenopetala* leaves powder contains 7.40% moisture content as compared with *Moringa oleifera* leaves 6.73% (Ntshambiwa et al., 2023). Moisture content or humidity of the powders is the main root cause of the bad followability of the powder samples. In food industries, moisture content plays a crucial role because it is associated with the cohesiveness and inter-particle liquid bridges of the powders, and in grinding processes, it is imperatively preferred to attain low moisture content to achieve high energy efficiencies. Secondly, higher the moisture content of powders maximum will be the chances of contamination in the formulations that's why the low water content is preferred for the powders.

6.4.3 Bulk Density

Bulk density is the mass of the powder which is dependable on particle size and the interparticle forces. The bulk density of *Moringa oleifera* flower powder was determined significantly ($p<0.05$) higher 0.44 g/m^3 than the bulk density of defatted *Moringa oleifera* flower powder 0.38 g/m^3. Bulk density of the flower powder gets decreased because of the low-fat content of the powder. As compared with the bulk density of the banana flower powder was observed 0.33 g/m^3 (Jha et al., 2021). Similarly in a study by George et al. (2021) bulk density of the *Moringa oleifera* leaves was found 0.29 g/m^3. The bulk density of the powders is measured to know the flow properties of the powders. The bulk density of the flower powder gets decreased because of the low-fat content of the powder.

6.4.4 Tapped Density

Tapped density is also known as true density which indicates about the volume of the powder on tapping because on tapping interparticle spaces between the particles get reduced. *Moringa oleifera* flower powder showed significantly ($p<0.05$) higher tapped density of 0.30 g/m^3 as compared to defatted *Moringa oleifera* flower powder 0.23 g/m^3. In comparison with another study *Moringa oleifera* leaves powder showed 0.26 g/m^3 and 0.30 g/m^3 tapped density (George et al., 2021). Another study by Kolo et al. (2018) reported that tapped density of *Moringa oleifera* leaves was 0.36 g/m^3 whereas Vonghirundecha et al., (2022) reported that tapped density of *Moringa oleifera* leaves was 0.35g/m^3. The same study also stated that tapped density of the flower powder decreased due to presence of the low-fat content of the powder. Particle size also affects the tapped density because the greater the particle size, the lesser will be the surface area, and when the particle is tapped, they become compact easily which causes a decrease in tapped density of the powders.

6.4.5 Hausner ratio

Hausner's ratio of *Moringa oleifera* flower powder was significantly ($p<0.05$) higher 0.68 g/m^3 than of the defatted flower powder 0.60 g/m^3. According to the literature, it was reported that the Hausner ratio value of less than 1.11 is considered to be an excellent flow. Premi and Sharma, 2017 reported that Hausner ratio values for the *Moringa oleifera* leaves powder ranged from 1.10 to 1.41 g/m^3. Similarly, a higher value of the Hausner ratio (<1.40 g/m^3) indicates the poor flowability of the powders (Schlick-Hasper et al., 2022). According to Kolo et al. (2018), study Hausner ratio of *Moringa oleifera* leaves was found 1.26 g/m^3.

6.4.6 Porosity

Porosity is the percentage of the void spaces in the powder sample which is defined as the volume of voids divided by the total volume. It is determined to know the ultimate volume of the flour/powder. The porosity of both the sample powders was found 0.14 g/m^3 and 0.15 g/m^3 so, there was non-significant difference between both of them. Poornima et al. (2023) reported the porosity of *Moringa oleifera* pods was found 0.31 g/m^3. The porosity of the powders is determined by surface area, high the surface area of the powder higher will be the porosity. High porosity determines the higher absorption capacity of the powders. In neem nuts as well as soya beans porosity is reduced from 54.16% to 40.36% with the inclined moisture content whereas in okra seeds and hemp seeds, porosity is directly proportional to the moisture content which means with the increase in moisture content porosity is also increasing (Aviara et al., 2013).

6.4.7 Angle of Repose

Moringa oleifera flower powder was observed 22.27˚ angle of repose that showed non-significant difference when compared with the defatted *Moringa oleifera* flower powder 23.21˚. The angle of repose of *Moringa oleifera* leaves powder was reported 34.54 (George et al., 2021). In a study, it was revealed that the angle of repose of *Moringa oleifera* seeds and kernels escalate from 17.1° to 23° and 13.1° to 21.8° and showed a significant ($p<0.05$) difference in them. Another study by Rai et al. (2022) reported the angle of repose of *Moringa oleifera* leaves powder was 24.00◦ whereas the angle of repose of nutraceutical tablets made with *Moringa oleifera* leaves was 15.07◦. Moisture content of powders plays an imperative role in angle of repose, whereas, *Moringa oleifera* seed powder showed significant ($p<0.05$) higher angle of repose with an increase in moisture content (Aviara et al., 2013).

6.4.8 Foaming Capacity and Foam Stability

The foaming capacity of the *Moringa oleifera* flower powder showed a non-significant ($p>0.05$) difference of 15.20 ml/L as compared with defatted flower powder of 15.13ml/L. The foaming capacity of corn silk was reported at 15.61ml/L (Chavhan, 2017). Zhao et al. (2022) reported that foaming capacity of the *Moringa oleifera* leaves powder was increased due to the presence of proteins, as proteins increase the ability of the powders to spread on an air-water interface and result in improved foaming capacity. The foaming capacity of the food will change on the increased rate of protein adsorption (Sun et al., 2022). Singh et al. (2022) reported that presence of flexible proteins is responsible for the foaming capacity of the

powders. The foaming stability of the *Moringa oleifera* flower powder was determined non-significant at 56.34ml/L in comparison to *Moringa oleifera* flower defatted powder at 56.23ml/L. Whereas, Singh et al. (2022) revealed the foaming stability of corn silk at 57.24ml/L. The reason behind the potential foaming stability of corn silk is the protein content present in it.

6.4.9 Oil absorption capacity

Oil absorption capacity depends on the hydrophobic nature of the flower powder, particle size, and charge density along with the drying methods used. Oil absorption capacity is an important factor that decides the texture as well as flavour retention capacity of the powders and also gives sponginess to the products. The oil absorption capacity of the *Moringa oleifera* flower powder was determined 6.10 % whereas *Moringa oleifera* flower defatted powder showed a significantly higher ($p<0.05$) oil absorption capacity at 7.80%. Dried corn silk powder showed an oil absorption capacity of 43.80% (Singh et al., 2022) whereas the oil absorption capacity of *Moringa oleifera* leaves powder was reported at 1.95% (Fapetu et al., 2022). The oil absorption capacity of the powders depends on the particle size, density, and chemical structure of the plant polysaccharides.

Table 6.2 Physical properties of *Moringa oleifera* flower powder

Properties of flower	***Moringa oleifera* Flower powder**	***Moringa oleifera* Defatted flower powder**
Water absorption Index (%)	10.60 ± 0.10^{a}	$11.40.10\pm0.02^{b}$
Water solubility Index (%)	8.70 ± 0.20^{a}	9.20 ± 0.01^{b}
Moisture Content (%)	6.91 ± 0.60^{b}	4.31 ± 0.01^{a}
Tapped/True Density (g/m^3)	0.30 ± 0.01^{b}	0.23 ± 0.01^{a}
Bulk Density (g/m^3)	0.44 ± 0.03^{b}	0.38 ± 0.01^{a}
Hausner ratio (g/m^3)	0.68 ± 0.03^{b}	0.60 ± 0.03^{a}
Porosity (g/m^3)	0.14 ± 0.01^{a}	0.15 ± 0.01^{a}
Angle of Repose (°)	22.27 ± 0.02^{a}	23.21 ± 0.01^{b}
Foaming Capacity (ml/L)	15.20 ± 0.20^{b}	15.13 ± 0.06^{a}
Foaming Stability (ml/L)	56.34 ± 0.10^{b}	56.23 ± 0.01^{a}
Oil absorption capacity (%)	6.10 ± 0.10^{a}	7.80 ± 0.01^{b}

Data are represented as mean ±SD (n=3)

[a-b] Means with the same superscript in a column do not vary significantly ($p<0.05$) from each other

6.5 CHARACTERIZATION OF *MORINGA OLEIFERA* FLOWER

6.5.1 Fourier transform infrared Spectroscopy (FTIR)

FTIR was used for the qualitative estimation of the alcohol, aromatic, carbonyl, alkyl, alkyne, and alkyl halide, compounds. The FTIR spectra of the *Moringa oleifera* flower extract were observed at a range of 4000-400cm^{-1} as shown in figure 6.1. FTIR spectroscopy absorption from the figure at 3329 shows stretched, H-bonds with a strong and broad intensity which belongs to an alcoholic group, the alkyne group is present at absorption 2132 with variable and non-symmetrical alkynes, absorption at 1637 shows stretched vibration with variable intensity and absorption pattern at 1338, 1215, 1107, 1040 and 590 showed stretched vibration with strong intensity as shown in Table 6.3. Table clearly revealed that *Moringa oleifera* flower consists of the huge number of functional groups. A study revealed the presence of the broad range of functional groups in the aquous leaf extract of *Moringa oleifera* leaves, these groups were aryl disulfide, aliphatic bromo compound, alkene, alcohol, carboxylic acid, esters, alkane, alkyl, phenol, and aromatic (Khalid et al., 2023). Another study done on *Mentha spicata* revealed the presence of alcohol at 3349.81, alkanes at 2927.23, alkene at 1633.44, 1537.09, carbonyl at 1253.97, 1054.89, and aromatic group at 599.76 wavenumbers (Ojewumi et al., 2021). Simon et al. (2022) revealed presence of the phenolic group at 3262.78, alkanes at 2921.05, ketones at 1580.97, alcohols at 1395.43, carbonyl at 1235.24, and aromatic group at 782.04 wavenumber in *Moringa oleifera* leaves extract. A list of the characteristics of IR absorption frequencies of organic functional groups is shown in the appendices.

Table 6.3 Representation of functional groups of FTIR

Functional Group	Type of vibration	Characteristic absorption (cm-1)	Intensity
Alcohol			
O-H	(Stretch)	3329	Broad, strong
Alkyne			
-C≡C-	Stretch	2132	variable, not present in symmetrical alkynes
Alkene			
C=C	Stretch	1637	Variable
Alkyl Halide			
C-F	Stretch	1338,1215,1107,1040	Strong
C-Br	Stretch	590	Strong

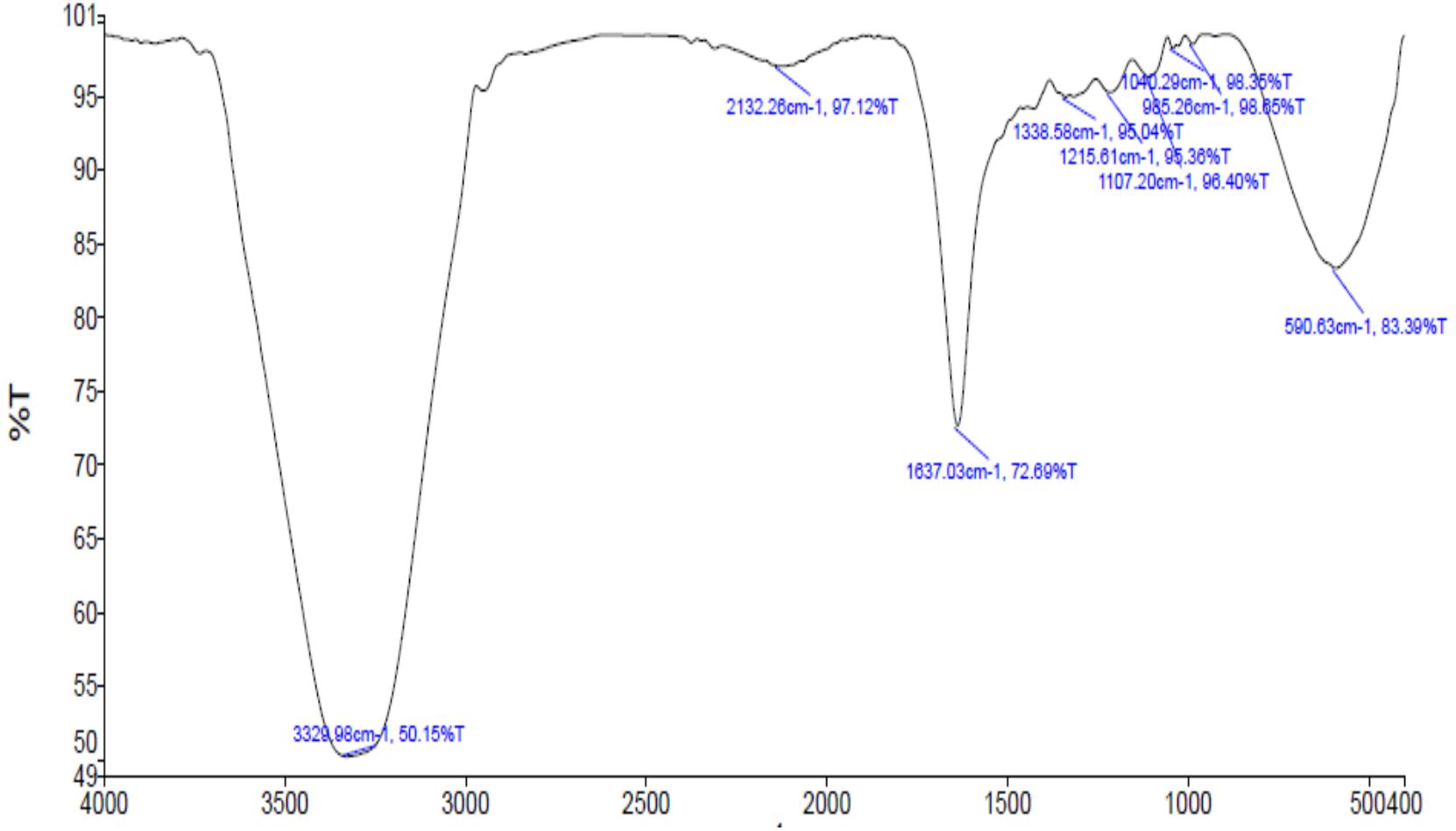


Figure 6.1 FTIR spectra of *Moringa oleifera* flower extract

6.5.2 Gas Chromatography-Mass Spectrometry (GC-MS)

GCMS identification of *Moringa oleifera* flower extract showed 20 bioactive compounds that are shown in Table 6.4 with retention time and peak area. Figure 6.2 given below shows presence of the bioactive compounds in *Moringa oleifera* flower. The bioactive compounds showed peaks at very minute differences in retention time because the sample contains a huge amount of bioactive compounds. Hexadecenoic acid showed the largest peak at a retention time of 19.50, and it is a specific inhibitor of phospholipase and acts as anti-inflammatory agent (Aparna et al., 2012). Hexadecenoic acid is a long chain monounsaturated fatty acid which is found in plants and microorganisms. 9-Octadecadienoic acid is a saturated fatty acid that is analgesic and shows an anti-inflammatory effect whereas 10,13-Eicosadienoic acid (omega-6 fatty acid) is a cryogel with antimicrobial properties (Hadi et al., 2016). Methyl stearate is a fatty acid and a non-ionic surfactant and helps in the solubility of the chemicals and unfolding of the proteins (NIST). Methyl 10-trans, and 12-cis-octadecadienoate show various biological properties such as anti-carcinogenic (by suppressing the growth of melanoma) and anti-atherogenic activity. Glycerolcidyl palmitate is a combination of glycerine and palmitic acid, and it exhibits antibacterial and bactericidal properties along with anti-inflammatory activity (Weimann et al., 2018). Tetracosamethyl-cyclododecasiloxane shows a cytotoxic effect and antimicrobial properties (Bratty et al., 2020) whereas Myristic acid Glycerolcidyl are saturated fatty acids generally used in the cosmetic products. They help to combat with cancer cells in pancreatic as well as prostate cancers (Cheng et al., 2017). Glycerolcidyl palmitate is used in the preparation of lysophosphatidic acids which help in the inhibition of apoptosis (Appel et al., 2013).

Methyl esters are type of fatty acid esters that had been reported to show the ability to inhibit the Kupffer cells that are resistant to macrophages present in the liver for the regulation of the inflammatory process by selecting TNF-alpha (Wang et al., 2018). There are other bioactive compounds which are found *in Moringa oleifera* flowers are Methyl stearate, Linoleyl acetate, and Methyl 5,11, 14-eicosatrienoate. A GC-MS study on *Moringa oleifera* leaf extract reported presence of the bioactive compounds *viz.* monomethyl malonate, dihydroxyacetone, propanoic acid, octyl ester, sorbitol, inositol, cyclohexane methanol, 9-Octadecenoic acid, and alpha-methyl-4-(methyl ethyl), etc. (Kandeepan et al., 2022).

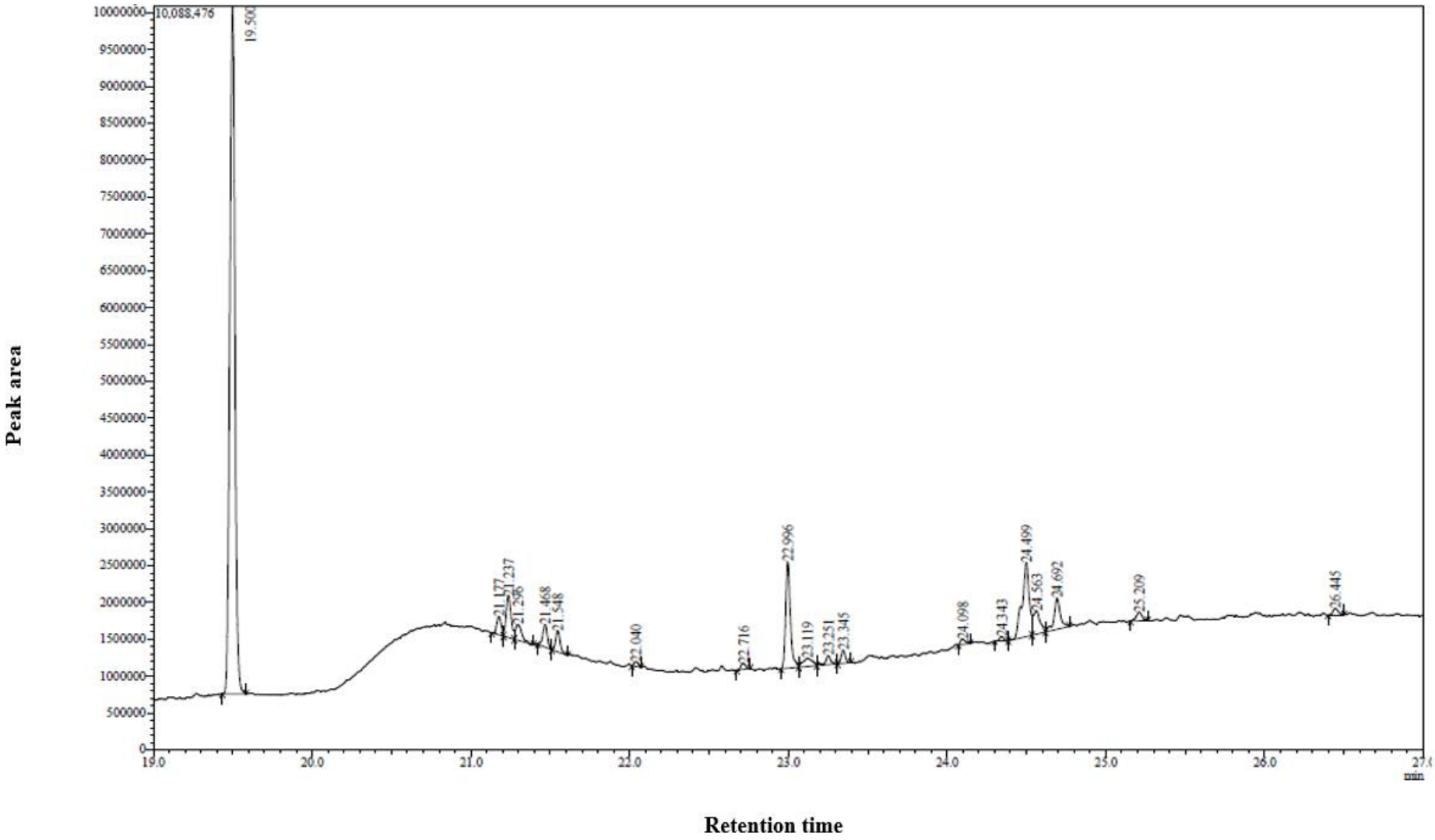


Fig.6.2 Representation of GCMS Chromatogram of *Moringa oleifera* flower extract

Table 6.4 Identification of bioactive compounds in the *Moringa oleifera* flower by GC-MS identification

Compounds	Retention time (RT)	Peak area (%)	Class of compounds
Hexadecenoic acid	19.500	49.54	Monounsaturated fatty acid
9-Octadecenoic acid	21.237	2.97	Saturated fatty acid
10,13-Eicosadienoic acid	21.296	1.32	Omega-6 fatty acid
Methyl stearate	21.468	1.44	Fatty acid
12-cis-octadecadienoate	22.040	0.28	Omega-6 fatty acid
Glycerolcidyl palmitate	22.996	7.22	Fatty acid
Eicosanoic acid	23.251	0.70	Saturated fatty acid
Oxiranyl methyl ester	24.499	7.53	Fatty acid
Methyl 5,11,14-eicosatrienoate	24.563	2.37	Fatty acid esters
Myristic acid Glycerolcidyl ester	24.692	2.87	Saturated fatty acids
Linoleyl acetate	25.209	0.72	Acyclic monoterpenoids

6.5.3 Thermogravimetric analyser (TGA)

Thermogravimetric analyser curve represents that in *Moringa oleifera* flower powder the weight of the sample was decreasing with an increase in temperature, which directly impact on the mass of the sample. In study it was observed that 67.97 was the glass transition temperature of the *Moringa oleifera* flower powder as shown in Figure 6.3. It was observed that at 150℃ the loss of the sample starts due to degradation of cellulose and lignin. The maximum loss in weight of *Moringa oleifera* flower powder was observed at 596℃. Hani et al. (2016) revealed that with the increase in Tg solubility as well as dissolution of the samples improved. Koley et al. (2023) revealed the effect of the temperature on the cellulose present in the *Moringa oleifera* fruit that thermal degradation of the cellulose starts at 150℃. Erol et al. (2022) revealed that *Moringa oleifera* leaves nanocomposite started decomposition at 242℃ and 50% of the mass was decomposed at 355℃ whereas the maximum weight loss occurred at 450℃. Shashiraj et al. (2023) studied the TGA curve of *Lagerstroemia speciosa* flower buds and depicted that flower demonstrates significant level of stability at temperature range of 27℃ to 800℃ with a minute weight loss in the sample. This study revealed that initial weight loss of the sample was associated with the evaporation of moisture content whereas later it was associated with desorption of the bio-organic constituents which acted as conjugated biomolecules on the surface.

A study by Manjari et al. (2017) resulted that *Aglaia elaeagnoidea* flower powder was used to carry out the thermogravimetric analysis and it showed that almost 22% of the sample weight was reduced at 30-200℃ whereas due to the evaporation of the water 40% of the weight reduction was seen at 200-800℃. These results almost match with the results obtained in this study with *Moringa oleifera* flower powder. This weight reduction corresponds to the decomposition of the organic matter of the sample powder.

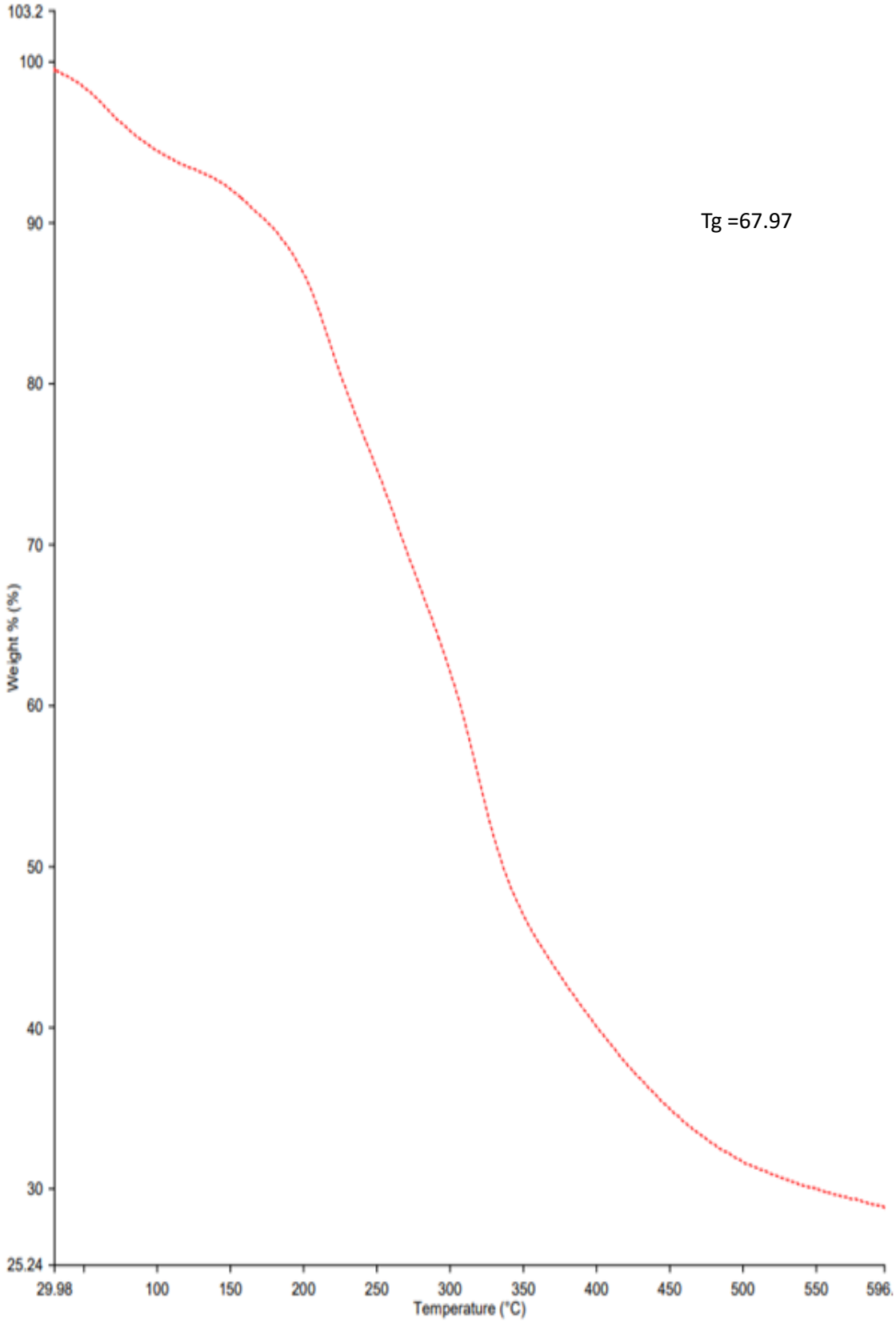


Fig.6.3 TGA Thermogram of *Moringa oleifera* flower

6.5.4 Field Emission Scanning Electron Microscopy (FESEM)

Field Emission Scanning Electron Microscop***y*** is used for the identification of the morphology of compounds such as cellulose, lignin, and hemicellulose. In this study, morphology of the *Moringa oleifera* flower powder was checked at various resolution powers A) 100X, B) 1,000X, C) 2,000X, D) 10,000X, E) 25,000X, F) 50,000X as shown in figure 6.4. FESEM results showed that the powder particles are rectangular in shape and exhibit a microporous structure. These structures represent the presence of cellulose-based materials in *Moringa oleifera* flower powder that is used for the binding of the polysaccharides as well as flavonoids. Cellulose is linked with the help of hydrogen bonds and van der wall force of attraction to form long thread like structures which are crystalline in nature and called as cellulose microfibril (Rongpipi et al., 2019). Yellow highlights in figure 6.4 showing cellulose structure of *Moringa oleifera* flower powder. Guo et al., 2018 reported the same results during their study on the binding properties of polysaccharides, they revealed the presence of lignin and cellulose in their study. A study by Koley et al. (2023) resulted in the presence of fibers in the rubber matrix where the cellulose microfibers were uniformly dispersed all over the matrix. FESEM study of the *Moringa oleifera* seed showed that biochar surface's macropores were rougher than the *Moringa oleifera* leaf's char surface macropores. Vesicles formed on the smooth surface of *Moringa oleifera* leaves because of the volatile gases in the softened biomass matrix being released during plasma furnace processing. CaF2 and AlF3 have very weak solubilities in water because of the strong metal-fluoride bonds. However, since fluoride is known to interact with hydrous aluminium oxides and alumina, the presence of Al on the biochar of *Moringa oleifera* leaves also facilitates fluoride adsorption in polluted drinking water. (Gourai et al., 2023).

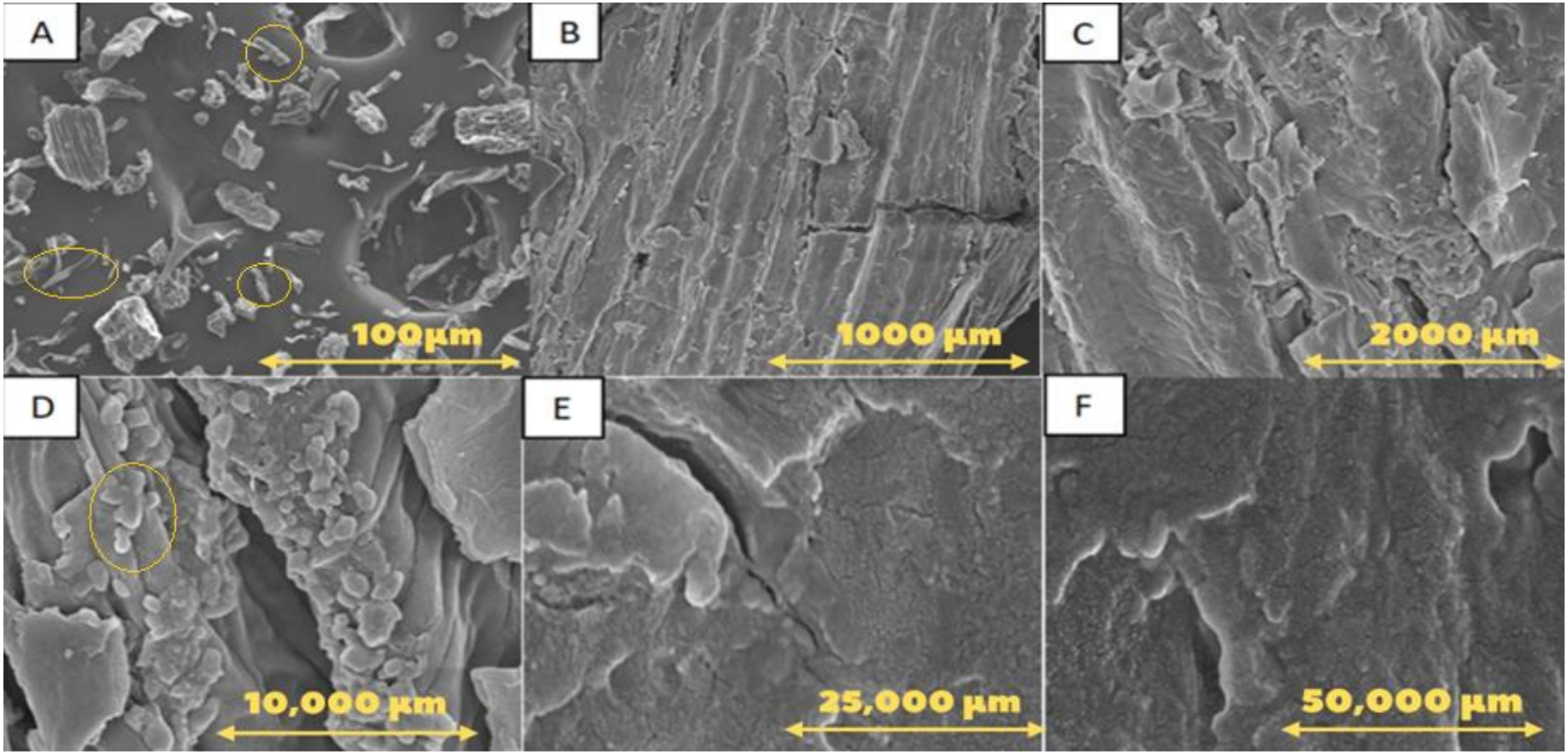
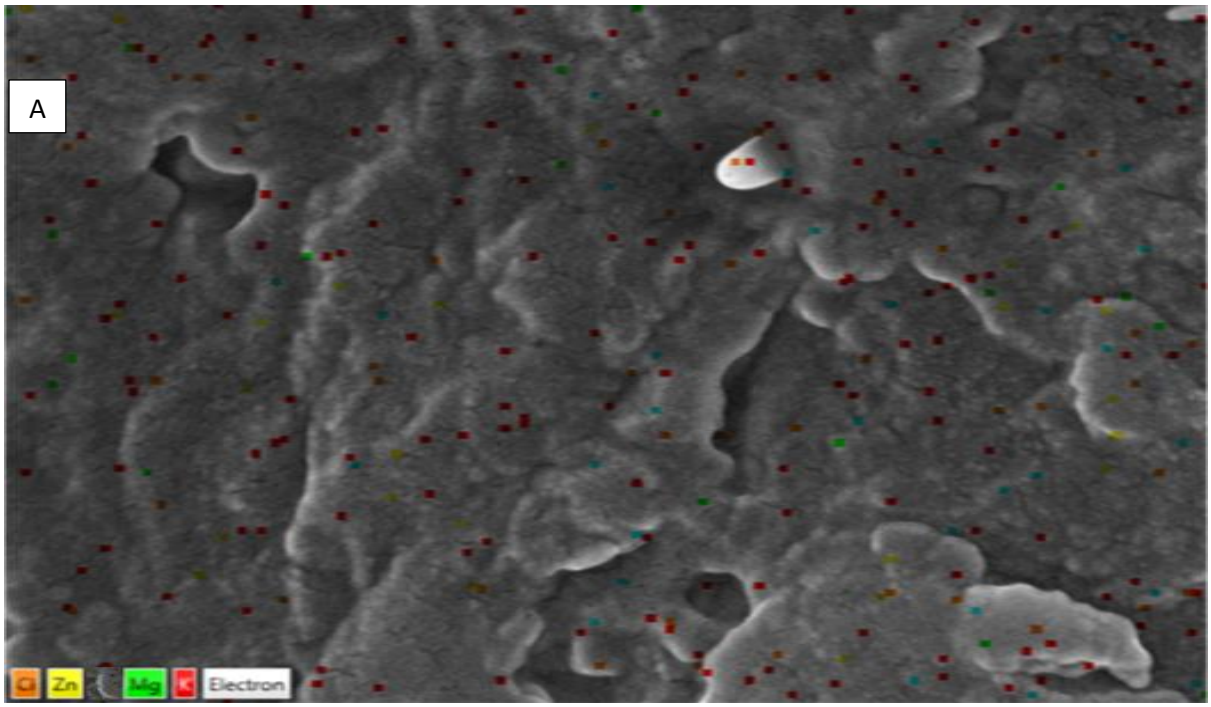


Fig.6.4 Surface morphology images of *Moringa oleifera* flower powder (Yellow highlights showing structure of cellulose)

6.5.5 Energy-dispersive X-ray Spectrometer (EDS)

Energy-dispersive X-ray Spectrometer study of *Moringa oleifera* flower powder showed the presence of various elements such as ZN, Mg, Fe, Cl, and K as shown in figure 6.5 A& B. Results revealed that the amount of potassium was significantly higher ($p<0.05$) than other elements (Cl, Zn, Mg, Fe), however Cl and Zn were almost equally present whereas, magnesium is present in trace amounts, whereas chlorine was 5%, potassium 89.07%, and zinc 4.98%. The higher level of potassium in *Moringa oleifera* flower help to maintain the fluids in the cells, contraction of muscles and normal blood pressure (Amrulloh et al., 2021). According to Ahmed et al., 2023 *Moringa oleifera* leaves are consist of magnesium 147.5ppm, Ca, 1110 ppm, potassium 559 ppm, and zinc 0.125 ppm respectively. Jadhav et al. (2022) reported that Moringa oleifera leaves powder showed the presence of Ca, O, and Na elements. Where O was present in highest % (59.81), Na (23.45%), and Ca was (16.74%). Oladele et al. (2022) reported the presence of C (59.78%), O (32.14%), Mg (1.16%), Al (0.30%), K (4.50%), and Ca (1.87%).



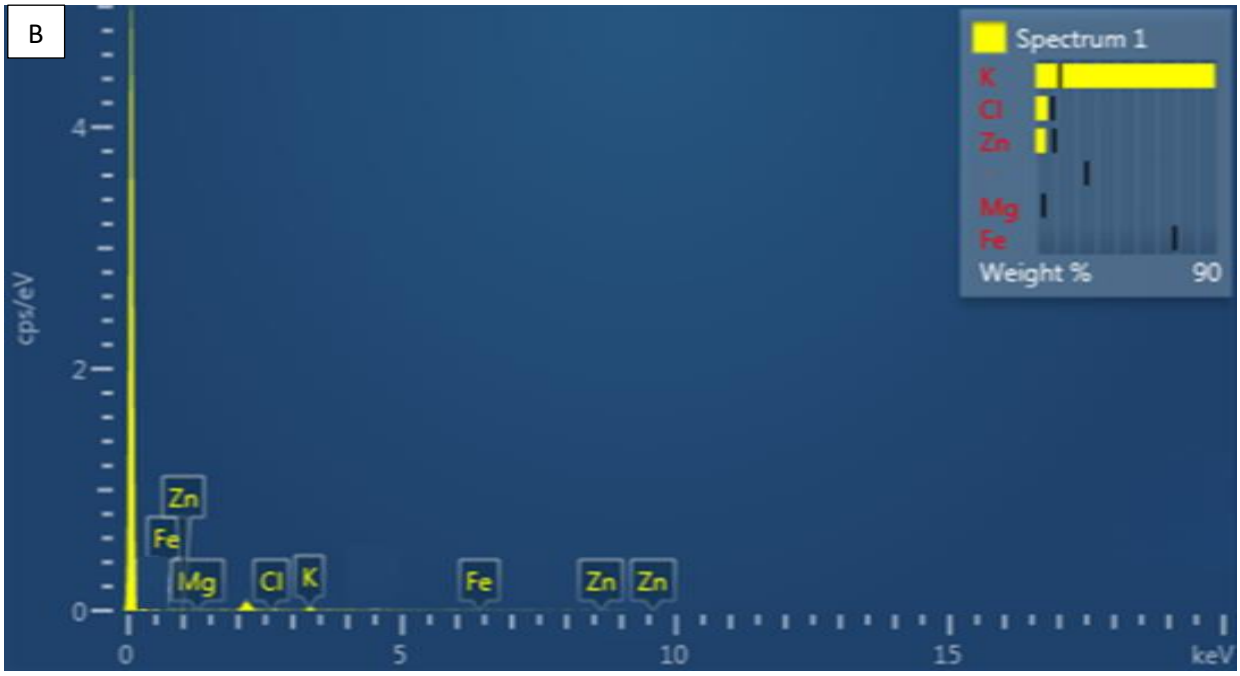


Figure 6.5 A & B Representation of elements in *Moringa oleifera* flower powder

6.5.6 High-Performance Liquid Chromatography (HPLC)

In present study High-Performance Liquid Chromatography was used for the identification of the individual compounds present in the *Moringa oleifera* flower extract. In this study, HPLC results showed that *Moringa oleifera* flower extract consists of an ample number of bioactive compounds such as Gallic acid, Vanillic acid, chlorogenic acid (phenol) as well as Quercetin, and Rutin (flavonoid). There were peaks in the chromatogram for Gallic acid (3.65), Chlorogenic acid (9.67), Vanillic acid (13.6), p-Coumaric acid (19.1), Ferulic acid (22.5), Rutin (24.7), Sinapic acid (25.5), Caffeine (26.2), Quercetin (27.07), Kaempherol-3-o-acetyl, (28.3), Epigalocatechin-3-gallate (30.02), Gallocatechin-3-gallate (31.05), and Epicatechin (35.3). Figure 6.6 showing HPLC chromatogram of *Moringa oleifera* flower extract. Results obtained from HPLC analysis show peaks at various time intervals, and their peaks area was used for the calculation of the concentration of the compound, for example here Quercetin had a peak area of 1974515 so the concentration of quercetin was 1974.515 mg/l whereas the peak area of Rutin was 1366452 so the concentration was 1366.452 mg/l. HPLC results showed that *Moringa oleifera* flower extract contained Rutin with a retention time of 24.75 and Quercetin at 27.01 with reference to standards and extracted samples of flowers. In study Ahmed *et al.* (2021) reported that bioactive compounds present in the *Moringa oleifera*

leaves extract were catechin hydrate (20.19 mg/g), rutin (60.38 mg/g), and quercetin (137.81 mg/g). However, the results shown by Ademiluyi *et al.* (2018) depicted the difference in concentration of compounds such as Catechin (6.08 mg/g), rutin (91.05 mg/g), and Quercetin (17.83 mg/g). A study by Salem et al. (2021) stated that *Moringa oleifera* leaves are potential source of multiple phytochemical compounds out of which vanillic acid, benzoic acid, chlorogenic acid, syringic acid, ellagic acid, kaempherol, and gallic acid are polyphenols whereas naringenin, , rutin and myricetin are flavonoid compounds.

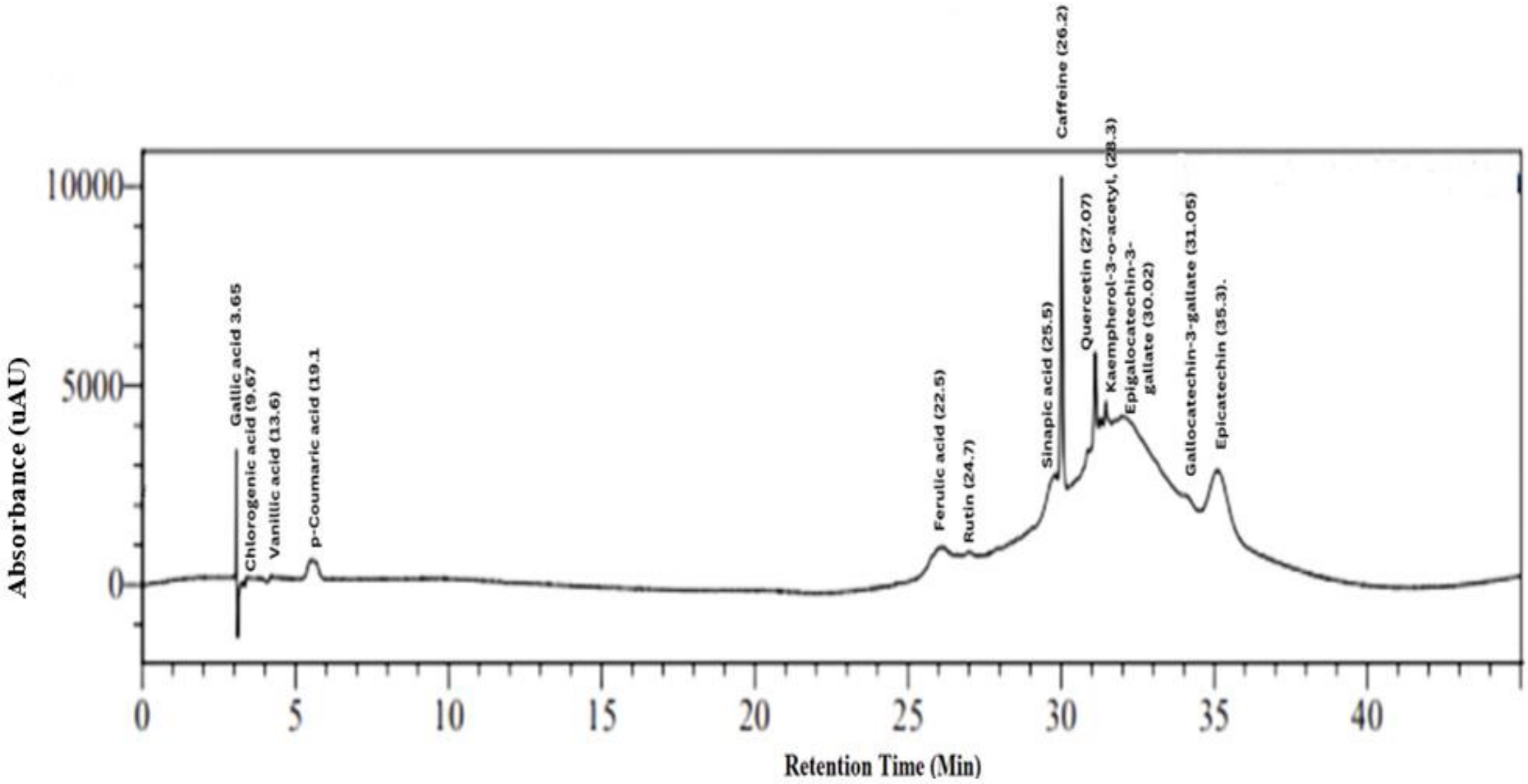


Fig 6.6 HPLC chromatogram *Moringa oleifera* flower extract

6.6 OPTIMIZATION OF EXTRACTION PROCESS BY USING DEEP EUTECTIC SOLVENTS (DESs)

6.6.1 *Extraction yield*

Six different types of DESs Glycerol, lactic acid, malic acid, L-proline, Choline chloride, and glucose were used in this study for extraction of the *Moringa oleifera* flower powder. In this study Choline chloride, and L-proline were used as Hydrogen Bond Acceptors whereas Glycerol, malic acid, lactic acid, and glucose (sugar alcohol, amide, acid, and sugar-based respectively) were used as Hydrogen Bond Donors. Table 6.5 below shows the extraction yield of the *Moringa oleifera* flower by using all of these extraction solvents (DESs) along with their molar ratio (1:1, 1:2, 1:3). All six combinations of solvents used were Choline Chloride-Glycerol, Choline chloride-Malic acid, Choline Chloride-Lactic acid, Choline Chloride-Glucose, L-proline- Glycerol, L-proline- Lactic acid. Out of all the solvent combinations, L-proline-Glycerol showed significantly ($p<0.05$) higher extraction yield 9.13%, and the second highest yield was obtained by L-proline- Lactic acid 8.33% after that Choline chloride -Glucose 7.16%, afterward Chloride-Lactic acid 6.86%, the second lowest yield was obtained with Choline chloride-Malic acid 5.93% and 5.33% with Choline Chloride-Glycerol. The results showed that the maximum yield of *Moringa oleifera* flower extract was obtained with a molar ratio of 1:2 in all the solvent combinations. This ratio showed a significant difference ($p<0.05$) between the yield obtained from ratios 1:1 and 1:3 in all the solvent combinations. The results obtained with molar ratio 1:2 showed maximum yield because of the viscosity and polarity of the DESs. So, the molar ratio of 1:2 showed maximum amount of yield, and it was decided to further go with this concentration of the solvent to check the phenolic, flavonoid content, and antioxidant activity of the *Moringa oleifera* flower extract. Table 6.5 shows the results of optimization of the extraction yield of *Moringa oleifera* flower extract.

The molar ratio between the hydrogen bond donor and hydrogen bond acceptor showed a remarkable effect on physical and chemical properties of the DESs (Ge et al., 2019). It was revealed in the literature survey that the extraction efficiency of acid-based DESs, amide-based DESs, and alcohol-based DESs is significantly ($p<0.05$) higher than that of sugar-based DESs (Wan Mahmood et al., 2019). Wu et al., 2020 showed almost the same results when they used the same combination of solvents to make DESs and revealed that amide-based and acid-based DESs showed maximum extraction yield. Manurung et al., 2019

reported that the freezing point of the DESs plays a significant ($p<0.05$) role in the extraction yield because the DESs with lower melting points were more viscous in nature and to reduce the viscosity of the DESs more water was added which affect the extraction process. The addition of more water means only hydrophilic compounds will be extracted and hydrophobic compounds extraction will not be reduced significantly ($p<0.05$) which reduce the extraction yield from 12.46% to 8.92% (Omar and Sadeghi, 2022). Furthermore, water can interact with both HBDs and HBAs which can break down the bonds between them and is also responsible to produce multi-hydrogen bonds with the solvents (Passos et al., 2016). Dai et al., 2015 revealed with the increase in water content in DESs declines the melting point, viscosity as well as density of the solvents by disrupting the hydrogen bonding within the components of DESs and by aggregating the ionic mobility. Similarly, with the addition of water polarity of the DESs decrease and extraction yield also subsequently decreases.

Do et al., 2014 reported that extraction efficiency of the solvents is affected by the physiochemical properties, the particle size of the solvents, and the interfering substances. So, high the polarity of the extraction solvent maximum will be the yield of extract and same goes with the number of bioactive compounds extracted. Airouyuwa et al. (2022) reported that viscosity of the Choline chloride and 1,4-Butanediol significantly ($p<0.05$) decrease (1710cP to 480cP) with an increase in temperature from 40℃ to 60℃. This indicates the expansion of molar volume which is resulted by increase in kinetic energy as well as free molecular movement of the hydrogen bond donors as well as hydrogen bond acceptors.

Table 6.5 Optimization of the extraction yield of *Moringa oleifera* flower by Deep Eutectic Solvents

Extraction Solvents	Temperatures (℃)	Extraction yield at different molar ratio (%)		
		1:1	1:2	1:3
Choline chloride /Glycerol	50	4.61 ± 0.21^{cF}	4.85 ± 0.16^{aF}	4.63 ± 0.07^{bF}
	60	4.94 ± 0.10^{bF}	5.05 ± 0.10^{aE}	4.84 ± 0.10^{cF}
	70	5.16 ± 0.19^{cE}	5.33 ± 0.11^{aD}	5.25 ± 0.09^{bE}
	80	4.92 ± 0.08^{cF}	5.12 ± 0.09^{aE}	5.02 ± 0.07^{bE}
Choline chloride /Malic acid	50	4.93 ± 0.11^{bF}	5.04 ± 0.11^{aE}	5.04 ± 0.19^{aE}
	60	5.11 ± 0.13^{cE}	5.35 ± 0.11^{aD}	5.15 ± 0.21^{bE}
	70	5.52 ± 0.08^{cD}	5.93 ± 0.09^{aD}	5.65 ± 0.11^{bD}
	80	5.29 ± 0.10^{cE}	5.65 ± 0.11^{aD}	5.46 ± 0.06^{bD}
Choline chloride /Lactic acid	50	5.36 ± 0.11^{cD}	5.93 ± 0.08^{aD}	5.62 ± 0.09^{bD}
	60	5.77 ± 0.95^{cD}	6.25 ± 0.10^{aC}	5.84 ± 0.08^{bD}
	70	6.84 ± 0.90^{bB}	6.86 ± 0.12^{aB}	6.42 ± 0.07^{cC}
	80	5.86 ± 0.13^{cD}	6.57 ± 0.08^{aB}	6.11 ± 0.11^{bD}

Choline chloride/Glucose	50	5.80 ± 0.09^{cD}	6.15 ± 0.20^{aC}	6.01 ± 0.06^{bD}
	60	6.21 ± 0.19^{bC}	6.66 ± 0.07^{aB}	6.14 ± 0.09^{cC}
	70	6.85 ± 0.07^{cB}	7.16 ± 0.10^{aB}	6.94 ± 0.01^{bB}
	80	6.53 ± 0.09^{bC}	6.87 ± 0.11^{aB}	6.68 ± 0.09^{bB}
L-proline/Glycerol	50	6.27 ± 0.08^{cC}	7.38 ± 0.08^{aA}	7.25 ± 0.11^{bB}
	60	7.43 ± 0.03^{bA}	8.53 ± 0.09^{aA}	7.36 ± 0.11^{cA}
	70	8.17 ± 0.10^{cA}	9.13 ± 0.10^{aA}	8.34 ± 0.01^{bA}
	80	7.93 ± 0.12^{cA}	8.84 ± 0.09^{aA}	8.26 ± 0.20^{bA}
L-proline/Lactic acid	50	6.08 ± 0.09^{cC}	6.97 ± 0.02^{aA}	6.63 ± 0.10^{bB}
	60	6.99 ± 0.12^{cB}	7.51 ± 0.09^{aA}	7.11 ± 0.09^{bB}
	70	7.63 ± 0.08^{cA}	8.33 ± 0.09^{aA}	7.93 ± 0.05^{bA}
	80	7.56 ± 0.11^{cA}	7.81 ± 0.09^{aA}	7.64 ± 0.10^{bA}

Data are represented as mean ±SD (n=3)

$^{a-c}$ Means within a column with different superscripts are significantly different ($P < 0.05$)

$^{A-F}$ Means within a row with different superscripts are significantly different ($P < 0.05$)

6.7 Total phenolic content (TPC)

The results revealed that out of 3 solvent ratios 1:2 showed significantly ($p<0.05$) higher extraction yield so, this ratio was carried forward to perform further experimentation. The TPC content of *Moringa oleifera* flower extract was measured by an FC reagent. Table 6.6 represent the phenolic content of all 6 DESs at four temperature ranges of 50℃, 60℃, 70℃, and 80℃.In this study, it was observed that different DES extraction solvent samples showed significant ($p<0.05$) differences ($p<0.05$) with each other at all the temperature ranges (50℃, 60℃, 70℃, and 80℃). The total phenolic content of the extract was increasing with the increase in temperature, till the temperature was 70℃, and after that, it starts decreasing at a significant difference ($p<0.05$) (32.4 to 30.7 mg GAE/g DW). The reason behind decrease in phenolic content at 80 ℃ was the degradation of the chemical structures of the bioactive compounds due to which the extraction efficiency decreased significantly ($p<0.05$). At 50 ℃ the TPC content of *Moringa oleifera* flower extract was 24.41 mg GAE/g, which was significantly ($p<0.05$) lower than 60℃. At, 60℃ it was significantly higher 29.32 mg GAE/g, at temperature 70℃ it was 32.46 mg GAE/g, whereas at 80℃ it was significantly ($p<0.05$) lower 30.74 mg GAE/g.

This study, showed that significantly ($p<0.05$) higher number of polyphenols was extracted at 70℃ with a solvent combination of L-proline and Glycerol (32.4 mg GAE/g DW), as compared with Choline chloride and lactic acid 23.1 mg GAE/g. The minimum extraction yield was obtained with the combination of Choline chloride and malic acid at 50℃ (3.8 mg GAE/g DW) however, it is quite evident from the results that Choline chloride and the malic acid combination gave minimum yield at all the temperatures due to less polarity between both the solvents. At 50℃ it showed 3.84mg GAE/g, which was significantly lower than 4.91mg GAE/g at 60℃. At 70℃ it showed significantly ($p<0.05$) higher phenolic content (5.82mg GAE/G) than 80℃ (5.22mg GAE/g).

The extraction efficiency of the phytochemicals is determined by various factors such as the nature of the phytochemicals, the particle size of the sample, solvents used for extraction, and last but not least and an interesting part presence of the interfering substances (Stalikas, 2007). Secondly, the temperature of extraction also plays an imperative part in phytochemicals extraction because there are phytochemicals that need a particular range of temperatures to be extracted. It has been proven in research by Elboughdiri et al. (2020) that if the extraction temperature was raised after a particular range (80℃) viscosity of the

extraction solvent gets reduced (380cP to 260cP) which increases the ability to solubilize the target analytes and the increased temperature is also responsible for breaking the bonds and enhancing the surface area of the diffusion. The same thing was happening here in this study increasing the temperature beyond 70℃ is increasing the surface area and decreased the amount of polyphenols content and below 70℃ temperature fewer amount polyphenols are getting extracted so the results revealed that polyphenolic content was significantly higher ($p<0.05$) at 70℃. Almost the same results were reported by Elboughdiri et al., 2020 when they get maximum polyphenolic content at 70℃ (28.51mg GAE/g). In that study, they revealed that increasing the temperature range from 55℃ to 70 ℃ resulted in the maximum amount of polyphenols extracted where at 50℃ the polyphenolic content was only 23.10mg GAE/g and at 70℃ it significantly ($p<0.05$) increased to 28.51mg GAE/g.

Duy et al., 2020 reported that increasing the temperature from 50℃ to 70 ℃ resulted in increased content of polyphenolic content as it leads to the maximum amount of polyphenols to be extracted. Further, Antony and Farid, 2022 reported that a temperature range of 60-80 ℃ showed the maximum amounts of polyphenols extracted. At temperatures beyond this range, a new type of reaction takes place (Maillard reaction) which could affect the extraction process of the polyphenols. Even in traditional methods of extraction the extraction temperature of 60-80 ℃ is said to be the most preferred temperature range, because at these temperatures there is a lesser amount of solvent loss, beyond this temperature range solvent loss is higher and can negatively affect the extraction process. Studies also reported that drying temperature also plays an important role in the number of polyphenols extracted because if the plant material is dried at a temperature range of more than 80 ℃ it resulted to diminish the polyphenolic content (heat labile compounds) (Al Juhaimi et al., 2018). It has been reported by Wu et al., 2020 that amide-based DESs (L-proline-Glycerol) had a great extraction efficiency for the phenolic and flavonoid compounds. Among all the extraction solvents L-proline-Glycerol had the highest total phenolic content with a significant range of polarity so this combination was selected as the best green solvent.

Table 6.6 Total phenolic content of *Moringa oleifera* flower extract at 50-80°C with six extraction solvents

Extraction Solvents	Total phenolic content (mg GAE/g)			
	50°C	**60°C**	**70°C**	**80°C**
Choline chloride: Glycerol	10.11 ± 0.39^{aB}	13.52 ± 0.36^{bB}	17.28 ± 0.004^{dC}	15.94 ± 0.35^{cC}
Choline chloride: Malic acid	03.84 ± 0.14^{aA}	04.91 ± 0.35^{bA}	05.82 ± 0.003^{dA}	05.22 ± 0.61^{cA}
Choline chloride: Lactic acid	17.41 ± 0.53^{aE}	20.63 ± 0.34^{bE}	23.15 ± 0.002^{dE}	21.74 ± 0.38^{cE}
Choline chloride: Glucose	11.72 ± 0.32^{aC}	13.86 ± 0.64^{bC}	15.11 ± 0.003^{dB}	14.83 ± 0.16^{cB}
L-proline: Glycerol	24.41 ± 0.26^{aF}	29.32 ± 0.43^{bF}	32.46 ± 0.002^{dF}	30.74 ± 0.28^{cF}
L-proline: Lactic acid	14.66 ± 0.35^{aD}	17.81 ± 0.41^{bD}	20.14 ± 0.004^{dD}	19.21 ± 0.13^{cD}

Data are represented as mean ±SD (n=3)

$^{a-d}$ Means with the same superscript in columns vary significantly ($p < 0.05$) from each other

$^{A-F}$ Means with the same superscript in rows vary significantly ($p < 0.05$) from each other

6.8 Total flavonoid content (TFC)

The total flavonoid content of *Moringa oleifera* flower extract was determined by using six different extraction solvents, at different temperatures 50℃, 60℃, 70℃, and 80℃. From the results it was observed *Moringa oleifera* flower extract is a rich source of flavonoids, whereas all the solvents showed significant ($p<0.05$) differences in TF content with each other at different temperature ranges as shown in table 6.7. Quercetin, kaempferol, apigenin, and rutin are flavonoids that are found in *Moringa oleifera* flowers at 50℃, 60℃, 70℃, and 80℃ extraction temperatures. Extraction solvent L-proline and Glycerol combination showed significantly ($p<0.05$) higher amount of flavonoids 44.13 mg QE/g at 70℃ temperatures as compared with Choline chloride and glucose combination 30.14 mg QE/g. Choline chloride and malic acid combination showed significantly ($p<0.05$) lower flavonoid content 26.6 mg QE/g as compared to L-proline-Glycerol 44.13mg QE/g and Choline chloride-glucose 30.11mg QE/g. L-proline and Lactic acid combination gave significantly ($p<0.05$) lower flavonoid content of 23.14 mg QE/g whereas the lowest amount of flavonoids was extracted by Choline chloride and Glycerol solvents 26.40 mg QE/g at 70℃. With the increase in temperature from 70℃ to 80 ℃ all the solvents showed a significant decrease ($p<0.05$) in flavonoid content. At this temperature Choline chloride and glycerol showed significantly($p<0.05$) lower amount of flavonoids.

According to research, this study revealed that 70℃ was the optimized temperature for the maximum amount of flavonoid content with extraction solvent L-proline and Glycerol (44.13 mg QE/g). The specific reason for the high flavonoid content with these solvents was the high polarity between the solvents (Zarrinmehr et al., 2022). Zhou et al. (2018) stated that extraction temperature and liquid-solid ratio play a significant role in the extraction of flavonoids on increasing temperature from 70℃ to 80℃ whereas, the flavonoid content of the *Moringa oleifera* leaves decreased significantly ($p<0.05$) from 24.82 mg GAE/g to 19.71 mg GAE/g. In another study Cui et al. (2019) revealed that optimum temperature for flavonoid extraction is between 60-80℃ and beyond that temperature range denaturation of flavonoid compounds start due to which flavonoid content significantly ($p<0.05$) decreased upto 30%.

Zhang et al., (2022) investigated how temperature affected the flavonoid content of the *Acanthopanax senticosus* and observed that both physical (thermal stability, sustainability, and biodegradability) and chemical properties (biocompatibility, melting point, and structure)

along with the functions of the DESs were affected by the increase in the temperature. Their study concluded that on increasing the temperature, the conductivity of the DESs gets elevated and the surface tension get decreased. As the extraction temperature is increased gradually the extraction rate of the flavonoids was also increased (11.24 mg QE/g to 17.34 mg QE/g) significantly ($p<0.05$). Above that temperature it showed a downward trend that was due to the increase in temperature could lead to speed up of the molecular movement and decrease in the viscosity. Increased flavonoid extraction is the result of the compounds' increased solubility in the DESs.

Table 6.7 Total flavonoid content of *Moringa oleifera* flower extract

Extraction Solvents	Total flavonoid content (mg QE/g)			
	50 °C	60°C	70°C	80°C
Choline chloride: Glycerol	11.83 ± 0.33^{cA}	11.32 ± 0.17^{aA}	26.40 ± 0.003^{dC}	11.64 ± 0.18^{bA}
Choline chloride: Malic acid	17.82 ± 0.37^{aC}	25.14 ± 0.27^{bD}	26.62 ± 0.001^{dC}	25.75 ± 0.25^{cD}
Choline chloride: Lactic acid	15.44 ± 0.31^{aB}	18.50 ± 0.35^{cB}	18.64 ± 0.003^{dA}	18.31 ± 0.33^{bB}
Choline chloride: Glucose	24.44 ± 0.29^{aD}	29.80 ± 0.13^{bE}	30.11 ± 0.005^{dD}	29.93 ± 0.13^{cE}
L-proline: Glycerol	39.71 ± 0.40^{aE}	41.31 ± 0.51^{bF}	44.13 ± 0.002^{dE}	43.91 ± 0.55^{cF}
L-proline: Lactic acid	17.75 ± 0.26^{aC}	22.82 ± 0.32^{bC}	23.14 ± 0.002^{dB}	22.90 ± 0.38^{cC}

Data are represented as mean ±SD (n=3)

$^{a-d}$ Means with the same superscript in columns vary significantly ($p < 0.05$) from each other

$^{A-F}$ Means with the same superscript in rows vary significantly ($p < 0.05$) from each other

6.9 DPPH radical scavenging activity

Antioxidants are free radical scavengers and play an imperative role in food preservation by oxidation inhibition process and also contribute to health promotion. Antioxidant activity could be determined using various assays and different methods such as DPPH scavenging, single electron transfer method, hydrogen atom transfer, metal chelation, and reducing power. Table 6.8 represent the antioxidant activity of *Moringa oleifera* flower extract by using six DESs solvents at varying temperatures from 50-80 ℃. Results obtained in this study showed that extract obtained from extraction solvent L-proline-Glycerol showed significantly ($p<0.05$) higher antioxidant activity (74%) at 70℃. The minimum antioxidant activity of flower extract was shown by Choline chloride-lactic acid (42%) extract. L proline and Glycerol, Choline chloride and glucose showed significantly ($p<0.05$) higher antioxidant activity 65.82% as compared with the Choline chloride and Glycerol combination 63.44%. Choline chloride and malic acid showed 51.41% antioxidant activity at 70℃ and the solvent mixture of choline chloride and lactic acid had the least amount of antioxidant activity 47.01%. All the DESs extracted extracts showed a significant ($p<0.05$) amount of antioxidant activity but due to the highly polar nature of the L-proline and Glycerol, the extract obtained from this solvent showed the highest antioxidant activity (68.34% at 50℃, 70.28% at 60℃, 74.83% at 70℃, and 72.62% at 80℃.

Duy and co-workers, (2020) showed the effect of extraction temperature on the antioxidant activity of the *hibiscus* extract. They found that the antioxidant activity increase with an increase in temperature up to 70℃ but once it goes higher than 70℃ it starts decreasing significantly from 72.58% to 67.74%. Moreover, Li et al., 2012 examined antioxidant activity of the tomatoes by using two factors, the extraction time and temperatures (50-90℃), and resulted that on increasing the temperature antioxidant activity of tomato extract also increased significantly ($p<0.05$) up to 70℃ (97.41%) and decreased at 80℃ (82%). Studies reported a significant ($p<0.05$) correlation in polyphenolic content and antioxidant activity. The extracts' antioxidant activity rises with polyphenolic concentration. On comparing the results of current study with previously done studies one needs to consider the usage of different extraction solvents and the methodology used for extraction. However, agroclimatic locations and the influence of season play an imperative part in the process (Llorent-Martinez et al., 2023).

In a study scientists revealed that administration *Moringa oleifera* extract in rats at a concentration of 50 and 100 mg/day for two weeks showed a significant ($p<0.05$) reduction in lipid peroxides as well as rise in glutathione concentration in the liver and kidneys, which occurred due to the presence of high antioxidant activity (Ma et al., 2018). Shady et al. (2022) revealed peroxide scavenging effect of *Moringa oleifera* extract observed in the mice model for four weeks of studies, resulting in great wound healing in mice at the rate of 49.26% with 1000µg/ml dose.

Table 6.8 Antioxidant activity of *Moringa oleifera* flower powder extract

Extraction Solvents	% Inhibition (DPPH)			
	50°C	**60°C**	**70°C**	**80°C**
Choline chloride: Glycerol	59.36 ± 0.24^{aD}	61.48 ± 0.40^{cE}	63.44 ± 0.05^{dD}	60.86 ± 0.12^{bC}
Choline chloride: Malic acid	43.71 ± 0.15^{aA}	46.32 ± 0.12^{cB}	51.41 ± 0.22^{dB}	46.21 ± 0.1^{bA}
Choline chloride: Lactic acid	42.84 ± 0.06^{aA}	44.35 ± 0.03^{bA}	47.01 ± 0.17^{dA}	45.42 ± 0.07^{cA}
Choline chloride: Glucose	55.42 ± 0.07^{aC}	57.46 ± 0.20^{bD}	65.82 ± 0.29^{dE}	62.43 ± 0.05^{cC}
L-proline: Glycerol	68.34 ± 0.78^{aE}	70.28 ± 0.03^{bF}	74.83 ± 0.16^{dF}	72.62 ± 0.03^{cD}
L-proline: Lactic acid	51.18 ± 0.14^{aB}	53.13 ± 0.15^{bC}	59.93 ± 0.19^{dC}	56.17 ± 0.04^{cB}

Data are represented as mean ±SD (n=3)

$^{a-d}$ Means with the same superscript in columns vary significantly ($p < 0.05$) from each other

$^{A-F}$ Means with the same superscript in rows vary significantly ($p < 0.05$) from each other

6.*10 IN VITRO* ANALYSIS

6.10.1 Lipase inhibition assay

Table 6.9 presented the results of the lipase inhibition assay and the Ic50 of the *Moringa oleifera* flower extract. In this study, different concentrations of *Moringa oleifera* flower extract *viz*.25, 50, 75, and 100 μgmL^{-1} were used to check the inhibition assay of lipase as compared to positive control. The study revealed that 100 μgmL^{-1} concentration of *Moringa oleifera* flower extract showed significantly ($p<0.05$) higher percentage of lipase inhibition as well as Ic50 62.01 as compared to 75 μgmL^{-1} (64.79), 50 μgmL^{-1} (59.34), and 25 μgmL^{-1} (52.96). Similarly, it was observed that *Moringa oleifera* flower extract showed significantly ($p<0.05$) lower Ic50 (62.01) as compared to positive control Ic 50 (74.32). However positive control showed significantly ($p<0.05$) higher difference at all the concentrations 25 μgmL^{-1} (71.38), 50 μgmL^{-1} (75.38), 75 μgmL^{-1} (78.47), and 100 μgmL^{-1} (82.57) as compared with *Moringa oleifera* flower extract 52.96%, 59.34%, 64.79%, and 67.10% respectively.

Ogundipe et al. (2022) reported that the ethanolic extract of the *Moringa oleifera* leaves showed a significantly ($p<0.05$) higher (108.77) inhibitory effect in comparison to the Orlistat drug (81.70). *In vivo,* and *in vitro* studies have reported *Moringa oleifera* leaf extract has antiobesity properties due to the existence of bioactive compounds in the extract such as polyphenols and flavonoids they inhibit the pancreatic lipase enzyme activity (Ogundipe et al., 2022). All these studies validate/support the lipase-inhibitory properties of *Moringa oleifera* flower extract. In a study Motallebi et al. (2023) reported that hydroalcoholic extract of the *Moringa oleifera* leaves had pancreatic lipase inhibition activity with Ic 50=437.1 µg/mL. The specific mechanism of action behind this was the level of resistin and leptin that was increased by the intake of *Moringa oleifera* leaves that also decreased the inflammatory cytokines level. It directly helps in the reduction of the fat which was the main source of the adipokines. Pancreatic lipase is one of the digestive enzymes which is responsible for the metabolism and absorption of complex fatty acids (triglycerides) to simpler ones (monoglycerides). If the lipase enzyme gets inhibited in the body, it can reduce the systematic absorption of fat, which will contribute to the caloric deficit as well as reduction of the total cholesterol in body (Vangoori et al., 2019).

Table 6.9 Lipase inhibition assay *of Moringa oleifera* flower extract

Concentration (µgmL^{-1})	% Lipase inhibition	
	Positive control	*Moringa oleifera* flower extract
25	71.38±0.18^{bA}	52.96±0.08^{aA}
50	75.28±0.26^{bB}	59.34±0.33^{aB}
75	78.47±0.45^{bC}	64.79±0.37^{aC}
100	82.57±0.32^{bD}	67.10±0.31^{aD}
Ic50	74.32	62.01

Data are represented as mean ±SD (n=3)

$^{a-b}$ Means with the same superscript in columns vary significantly ($p < 0.05$) from each other

$^{A-D}$ Means with the same superscript in rows vary significantly ($p < 0.05$) from each other

6.10.2 Amylase Inhibition Assay

Table 6.10 represented the amylase percentage inhibition assay and Ic 50 value of the *Moringa oleifera* flower extract. This study, resulted that *Moringa oleifera* flower extract has a significantly ($p<0.05$) higher inhibition activity (35%) at 100 µg/ml, as compared to 80 µg/ml (27.80%), 60 µg/ml (20.31%), 40 µg/ml (13.60%), and 20 µg/ml (6.50%). Similarly, it was observed that *Moringa oleifera* flower extract showed significant ($p<0.05$) higher Ic 50 (46.72) as compared to a study done by Kumar et al. (2020) *Bergenia ciliate* reported to be 18.27% of amylase inhibition and the Ic50 value is 4.47. Ogundipe et al. (2022) reported that hydroalcoholic extract from *Moringa oleifera* leaves has amylase inhibition activity (18.02%). Similarly, it showed that *Moringa oleifera* leaves extract showed 34% inhibitory activity at 100 µg/ml, 29% inhibition at 80 µg/ml, 27% inhibition at 60 µg/ml, 22.58% inhibition at 40 µg/ml.

Table 6.10 Amylase inhibition assay of *Moringa oleifera* flower extract

Concentration of flower extract (µg/ml)	% of Amylase Inhibition	Ic50
20	6.50±0.003	46.72
40	13.60±0.004	
60	20.31±0.004	
80	27.80±0.003	
100	35.1±0.004	

Data are presented as mean ± S.D (n=3)

6.10.3 Glucose uptake assay

Glucose uptake assay is essential in multiple ailments and cardiometabolic conditions *viz.* diabetes, obesity, and cancer (Yamamoto et al., 2015). A glucose uptake assay was observed for *Moringa oleifera* flower extract to check the role in ameliorating high glucose levels. In this research, glucose level was observed at 0.5 mg/dL, 1.0 mg/dL, 1.5 mg/dL, 2.0 mg/dL, and 2.5 mg/dL with different time period (15 min, 30 min, 60 min, 120 min, 240 min, 360 min, 480 min. Figure 6.7 represents *Moringa oleifera* flower extract showed maximum higher glucose retention at 280 min (2.03 mg/dL) in the dialysis membrane whereas, control showed (2.32mg/dl) glucose retention at 360 min. Interestingly glucose retention of the *Moringa oleifera* flower extract reduces at 400 min. The results indicate that *Moringa oleifera* flower extract shows the quenching property of the glucose molecules and plays significant part in movement of higher glucose concentrations. *Moringa oleifera* flower extract showed the property to reduce the action of the alpha-glucosidase that significantly ($p<0.05$) reduce the levels of post-prandial hyperglycaemia (Nova et al., 2020).

Multiple studies favour the anti-diabetic properties of the *Moringa oleifera* plant parts. Studies performed on Streptozotocin (STZ) or alloxan-induced rats and a few of them have been executed on obese models that were fed on a high-fat diet (HFD). One of these models relies on the chemical devastation of the pancreatic beta cells and these chemicals are

retained as cytotoxic glucose analogues which tend to be accumulated in the pancreatic beta cells *via* glucose transporter 2 (GLUT2). Moreover, it also depends on the dose of the chemicals retained *via* intraperitoneal instillation (Gheibi et al., 2017). *Moringa oleifera* extract contains compounds like phenyl glycoside, carbamates, and thiocarbamate, that encourage pancreatic beta cells to secrete insulin and help the body to manage the blood glucose levels. Secondly, it also contains terpenoids that stimulate the beta cells of the pancreas and subsequently secret insulin (Razis et al., 2014). The hypoglycaemic property of *Moringa oleifera* extract has been studied in both standard and Streptozotocin-induced (STZ) diabetic rats. Oral administration of the extract reduces sugar levels by about 26.7%. Even continuous usage of the extract showed the decrease in blood glucose levels by 69.2% (Tang et al. 2017). Aqueous extract of the plant showed a great hypoglycaemic effect in alloxan-induced rats by reducing the rate of gluconeogenesis and regenerating the damaged hepatic cells and pancreatic β-cells (Abd El Latif et al., 2014). In a randomized study Anthanont et al., 2016 revealed that consuming 7gm *Moringa oleifera* leaves powder continuously for three months resulted in 13.5% of reduction in postprandial as well as fasting blood glucose levels. A study stated that on administration *of Moringa oleifera* leaves extract for 10 days animal models showed a significant ($p<0.05$) decrease in post-prandial glucose levels from 280 to 210 mg/dL (Barodia et al., 2021).

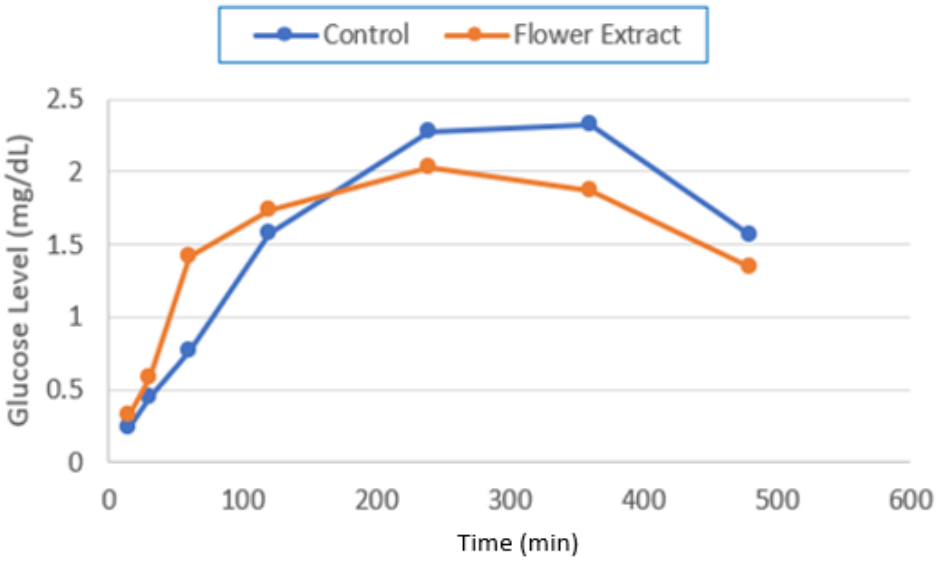


Fig. 6.7 Glucose level (mg/dL) *Moringa oleifera* flower extract

6.11 Antibacterial activity

The antibacterial activity of *Moringa oleifera* flower extract was assessed against two bacteria *Escherichia coli & Staphylococcus aureus* by the presence of a zone of inhibition. The antibacterial activity of the *Moringa oleifera* flower extract is shown in Table 6.11 and Figure 6.8 respectively. These results revealed that the *Moringa oleifera* flower extract had a zone of inhibition of 11 mm against *Escherichia coli* and an 8 mm against *Staphylococcus aureus*, whereas the antibiotic Streptomycin had a zone of inhibition of 20 mm. Al-Reza et al. (2022) reported that *Moringa oleifera* leaf extract had a zone of inhibition against *Escherichia coli* (11mm), Staphylococcus aureus (25 mm), and streptomycin (22mm). All of these results suggest that Moringa oleifera might have antibacterial properties. All of these results make *Moringa oleifera* a potential source of antibacterial activities.

Moringa oleifera flower extract exhibited an antibacterial effect against *Escherichia coli* with an inhibition zone of 11 mm which is significantly ($p<0.05$) higher than the activity against *Staphylococcus aureus* with an inhibition zone of 9 mm. Antibiotic streptomycin showed the maximum zone of inhibition by 21 mm and 19 mm respectively whereas negative control DMSO showed no activity against any of the bacteria. Talath *et al*. (2022), reported the inhibition zone of 14.5 and 12.7 mm, respectively when activity was checked with *Moringa oleifera* leaf extract. 1000 mg/ml concentration showed highest inhibition in both studies whereas there was no zone of inhibition in concentrations below 125 mg/ml. According to current research by Yang et al. 2020, the anti-bacterial action of quercetin includes breakdown of bacterial cell wall, change in permeability of the cell, reduction in enzymatic activities, and inhibiting nucleic acid synthesis. The isothiocyanates present in the Moringa are responsible for the antibacterial activity. Ahmed et al., (2023) revealed that *Moringa oleifera* flower extract exhibits great antimicrobial activity with an inhibition zone of 7.66, and 10.00, against *Bacillus cereus, and Escherichia coli* respectively. In ethanolic, methanolic, and aqueous extract of the leaves, the aqueous extract significantly ($p<0.05$) exhibits the higher antimicrobial activity against all three microorganisms.

Table 6.11 Antibacterial activity of *Moringa oleifera* flower extract

Microorganism	Zone of inhibition (mm)	
	***Moringa oleifera* flower extract**	**Positive control**
Escherichia coli	11±0.6	20±0.5
Staphylococcus aureus	08±0.7	18±0.7

Data are presented as mean ± S.D (n=3)

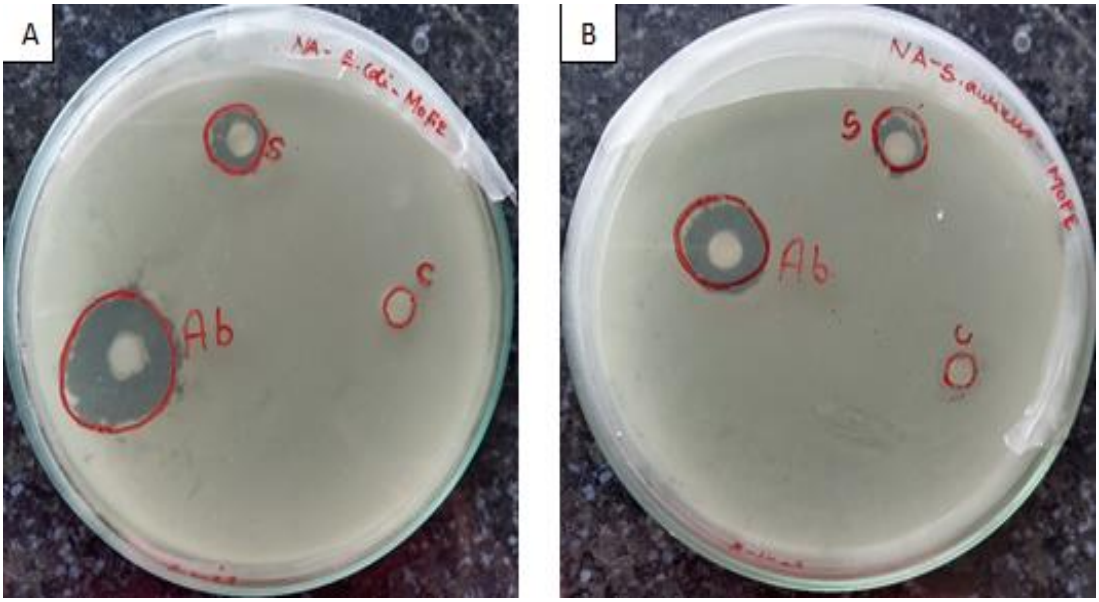


Figure 6.8 Zone of inhibition of *Moringa oleifera* flower extract A. *Escherichia coli*, B. *Staphylococcus aureus*

6.11.1 Minimum inhibitory concentration

The minimum inhibitory concentration of *Moringa oleifera* flower extract was observed with various pathogenic organisms ie. *Escherichia coli* and *Staphylococcus aureus* (Table 6.12). The findings demonstrated that minimum inhibitory concentration for both the bacteria were found at 250 mg/ml as compared with the study of by Raheem *et al.* (2015) whereas the MIC was reported to be 512 mg/ml and 256 mg/ml respectively for the same set of bacteria in *Moringa oleifera* leaves extract. Out of these two bacteria *Moringa oleifera* flower extract showed significantly ($p<0.05$) increased inhibition of *Escherichia coli* than the *Staphylococcus aureus.* These findings are also comparable to the results reported by Farhan

et al. (2021) where minimum inhibitory concentrations for food-borne pathogens were found 200 mg/ml.

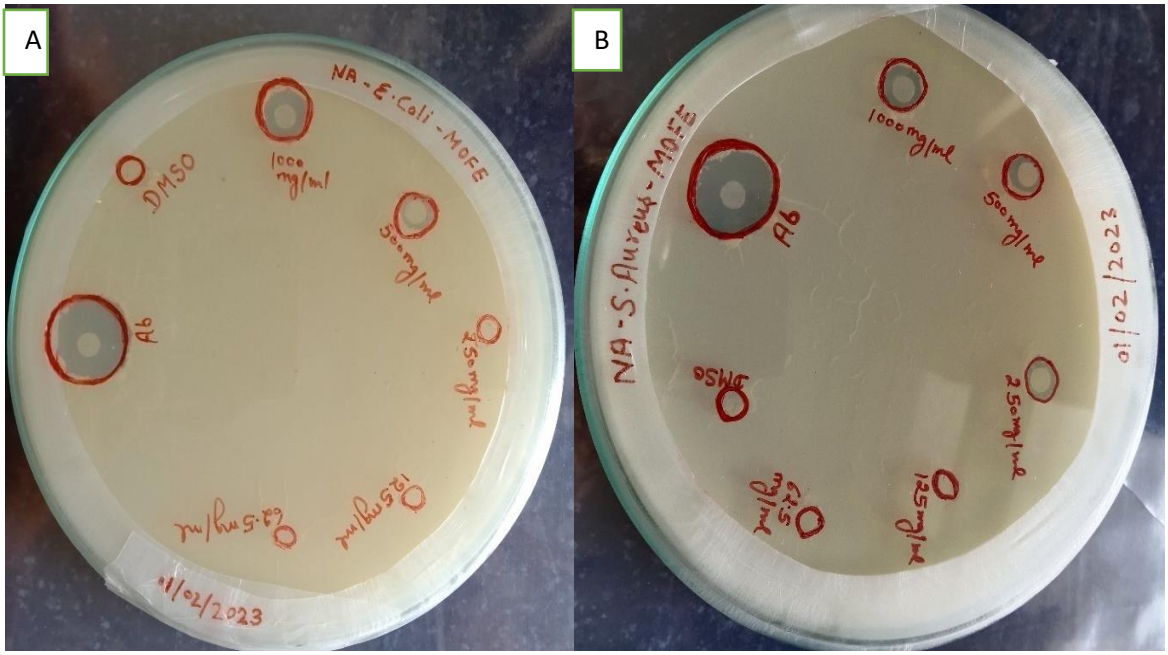


Fig 6.9 Minimum inhibitory concentration by zone of inhibition with different concentrations of *Moringa oleifera* flower extract against A) *Escherichia coli*, B) *Staphylococcus aureus*

Table 6.12 Minimum inhibitory concentration of *Moringa oleifera* flower extract

Bacteria	Concentrations of *Moringa oleifera* flower extract					Negative Control	Positive control
	1000 mg/ml	500 mg/ml	250 mg/ml	125 mg/ml	62.5 mg/ml		
Escherichia coli	11±0.65	9±0.45	3±0.67	Resistant	Resistant	Resistant	21±0.85
Staphylococcus aureus	9±0.72	6±0.64	2±0.71	Resistant	Resistant	Resistant	19±0.64

Data are represented as mean SD± (n=3)

6.12 PREPARATION OF YOGURT

Moringa oleifera flower extract-enriched yogurt samples were checked on the 0th, 7th, 14th, 21st, and 28 days for the viability of the bacteria, titratable acidity, pH, viscosity, and syneresis of the yogurt. The results for independent variables such as incubation time and flower extract concentration and all the responses are given in table no. 6.13.

6.12.1 Diagnostic checking for the fitted model

All the effects of the Central Composite Design (CSD) like quadratic, and linear were considered for each one of the models, and their adequacies were checked by using the F ratio, and the lack of fit test. All the responses *viz.* titratable acidity, protein, antioxidant activity, phenolic and total flavonoid content were fitted with the quadratic significant models. Table 6.14 shows estimated regression coefficients, the design summary, and their significance for the dependable variables.

Table 6.13 Levels of independent variables A. Starter culture (%), B. Flower extract concentrations (%) used in central composite design with all the measured responses

Run	Independent variables		Responses				
	A	B	Y_1	Y_2	Y_3	Y_4	Y_5
1	5.50	3.00	0.832	4.9	76.43	24.76	20.32
2	5.00	1.00	0.766	4.6	74.16	20.74	18.21
3	6.21	3.00	0.817	5.1	76.06	24.89	20.42
4	5.50	3.00	0.826	4.8	75.34	24.59	20.34
5	6.00	5.00	0.935	5.7	84.21	28.31	21.34
6	6.00	1.00	0.945	4.6	74.21	20.34	18.16
7	5.50	0.17	0.978	4.3	71.25	19.01	17.16
8	5.50	3.00	0.825	4.8	75.33	24.59	20.71
9	5.00	5.00	0.923	5.6	82.67	27.94	21.26
10	5.50	3.00	0.834	4.9	76.16	24.92	20.45
11	5.50	3.00	0.843	4.8	75.98	24.99	20.39
12	4.79	3.00	0.831	5.1	76.27	25.02	19.56
13	5.50	5.83	0.925	5.8	84.38	28.39	21.37

A Incubation time, B flower extract concentration, Y_1 titratable acidity, Y_2 Protein, Y_3 antioxidant activity, Y_4 total phenolic content, Y_5 total flavonoid content

Table 6.14 Design summaries, the estimated regression coefficients, and their significance for the dependable variables for *Moringa oleifera* flower extract-enriched yogurt

Factors	Titratable acidity (%)	Protein (%)	Antioxidant activity (%)	TPC (mg GAE/g)	TFC (mg QE/g)
Model	Quadratic significant	Quadratic significant	Quadratic significant	Quadratic significant	Quadratic significant
Intercept	0.038	4.84	75.85	24.77	20.44
A	3.64	0.012	-0.21	-0.027	0.16
B	6.47	0.53	5.01	3.55	1.52
AB	6.97	0.025	0.38	0.19	0.032
A^2	5.72	0.14	0.79	0.093	-0.20
B^2	5.72	0.12	1.63	-0.53	0.56
R^2 (%)	0.7596	0.9925	0.9347	0.9943	0.9855
Lack of fit	Significant	not significant	not significant	not significant	not significant

6.12.2 Response surface methodology model for titratable acidity

According to RSM, the model is found to be quadratic significant for the titratable acidity and the following equation expresses the significance of the model-

Titratable acidity = $0.83+0.021A+8.99B-0.042AB–2.851A^2+0.061B^2$ **(27)**

The coefficient of regression (R^2) for the titratable acidity is found to be 0.7596 which means the model for the same could account for 75.96% of the data. A was a coded factor for the incubation time of the yogurt whereas B was flower extract concentration. The titratable acidity of the yogurt ranged between 0.766 to 0.978. Table 6.13 represent that an increase in flower extract concentration is responsible for the increase in titratable acidity because high the *Moringa oleifera* flower extract higher will be the amino acid content of the yogurt which accelerates the lactic acid production and a rise in the acidity of the yogurt (Aznury et al.,

2020). Figure 6.10 shows the response surface plot for titratable acidity. Zhang et al., 2018 reported that adding the *Moringa oleifera* leaf extract to the yogurt enhances its fermentation process and increase the titratable acidity for the same (0.875 to 0.956). Flower extract is accelerating the process, and it was in an acceptable range (0.861 to 0.972) and improving the acceptability of the yogurt. A study done by Santoso et al., 2021 found that an increase in incubation time from 6 to 10 hrs which is also proportional to the production of lactic acid that led to a slight increase in the titratable acidity of the flower-extract enriched yogurt from (0.915 to 0.987).

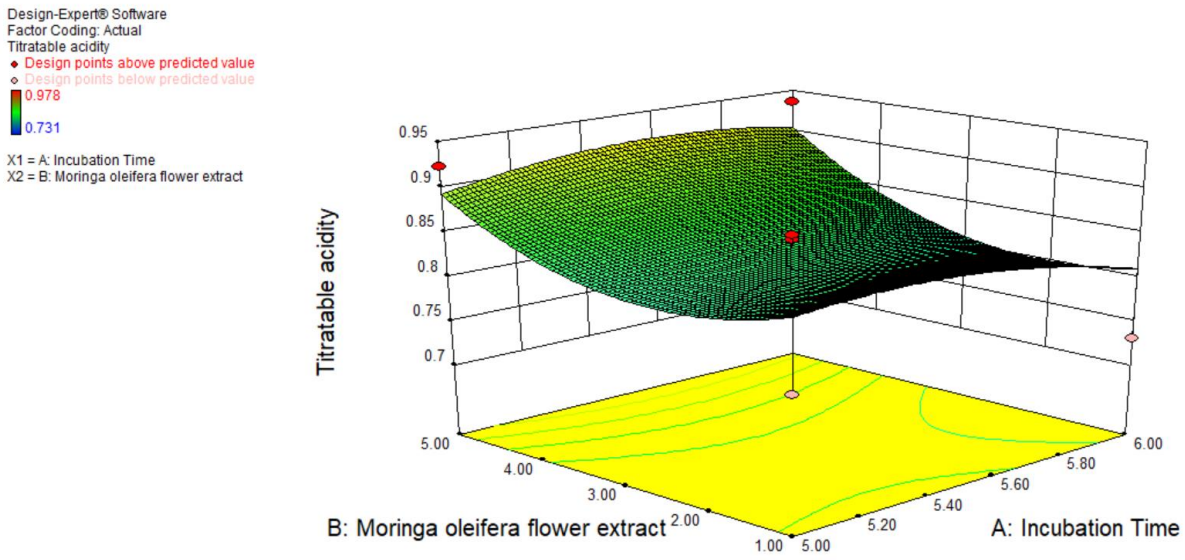


Fig.6.10 Response surface plot for titratable acidity

6.12.3 RSM Model for protein

RSM model for proteins is found to be quadratic significant with the protein values ranging between 4.3 to 5.8 gm. The following equation expresses the quadratic model for the proteins-

$$\textbf{Protein} = 4.84 + 0.012A + 0.53B + 0.025AB + 0.14A^2 + 0.12B^2 \quad (28)$$

The coefficient of regression (R^2) for the protein content is found to be 0.9925 which means the model for the same could account for 99.25% of the data. From the results, it is evident that on increasing the flower extract concentration protein content of yogurt automatically increase (4.6% to 5.8%) because *Moringa oleifera* flower is a potential source of protein

(Adepoju & Selezneva, 2020). Figure 6.11 shows the response surface plot for the protein content of yogurt. The same results were shown in a study done by Lisak Jakopovic et al., 2022 where by adding *Moringa oleifera* leaf powder to the yogurt enhances the protein of the yogurt from 3 to 3.8% because it had higher dry matter content, high protein content (5.2%) when compared with the control samples (4.9%). Mendoza-Taco et al., 2022 reported that when sheep were fed with the *Moringa oleifera* leaves the protein content of the milk increased from 4.36% to 4.80%. Similarly, Al-Juhaimi et al. (2020) reported that goats fed with Moringa leaves showed a significant ($p<0.05$) increase in the protein content (3.51 to 3.58%) of the milk. Quintanilha et al., 2021 reported that *Moringa oleifera* seeds are potential source of protein (44%) whereas, addition of seed extract to the yogurt increases the protein content of the yogurt. However, the increase in incubation time from 6 hrs to 10 hrs showed a decrease in the protein content of the yogurt (5.6% to 4.8%) because of the denaturation of the protein in acidic conditions (Santoso et al., 2021).

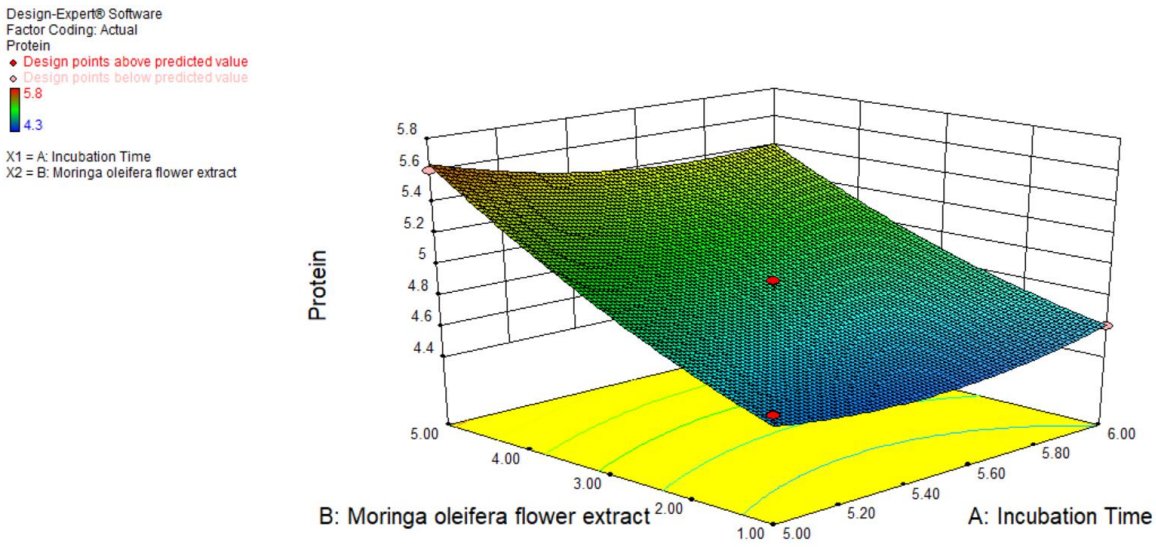


Fig. 6.11 Response surface plot for protein

6.12.4 *Model for antioxidant activity*

The quadratic model for antioxidant activity is significant and the following equation showed the significance of the model.

$$\textbf{Antioxidant activity} = 75.85 - 0.21A + 5.01B - 0.38AB + 0.79A^2 + 1.63B^2 \quad (29)$$

The coefficient of regression (R^2) for the antioxidant activity is found to be 0.9347 which means the model for the same could account for 93.47% of the data. The model shows that with the increase in the flower extract concentration from 1% to 5% antioxidant activity is significantly ($p<0.05$) increasing from 71.25% to 84.38% because *Moringa oleifera* flower extract is a potential source of antioxidants, and also increasing the activity of the yogurt. The antioxidant potential of the yogurt is increasing from 71.25 to 85.67%. Figure 6.12 shows the response surface plot for the antioxidant activity of yogurt. Lisak Jakopovic et al., 2022 reported the same results when *Moringa oleifera* leaf extract was added to the yogurt it increased the antioxidant potential of the yogurt significantly from 495 to 664 µmol TE L^{-1}. Sutakwa et al., 2021 resulted that the addition of blue pea flour to the various types of milk increases the total antioxidant potential of the yogurt from 7.03 to 69.36%. Whereas, incubation time does not affect the antioxidant activity of the yogurt.

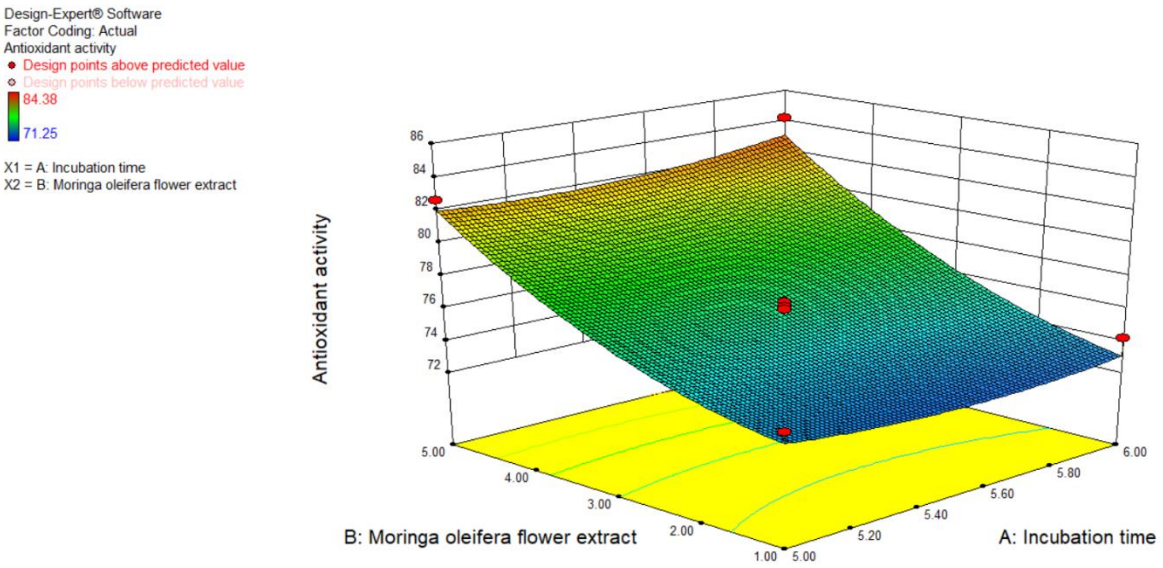


Fig. 6.12 Response surface plot for antioxidant activity

6.12.5 *Response surface methodology model for total phenolic content*

The quadratic model for TPC of the yogurt is significant and the following equation showed the significance of the model.

$$\textbf{Total phenolic content} = 24.77-0.027A+3.55B+0.19AB+0.093A^2-0.53B^2 \quad (30)$$

The coefficient of regression (R^2) for TPC was resulted to be 0.9943 which means the model for the same could account for 99.43% of the data. Figure 6.13 shows the response surface plot for the phenolic content of yogurt. Results obtained from the RSM for the total phenolic content showed that an increase in flower extract concentration from 1% to 5% was significantly increasing the total phenolic content of the yogurt from 19.01 mg GAE/g to 28.39 mg GAE/g. Milk and milk products barely contain any amount of phenolic compounds but when the flower extract is added to the yogurt, it improves the phenolic content by a significant amount. In a study, researchers conclude that *Moringa oleifera* leaf extract addition (1-4%) in cow's milk yogurt enhances the total phenolic content of the yogurt from 73 to 94 mg GAE/g DW (Lisak Jakopovic et al., 2022). Dhawi et al. (2020) reported that on addition of *Moringa oleifera* leaf extract and the fenugreek seeds extract in various proportions it resulted in significantly ($p<0.05$) higher amount of TPC of the yogurt (47.4 to 140.12 mg GAE/g DW).

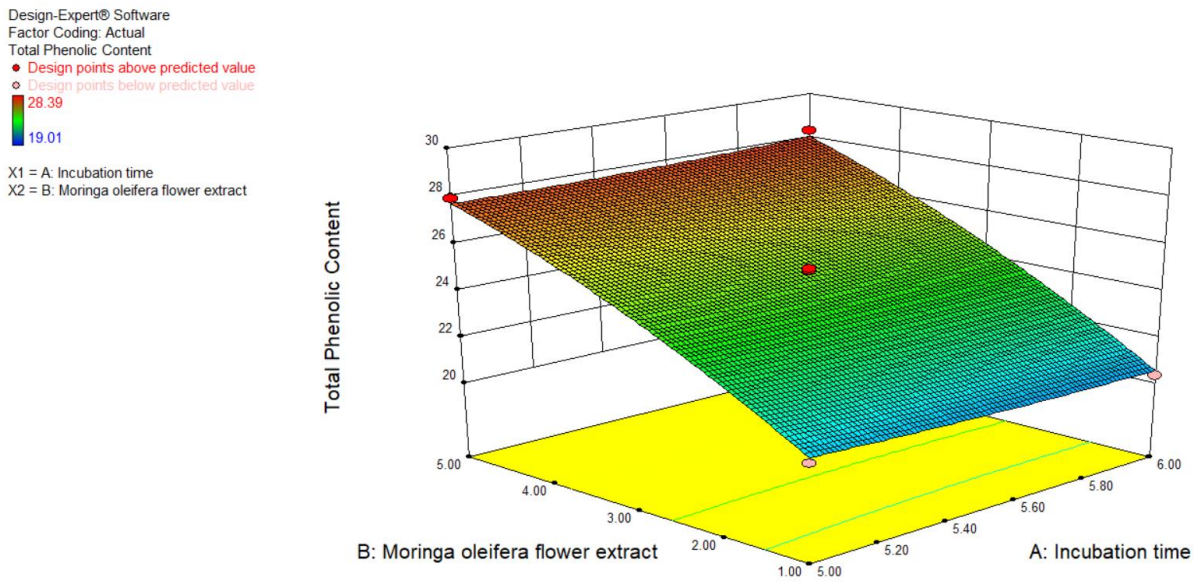


Fig. 6.13 Response surface plot for Phenolic content

6.12.6 Response surface methodology model for total flavonoid content

The quadratic model for the total flavonoid content of the yogurt is significant and the following equation showed the significance of the model.

$$\textbf{Total flavonoid content} = 20.44+0.16A+1.52B+0.032AB-0.20A^2-0.56B^2 \quad (31)$$

The coefficient of regression (R^2) for the total flavonoid content is found to be 0.9855 which means the model for the same could account for 98.55% of the data. The total flavonoid content of the *Moringa oleifera* flower extract-enriched yogurt is checked and the results obtained from response surface methodology showed an increase in flavonoid content from 17.16 mg QE/g to 21.37mg QE/g with the addition of flower extract (1-5%). Figure 6.14 shows the response surface plot for the flavonoid content of yogurt. Ao et al., 2022 reported that an increase in leaf extract concentration from 1% to 3% increased the flavonoid content of the yogurt from 13.31mg QE/g to 18.73mg QE/g and also accelerates the fermentation process because of the phytochemical components which are known for promoting the growth of the lactic acid bacteria.

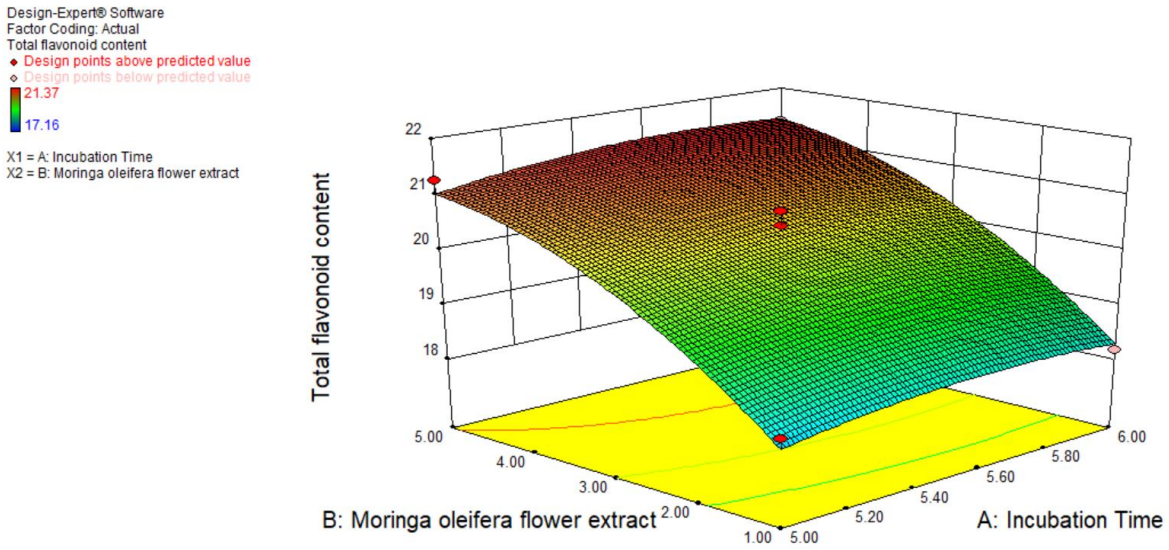


Fig. 6.14 Response surface plot for Flavonoid content

6.12.7 Optimization of the level of independent variables

For getting the optimized levels of the variables various responses, such as titratable acidity, protein content, antioxidant activity, total phenolic content, and total flavonoid content were assigned with an equal weightage so that the quality and palatability of the yogurt do not get affected and the best version of the yogurt could be obtained. The standards used for the predicted as well as actual responses are given in Table 6.15 below. The optimized value for the incubation time is 5 hrs whereas flower extract concentration is 5%. The calculated responses are near to the values which were predicted for the optimized independent variables. Titratable acidity value is found to be 0.923 %, protein 5.65%, antioxidant activity 82.67%, and total phenolic content is 27.94 mg GAE/g whereas TFC was 21.26mg QE/g.

Table 6.15 Criteria for the optimization of the process for *Moringa oleifera* flower extract enriched yogurt

Constraints	Goal	Lower limit	Upper limit	Importance	Solution	Actual response values
A. Incubation time	Minimize	5	6	3	5	-
B. Flower extract concentration	Maximize	1	5	3	5	-
Titratable acidity	Is in range	0.766	0.978	3	0.913	0.923
Protein	Maximize	4.3	5.8	3	5.6	5.6
Antioxidant activity	Maximize	71.25	85.67	3	82.47	82.67
TPC	Is in range	19.01	28.39	3	27.98	27.94
TFC	Maximize	17.16	21.37	3	21.17	21.26

6.13 PHYSIOCHEMICAL ANALYSIS OF THE YOGURT

6.13.1 Titratable acidity

The titratable acidity of the yogurt was increased with an increase in storage time, as shown in Figure 6.15. In this study, it was observed that *Moringa oleifera* flower extract-enriched yogurt titratable acidity was 0.91 g/L at 0^{th} day of the study which significantly ($p<0.05$) increased up to 1.43 by the 28^{th} day of the study. Similarly, the titratable acidity of the control yogurt was 0.85 at the 0^{th} day and it also increased significantly ($p<0.05$) by 28^{th} day of the study (1.31 g/L). However, it was observed that *Moringa oleifera* flower extract-enriched yogurt showed significantly ($p<0.05$) higher (1.43 g/L) titratable acidity as compared to control yogurt (1.31) up to 28^{th} day of the storage. However, control yogurt showed significantly ($p<0.05$) lower titratable acidity (0.85 g/L) as compared with *Moringa oleifera* flower extract enriched yogurt (0.91) at 0^{th} day of the study. On day 7^{th} titratable acidity of the control yogurt was monitored at 0.86 whereas for *Moringa oleifera* flower extract

enriched yogurt, it was 0.94 g/L which is significantly ($p<0.05$) higher than that of the control yogurt. The same pattern was noticed on day 14th and day 21st, as on day 14th titratable acidity of control yogurt was 0.89 g/L, and on day 21st it was 1.14 g/L whereas the titratable acidity of *Moringa oleifera* extract enriched yogurt was significantly ($p<0.05$) higher 1.01 g/L and 1.04 g/L respectively.

The increase in titratable acidity is due to the decrease in the pH of the yogurt and the results are consistent with the results reported by Seo et al., (2009). Titratable acidity was also reported to be increased in a study by Noh et al., (2013) where extending the storage of the yogurt after 14 days showed a significant ($p<0.05$) increase in the titratable acidity. Izadi et al., (2015) reported that the fat content of the milk also affects the titratable acidity of the yogurt and observed higher fat content in yogurt showed the lower acidity.

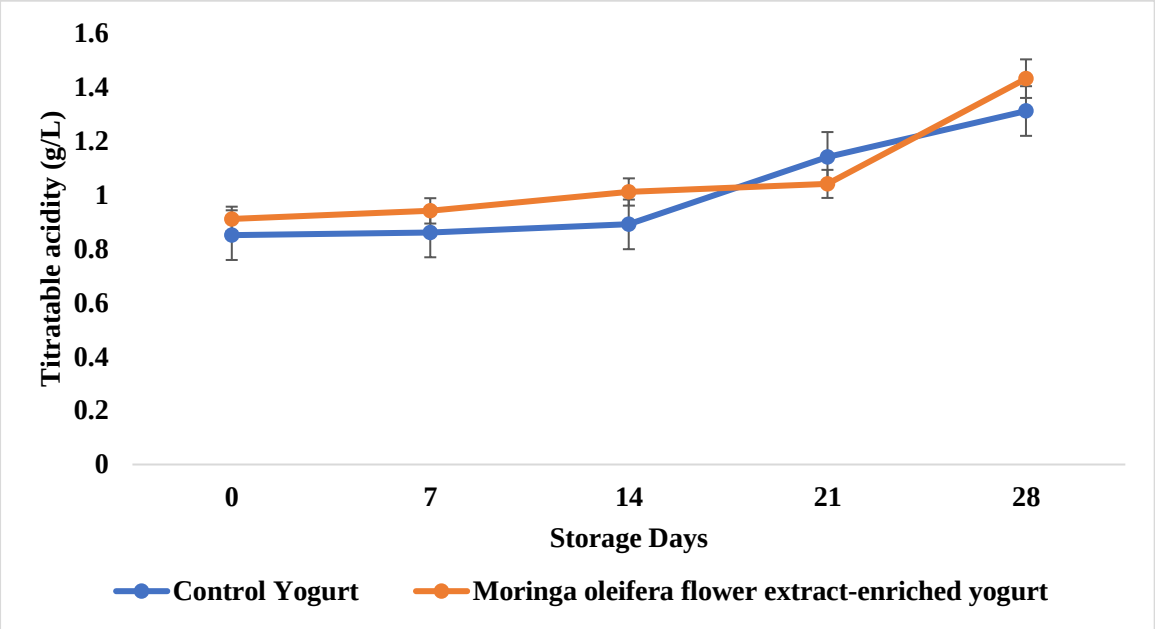


Figure 6.15 Representation of titratable acidity of the yogurt from day 0th to day 28th

6.13.2 pH

The pH of the yogurt was decreasing with the increase in storage period as shown in Table 6.16. In this study, it was observed that the pH of *Moringa oleifera* flower extract-enriched yogurt was significantly ($p<0.05$) lower by 4.61 on day 0th and it was 4.49 for the control yogurt whereas on day 7th pH of both the yogurt samples was decreasing with the increase in

time and it was observed 4.42 for the control yogurt and 4.52 for the *Moringa oleifera* flower extract-enriched yogurt. For day 14th control yogurt pH was observed at 4.37 and *Moringa oleifera* flower extract-enriched yogurt was at 4.48 which was significantly ($p<0.05$) higher than the pH of the control yogurt 4.37. The same trend of the pH was observed throughout the study the pH of control yogurt, as well as *Moringa oleifera* flower extract enriched yogurt, was decreased significantly ($p<0.05$) from 4.49 to 3.88. On day 21st control yogurt pH was observed 4.34 whereas the *Moringa oleifera* extract-enriched yogurt pH was 4.39, on day 28th at the end of the study pH of the control yogurt was 3.88. The pH of *Moringa oleifera* flower extract enriched yogurt was significantly ($p<0.05$) higher (3.91) as compared to the control yogurt which makes *Moringa oleifera* flower extract-enriched yogurt more suitable to consume and it also showed that *Moringa oleifera* extract enriched yogurt shelf life was improved in comparison to control yogurt.

Results obtained from the study indicate that the pH of the control yogurt and the flower extract-enriched yogurt is decreasing from 4.49 to 3.88 for control yogurt and 4.61 to 3.91 for the *Moringa oleifera* extract enriched yogurt as the storage time is increasing from day 0th to day 28th. The yogurt bacteria react with lactose for the production of lactic acid that is responsible to decrease the pH. Although pH of the *Moringa oleife*ra flower extract-enriched yogurt decreased comparatively slower than the control yogurt due to the alkaline nature of the *Moringa oleifera* flower extract. Saeed et al., (2021) reported almost same results when *Moringa oleifera* leaf extract was incorporated into the yogurt, the pH levels start decreasing from 4.38 to 3.96 after a period of storage. Ani et al., (2018) research resulted that after a period of storage, the pH of the control and *Moringa oleifera* leaves extract enriched yogurt drops from 4.12 to 3.85 due to increased acidity which restricts the growth of food poisoning bacteria (*Campylobacter, E. coli, Salmonella,* etc.).

6.13.3. Viscosity and syneresis

Viscosity and syneresis of the yogurt have opposite effects because as the syneresis decrease the viscosity of the yogurt increase and the same results are seen in this study where after some time syneresis of the yogurt decreased and vice versa viscosity of the yogurt increased over the storage period of 28 days as shown in table 6.16. Viscosity of the control yogurt was observed 377 on day 0th of the study whereas for *Moringa oleifera* flower extract-enriched yogurt it was monitored 336 which was lower than the control yogurt (377). Viscosity of

control yogurt was 345, 352, 359, and 364 on 7th, 14th, 21st, and 28th day of study whereas for *Moringa oleifera* extract-enriched yogurt viscosity was increasing as the storage of the yogurt was increasing from day 0th to day 28th. On day 7th it was 345, on day 14th 352, day 21st 359 whereas on last day of study 28th it was 364.

Syneresis was showing the opposite pattern to the viscosity, on day 0th it was (20ml), 7th day (25.23ml), 14th day (21.04ml), 21st day (20.24ml) and on the last 28th day it was (17.21ml) for *Moringa oleifera* flower extract-enriched yogurt whereas for control yogurt it was 23.40ml on 0th day, 27.33ml on 7th day, 22.02ml on 14th day, 21.21ml on 21st day and 18.11 on 28th day of the storage study. Sahan et al., (2008) reported that the viscosity of the yogurt increased from 320 to 359 over the period of storage (day 0th to day 21st) due to rearrangement of the protein. *Moringa oleifera* flower extract resulted in reduced fermentation time and increased viscosity of the yogurt may be due to the phytoconstituents (phenols, flavonoids, amino acids) present in it, which are responsible for promoting the lactic acid bacteria. Moreover, a few researchers also claim that Moringa extract is prebiotic and claimed that the addition of this extract to the milk accelerates the process of fermentation and reduced the incubation time to 6 hr (Ao et al., 2022).

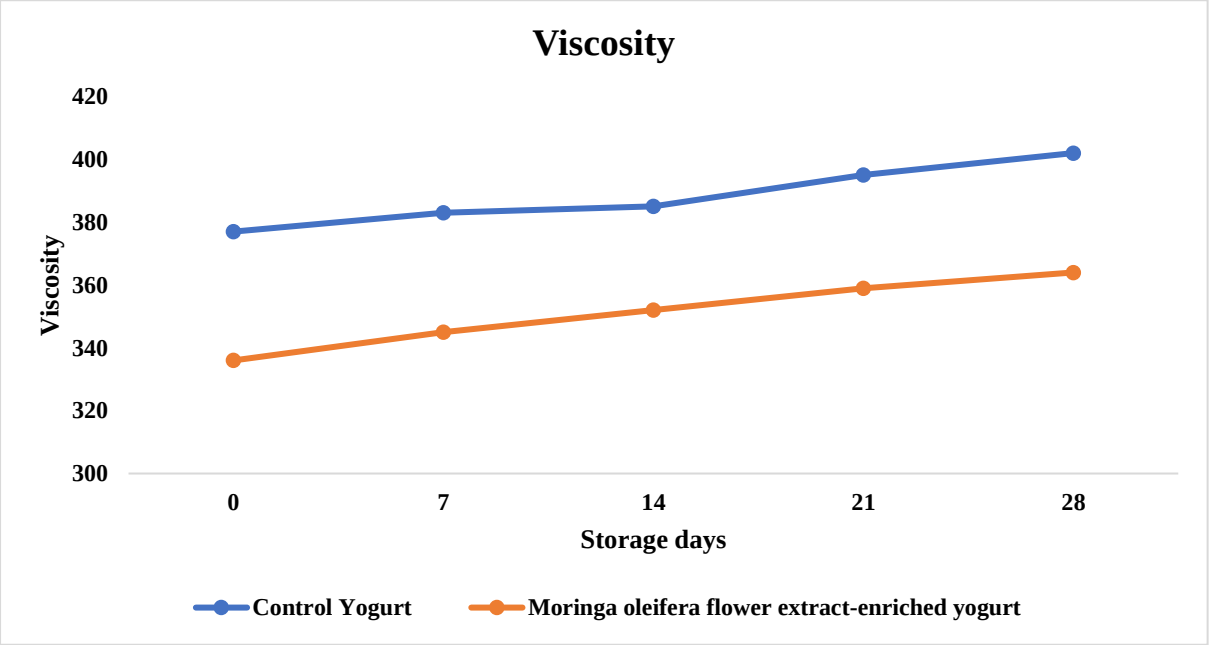


Figure 6.16 Representation of Viscosity of the yogurt from day 0th to day 28th

Table 6.16 Effect of storage period (0 to 28th day) on prepared *Moringa oleifera* flower extract-enriched yogurt

Type of Yogurt	Parameters	Days				
		0	7	14	21	28
Control Yogurt	Titratable acidity (%)	0.85±0.018[b]	0.86±0.015[b]	0.89±0.023[b]	1.14±0.167[b]	1.31±0.023[b]
	pH	4.49±0.066[b]	4.42±0.133[a]	4.37±0.026[b]	4.34±0.134[a]	3.88±0.080[a]
	Viscosity (cp)	377±0.493[a]	383±0.520[a]	385±0.493[a]	395±0.321[a]	402±0.467[a]
	Syneresis (ml)	23.40±0.435[a]	27.33±0.208[a]	22.02±0.339[a]	21.21±0.293[a]	18.11±0.577[a]
Moringa oleifera flower extract-enriched yogurt	Titratable acidity (%)	0.91±0.203[a]	0.94±0.315[a]	1.01±0.404[a]	1.04±0.360[a]	1.43±0.556[a]
	pH	4.61±0.095[a]	4.52±0.458[a]	4.48±0.063[a]	4.39±0.503[a]	3.91±0.550[a]
	Viscosity (cp)	336±1.001[b]	345±1.512[b]	352±0.387[b]	359±0.593[b]	364±0.671[b]
	Syneresis (ml)	20.00±0.261[b]	25.23±0.284[b]	21.04±0.146[b]	20.24±0.384[b]	17.21±0.517[b]

Data are represented as mean ±SD (n=3)

[a–b] Means within a row with different supsserscripts are significantly different ($P < 0.05$)

6.14 MICROBIOLOGICAL ANALYSIS

Table 6.17 represents the *Lactobacillus* and *Streptococcus* species that were counted during the storage period of over 28 days. From the results is quite evident that from day 0 to day 7 of the study. Whereas from day 14th of the study the viable cell count of the bacteria was increasing significantly ($p<0.05$). Similarly, the cell count was started to decrease from 21st day for both controls as well as *Moringa oleifera* flower extract-enriched yogurt due to decrease in pH of the yogurt and it gets acidified which was responsible for the growth of the bacteria diminishing in the yogurt. Figure 6.17 shows the cell count in both controls and *Moringa oleifera* flower extract-enriched yogurt. On day 0 control yogurt showed a 6.41 log ($CFUmL^{-1}$) cell count as compared with *Moringa oleifera* flower extract-enriched yogurt has a 6.84 log ($CFUmL^{-1}$) cell count and at the end of the storage period, the cell count decreased significantly ($p<0.05$) to 4.31 log ($CFUmL^{-1}$) and 5.24 log ($CFUmL^{-1}$). Mani-Lopez et al. (2014) reported that the viability of the bacteria starts diminishing after a storage period of 14 days because, lactose starts diminishing which affects the growth of the lactic acid bacteria due to the lack of nutrition is the main reason behind the decreased number of the viability of the bacteria. Sah et al., (2015) reported that the bacterial count decreases with the increase in time due to the lowering of the pH and an increase in the acidity of the yogurt. Control yogurt shows a lesser number of colonies than that *Moringa oleifera* extract-enriched yogurt because it determines the dose-dependent increase in cell viability at a concentration of 0.313mg/ml (Fernandes et al., 2016).

Table 6.17 Viable count of bacteria in control and *Moringa oleifera* flower extract-enriched yogurt (MOFEEY)

Storage time	Control yogurt log ($CFUmL^{-1}$)	*Moringa oleifera* extract-enriched Yogurt ($CFUmL^{-1}$)
0	6.41 ± 0.040^{cA}	6.84 ± 0.030^{cB}
7	6.55 ± 0.040^{dA}	7.08 ± 0.060^{dB}
14	6.67 ± 0.040^{eA}	7.27 ± 0.050^{eB}
21	5.62 ± 0.030^{bA}	6.60 ± 0.020^{bB}
28	4.31 ± 0.050^{aA}	5.24 ± 0.891^{aB}

Data are represented as mean ±SD (n=3)

$^{a–e}$ Means within a row with different superscripts are significantly different ($P < 0.05$)

[A–B] Means within a column with different superscripts are significantly different ($P < 0.05$)

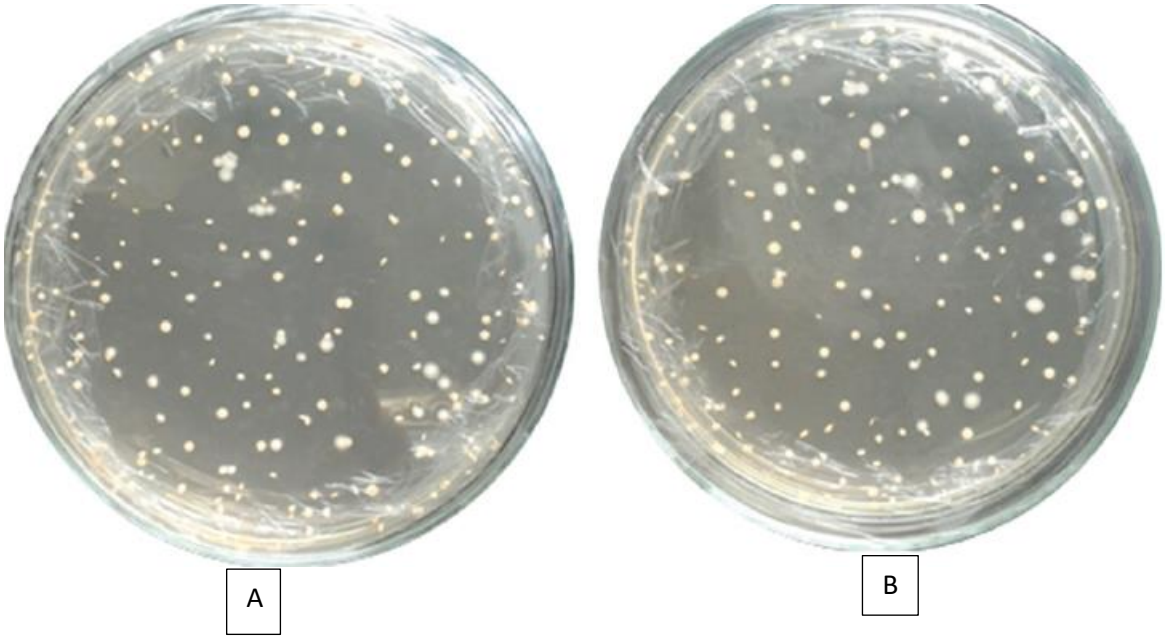


Figure 6.17 Representation of microbial analysis (A) Cell count of the control yogurt (B) *Moringa oleifera* flower extract-enriched yogurt

6.15 SENSORY EVALUATION

6.15.1 Flavour

All the results of the sensory analysis were evaluated based on parameters like flavor, body, texture, acidity, color and appearance, and containers and closures. In this study, yogurts were made in four different treatments- control yogurt, yogurt with 1% flower extract, 3% flower extract, and finally 5% flower extract. Table 6.17 represents the sensory analysis of control and *Moringa oleifera* flower extract-enriched yogurt. In general, the sensory properties grouped the yogurt treatments into two groups, the control yogurt was in the first group, and the *Moringa oleifera* flower extract-enriched yogurt was in the second group. The results obtained from sensory analysis of the yogurt revealed that yogurt with 3% *Moringa oleifera* flower extract got the maximum score for flavour on day 0^{th} (43.80) out of 45, on day 21^{st} yogurt enriched with 3% extract got 43.26 score, whereas control yogurt got 42.26 score on day 14^{th} and day 21^{st}. From the results it was evident *Moringa oleifera* flower (3%) extract-enriched yogurt got significantly ($p<0.05$) higher score in comparison to the control yogurt. Out of all the treatments yogurt enriched with 1% *Moringa oleifera* flower extract obtained

lower score. The specific reason was the alkaline nature of the *Moringa oleifera* flower extract which helps to maintain the acidity of the yogurt for a longer period and maintains the pH as well due to which its sensory properties stay acceptable for a longer period of time.

6.15.2 Body and Texture

Total score for body and texture was 30, out of that *Moringa oleifera* flower (3%) extract-enriched yogurt got significantly ($p<0.05$) higher score (28.46) in comparison to other yogurt, after 3% yogurt second highest score was obtained by yogurt enriched with 5% *Moringa oleifera* flower extract (27.66). Control yogurt got comparatively lower score for body and texture (26.06). Overall results for body and texture were maximum for yogurt enriched with 3% *Moringa oleifera* flower extract. A study by Saeed et al. (2021) reported that *Moringa oleifera* leaves powder enriched (0%, 0.5%, 1.0%, 1.5% and 2.0%) yogurt was studied for the body and texture, where it was revealed that yogurt with 2% *Moringa oleifera* leaves powder scored higher on day 0^{th} of the study (8.01 out of 9), on day 5^{th} it scored 7.40, whereas on day 15^{th} same yogurt scored 6.63. Whereas other yogurt types scored significantly ($p<0.05$) lower on all the days.

6.15.3 Acidity

In the Sensory analysis, it was monitored that the acidity of the yogurt was increasing over time and due to this the flavor of the yogurt was decreasing significantly ($p<0.05$). According to descriptive sensory analysis score for the acidity was 10, out of which the lowest score was obtained by yogurt enriched with 3% *Moringa oleifera* flower extract. Yogurt enriched with 3% flower extract showed a significant ($p<0.05$) difference in acidity due to which overall acceptability of the yogurt increased, in comparison with control and 1% extract yogurt. But when the results of all four yogurts were compared, it was observed that maximum score was obtained by the yogurt with 1% flower extract. Yogurt enriched with 5% flower extract showed maximum acidity on day 7^{th} (8.00) whereas control yogurt showed significantly ($p<0.05$) low acidity (7.13) on day 7^{th}. A study reported that yogurt enriched with *Moringa oleifera* leaves powder (0.5%) showed significant ($p<0.05$) increase in acidity from 1.08 on day 0^{th}, 1.13 on day 10^{th}, and 1.21 on day 15^{th}. Yogurt enriched with 1% *Moringa oleifera* leaves powder showed acidity 1.10 on day 0^{th}, 1.15 on day 10^{th}, and 1.23 on day 15^{th} which showed a significantly ($p<0.05$) increasing trend from day 0^{th} to day 15^{th}. Yogurt enriched with 2% *Moringa oleifera* leaves powder showed same increasing trend from day 0^{th} to day

15th. On day 0th it was 1.13, on day 10th 1.18, whereas on day 15th it was 1.26 (Saeed et al., 2021).

6.15.4 Colour and appearance

The colour and appearance of the yogurt were given 10 points out of which all the yogurt scored almost the same because there was no difference in the colour and appearance of the yogurt. *Moringa oleifera* flower extract showed no significant ($p<0.05$) effect on the colour and appearance of the yogurt because it was light pale in colour.

6.15.5 Container and closures

All the yogurt samples were kept in the same containers made with LDPE (Low-density polyethylene) so there was non-significant ($p<0.05$) difference in all the yogurt scores for all the days of storage.

6.15.6 Overall acceptability

The yogurt samples were checked for overall acceptability by using a descriptive scorecard. The overall acceptability of control yogurt was significantly ($p<0.05$) lower on the 0th day (85.29) than the yogurt enriched with *Moringa oleifera* flower extracts 86.73 for 1% enriched yogurt, 91.41 for 1% flower extract enriched yogurt whereas 87.98 for 5% flower extract-enriched yogurt. For day 7th control yogurt scored significantly ($p<0.05$) lower than 1% (87.46), 3% (91.00), and 5% (90.80). The same trend was seen for day 14th, day 21st, and day 28th. The results predicted that yogurt enriched with 3% *Moringa oleifera* flower extract scored significantly ($p<0.05$) higher in comparison to other yogurts.

In previous studies, *Moringa oleifera* leaf extract enriched yogurt showed almost the same results where the overall acceptability was increased due to addition of the *Moringa oleifera* extract and flavor of the yogurt and increased the shelf life of the yogurt due to which yogurt was acceptable for longer period of time in comparison to the control yogurt (Shokery et al., 2017; Adepoju & Selezneva, 2020). Vijay et al. (2022) resulted those sensory properties of the yogurt enriched with 0.5%, 1%, 1.5%, and 2% of *Moringa oleifera* leaves powder were significantly ($p<0.05$) lower (8.58, 8.33, 8.25, and 7.50 respectively) than the control yogurt (8.75). A study by Saeed et al. (2

021) reported that Yogurt sample enriched with 1% *Moringa oleifera* leaves powder showed significantly ($p<0.05$) higher overall acceptability on day 0th (8.11), on day 10th (7.63), and

day 15^{th} (5.20). Yogurt fortified with 2% Moringa oleifera leaves powder showed significantly ($p<0.05$) lower overall acceptability for all the study days. For day 0^{th} (6.40), day 10^{th} (6.20), whereas on day 15^{th} (5.20).

Table 6.18 Sensory analysis of *Moringa oleifera* flower extract enriched yogurt from day 0th to 28th

Parameters	Days	Control	1%	3%	5%
Flavour	**0**	42.13 ± 1.18^{bA}	41.20 ± 2.42^{dC}	43.80 ± 1.32^{aA}	41.86 ± 1.68^{cA}
	7	40.06 ± 1.48^{dB}	40.73 ± 2.28^{cC}	42.06 ± 1.27^{aB}	41.33 ± 2.41^{bC}
	14	42.26 ± 1.22^{aA}	41.13 ± 2.26^{dB}	41.93 ± 1.57^{bB}	41.66 ± 2.44^{cB}
	21	42.26 ± 0.96^{bA}	42.33 ± 1.23^{bA}	43.26 ± 1.70^{aA}	41.53 ± 0.91^{cB}
	28	39.66 ± 1.34^{cB}	39.33 ± 1.75^{cD}	40.40 ± 1.91^{bC}	41.33 ± 0.89^{aC}
Body & Texture	**0**	26.06 ± 2.01^{cA}	27.20 ± 2.45^{bA}	28.46 ± 0.91^{aA}	27.66 ± 1.39^{aA}
	7	26.53 ± 1.18^{bA}	26.46 ± 2.44^{bA}	27.00 ± 2.03^{aA}	27.26 ± 2.49^{aA}
	14	26.53 ± 1.88^{bA}	26.86 ± 2.38^{bA}	26.66 ± 2.05^{bA}	27.46 ± 2.38^{aA}
	21	26.40 ± 1.91^{bA}	26.86 ± 2.03^{bA}	28.26 ± 1.48^{aA}	26.06 ± 1.09^{cA}
	28	26.13 ± 1.68^{aA}	25.73 ± 1.03^{bA}	26.20 ± 0.77^{aA}	26.06 ± 1.09^{aA}
Acidity	**0**	7.46 ± 0.99^{bB}	8.00 ± 1.81^{bA}	9.06 ± 0.88^{aA}	8.26 ± 1.33^{aA}

	7	7.13 ± 1.18^{bC}	7.20 ± 1.20^{bC}	8.20 ± 1.20^{aB}	8.00 ± 1.81^{aB}
	14	7.80 ± 1.16^{aA}	7.80 ± 1.69^{aB}	7.53 ± 1.50^{aC}	7.46 ± 1.84^{aC}
	21	7.66 ± 1.04^{cB}	8.00 ± 1.30^{bA}	8.86 ± 1.06^{aA}	6.46 ± 0.63^{dD}
	28	6.46 ± 1.12^{aD}	6.40 ± 0.73^{aD}	6.73 ± 0.59^{aC}	6.42 ± 0.63^{aD}
Colour & Appearance	**0**	8.66 ± 0.48^{bB}	9.20 ± 0.77^{aA}	9.13 ± 0.74^{aA}	9.20 ± 0.56^{aA}
	7	8.26 ± 1.09^{cC}	8.86 ± 1.06^{bB}	8.86 ± 0.51^{bB}	9.20 ± 0.77^{aA}
	14	8.80 ± 0.56^{bAB}	9.06 ± 0.79^{aA}	8.86 ± 0.51^{bB}	9.40 ± 0.82^{aA}
	21	8.66 ± 0.48^{cB}	8.86 ± 0.51^{cB}	9.26 ± 0.70^{bA}	9.40 ± 0.73^{aA}
	28	8.26 ± 0.59^{bC}	8.40 ± 0.50^{bC}	8.46 ± 0.63^{bC}	9.33 ± 0.72^{aA}
Container & Closures	**0**	4.80 ± 0.41^{aA}	5.00 ± 0.00^{aA}	4.73 ± 0.45^{aA}	4.93 ± 0.25^{aA}
	7	4.73 ± 0.45^{aA}	4.86 ± 0.35^{aA}	4.86 ± 0.35^{aA}	5.00 ± 0.00^{aA}
	14	4.86 ± 0.35^{aA}	5.00 ± 0.00^{aA}	4.86 ± 0.35^{aA}	5.00 ± 0.00^{aA}
	21	4.86 ± 0.35^{aA}	4.86 ± 0.35^{aA}	4.73 ± 0.45^{aA}	4.73 ± 0.45^{aA}

	28	4.86±0.35^{aA}	4.80±0.41^{aA}	4.86±0.35^{aA}	4.73±0.45^{aA}
Overall acceptability	**0**	85.29±3.15^{dB}	86.73±5.95^{cB}	91.41±2.83^{aA}	87.98±3.83^{bB}
	7	85.86±3.64^{cB}	87.46±6.12^{bA}	91.00±3.64^{aA}	90.80±6.57^{aA}
	14	86.37±3.21^{aA}	85.86±6.08^{bB}	85.97±4.00^{bB}	86.93±6.46^{aC}
	21	85.97±2.83^{cB}	87.04±3.65^{bA}	90.61±3.93^{aA}	84.41±2.01^{dC}
	28	81.50±2.57^{cC}	80.82±1.89^{dC}	82.77±2.08^{bB}	84.08±1.78^{aC}

Data are represented as mean ±SD (n=15)

$^{a–d}$ Means within a column with different superscripts are significantly different ($P < 0.05$)

$^{A–D}$ Means within a row with different superscripts are significantly different ($P < 0.05$)

6.16 Cost analysis of Moringa oleifera flower extract enriched yogurt

The product computed cost was Rs. 22.05/100 ml and Rs. 220.50 per 1000ml. The net pack of a product was 250ml, with the cost Rs. 55.12 per pack. Whole buffalo milk cost Rs. 65/ ltr, packaging container Rs. 4/unit, 1kg L-proline Rs.1000, 1 Kg Glycerol Rs. 205*, Moringa oleifera* flowers Rs. 600, milk homogenization, refrigeration, incubation cost Rs. 0.85, GST (5%). There are an ample number of flavored yogurt available in the market, but this product is comparatively less expensive in comparison to others. For instance, Mother Dairy 100gm raspberry flavored yogurts cost Rs. 33/ 100gm whereas Milky Mist fruit yogurt costs Rs. 35/ 100gm and Epigemia flavored yogurt costs Rs. 40/100gm unit. The cost of papaya-based yogurt was Rs. 188.5/ltr, strawberry flavoured yogurt Rs. 320/ltr, whereas rice-based yogurt costs Rs. 398.50/ltr. On comparison of the *Moringa oleifera* flower extract enriched yogurt with the other flavoured yogurt available in marked it was found that Moringa-based yogurt was significantly ($p<0.05$) low in cost.

Table 6.19 Cost analysis of developed product

Type of product	Ingredients	Amount used (g)	Cost as the amount used (Rs.)	Total cost per Kg (Rs.)
***Moringa oleifera* flower extract-enriched yogurt**	*Moringa oleifera* flower powder	1	0.60	600
	Buffalo milk	100	6.50	65
	L-proline	1	2.56	2565
	Glycerol	2	0.40	205
	Culture	2	4.5	45
	Packaging		4	16
Total			18.56	
Overhead chargess	Electricity supply (for flower drying, grinding, and pasteurization of milk)	3	2.85	9.53
	GST charges (5%)		0.64	6.4
Total cost			22.05/100 ml	

Conclusion

Moringa oleifera has played a vital role in providing health benefits to all age group with cholesterol, immune problems, diabetes, hypertension, etc. The *Moringa oleifera* leaves, stems, flowers and extracted oil are worthy of medication as well as benefit the food industry. Many researchers have shown their support for the health benefits of *Moringa oleifera* flowers and leaves due to presence of higher amount of phytochemical compounds like alkaloids, ketones, alkanes, tocopherols, aldehydes, fatty acids, quercetin, kaempferol, D-mannose, D-glucose, protein, Glycoside niazirin, terpenoids, niaziminin A & B, and

niazirinin as they provide many functional nutrients that are necessary for the health of cardiac and metabolic diseases patients. The critical analysis has found that the combination of herbs can have a subsequent effect on the patient's health compared to taking them separately. Initial studies on the antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, and anticancer properties and investigations of several solvent extracts showed great results. There is scope for thorough research to identify, purify, and define the bioactive phytochemicals as well as to explain the mechanism underlying the remarkable therapeutic potential of Moringa flowers. *Moringa oleifera* flower powder was analyzed for its proximate composition, antioxidant activity, and flavonoid content. Results showed that it is a potential source of protein (18.10%), fiber (6.70%), phenols (32.46mg GAE/g), flavonoids (44.13mg QE/g), and antioxidants (74.83%) which means it has the potential for the prevention and treatment of various diseases. Techno-functional properties showed that the flower powder could be used for various supplements and food additives. For research where quality is the main priority, HPLC is very helpful in isolating and purifying plant-based products such as secondary metabolites and proteins. HPLC results showed that the sample contains numerous phytochemicals such as quercetin, rutin, ferulic acid, epigallocatechin, and catechin. In conclusion, results for antibacterial activity against test microorganisms revealed that *Moringa oleifera* flower extract is a potential source for antibacterial activity. In addition, this study also revealed an alternative source of treatment for infectious diseases along with minimum inhibitory concentration(250mg/ml). Food items made from flowers, therefore, have higher levels of protein, dietary fiber, minerals, and antioxidants. Moringa's bioactive secondary metabolites provide motivation for additional research to find innovative lead compounds for drug discovery and the creation of novel herbal pharmaceuticals that meet GRAS (Generally Recognised as Safe) standards. One of the objectives of this study was designed to optimize the extraction process of *Moringa oleifera* flower extract by using six combinations of Deep Eutectic Solvents at three ratios 1:1, 1:2, and 1:3. It was designed to optimize the extraction process to obtain the maximum amount of phenolic, flavonoid and antioxidants at a temperature range of (50-80℃). Based on the extraction yield 1:2 ratio was finalized because it gave the maximum amount of yield (9.13%), total phenolic content (32.46 mg GAE/g), flavonoid (44.13mg QE/g), and antioxidants (74.83%) at all four temperatures, and found that 70 ℃ is the optimum temperature to get the maximum number of phytochemicals and antioxidant activity. An enzyme inhibition assay was performed to check the *in vitro* effect of flower extract on lipid and glucose levels and results obtained favour the studies done by researchers that it has the potential to show hypoglycaemic and

hypolipidemic effects. This study provides an efficient and non-toxic method to extract the phytochemicals and to study their effects *in vitro* and *in vivo* models. The Response Surface Methodology model was used for optimizing the yogurt conditions (incubation time and flower extract concentration). The optimized Incubation time was 5 hrs, and the flower extract concentration was 5%. Due to potential health benefits yogurt is considered one of the most popular probiotic food choices all around the world. This model could be used for large-scale *Moringa oleifera* flower extract-enriched (1-5%) yogurt production. The results showed that flower extract has a significant ($p<0.05$) effect on the protein content (4.6 to 5.8%), antioxidant activity (71.25% to 84.38%), TPC (19.01mg GAE/g to 28.39mg GAE/g), and TFC content (17.16 mg QE/g to 21.37mg QE/g) of the yogurt. The addition of flower extract is improving the acceptability (81.50 to 91.41) of the yogurt as well as the storage of the yogurt. So, this research opens another opportunity for a plant-based yogurt with excellent antioxidant activity. *Moringa oleifera* flower extract also enhanced the shelf life of the yogurt, in comparison to control yogurt, flower extract enriched yogurt contains a significantly ($p<0.05$) high amount of protein, phytochemicals, and improved shelf life. Secondly, this product is also cost-efficient, on comparison of the product with the other products available in the market it is low in cost and showed significant health benefits.

REFERENCES

1. Abalaka, M. E., Daniyan, S. Y., Oyeleke, S. B., & Adeyemo, S. O. (2012). The antibacterial evaluation of Moringa oleifera sssss extracts on selected bacterial pathogens. *Journal of Microbiology research*, 2(2), 1-4. DOI: 10.5923/j.microbiology.20120202.01.
2. Abbott, A. P., Boothby, D., Capper, G., Davies, D. L., & Rasheed, R. K. (2004). Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids. *Journal of the American Chemical Society*, *126*(29), 9142-9147. https://doi.org/10.1021/ja048266j.
3. Abbott, A. P., Capper, C., Davies, D. L., Rasheed, R. K., Rajah, T. V. (2003). Novel solvent properties of choline chloride/urea mixtures. *Chemical Communications*, 9, 70-71.
4. Abd Eldaim, M. A., Shaban Abd Elrasoul, A., & Abd Elaziz, S. A. (2017). An aqueous extract from Moringa oleifera leaves ameliorates hepatotoxicity in alloxan-induced diabetic rats. *Biochemistry and Cell Biology*, *95*(4), 524-530. https://doi.org/10.1139/bcb-2016-0256.
5. Abd El-Hack, M. E., Alagawany, M., Elrys, A. S., Desoky, E. S. M., Tolba, H. M., Elnahal, A. S., ... & Swelum, A. A. (2018). Effect of forage Moringa oleifera L.(moringa) on animal health and nutrition and its beneficial applications in soil, plants, and water purification. *Agriculture*, 8(9), 145. https://doi.org/10.3390/agriculture8090145.
6. Abdel Hamid, S. M., Murad, H. A., El-Dairouty, R. K., Ayokda, A. Y., Naeem, M. A., & Shaheen, M. N. (2022). Preparation of Flavored Yoghurt with Orange Peel-Oil and Evaluation its Potential Antioxidant, Antimicrobial, and Antiviral Properties. Egyptian Journal of Chemistry, 65(132), 41-49. DOI: 10.21608/EJCHEM.2022.109170.5127.
7. Abdulkarim, S. M., Long, K., Lai, O. M., Muhammad, S. K. S., & Ghazali, H. M. (2005). Some physico-chemical properties of Moringa oleifera seed oil extracted using solvent and aqueous enzymatic methods. *Food chemistry*, *93*(2), 253-263. https://doi.org/10.1016/j.foodchem.2004.09.023.
8. Abdull Razis, A. F., Ibrahim, M. D., & Kntayya, S. B. (2014). Health benefits of Moringa oleifera. *Asian pacific journal of cancer prevention*, *15*(20), 8571-8576. http://dx.doi.org/10.7314/APJCP.2014.15.20.8571.

9. Abdull Razis, A. F., Ibrahim, M. D., & Kntayya, S. B. (2014). Health benefits of Moringa oleifera. *Asian pacific journal of cancer prevention*, *15*(20), 8571-8576. http://dx.doi.org/10.7314/APJCP.2014.15.20.8571.
10. Abuhajar, E., Ali, K., Zulfiqar, G., Al Ansari, K., Raja, H. Z., Bishti, S., & Anweigi, L. (2023). Management of Chronic Atrophic Candidiasis (Denture Stomatitis)—A Narrative Review. *International Journal of Environmental Research and Public Health*, *20*(4), 3029. https://doi.org/10.3390/ijerph20043029.
11. Acuram, L. K., & Chichioco Hernandez, C. L. (2019). Anti-hypertensive effect of Moringa oleifera Lam. *Cogent Biology*, *5*(1), 1596526.https://doi.orgs/10.1080/23312025.2019.1596526
12. Adebowale, A. A., M. T. Adegoke, S. A. Sanni, M. O. (2012). "Functional properties and biscuit making potentials of sorghum-wheat flour composite." *American Journal of food technology* 7, no. 6: 372-379. DOI: 10.3923/ajft.2012.372.379.
13. Ademiluyi, A. O., Aladeselu, O. H., Oboh, G. and Boligon, A. A. (2018). Drying alters the phenolic constituents, antioxidant properties, α-amylase, and α-glucosidase inhibitory properties of Moringa (Moringa oleifera) leaf. *Food science & nutrition.* 6: 2123-2133. https://doi.org/10.1002/fsn3.770.
14. Adepoju, F. O., & Selezneva, I. S. (2020). Comparative study of yoghurt enriched with Moringa powder in different concentrations. In *AIP Conference Proceedings* (Vol. 2280, No. 1, p. 030001). AIP Publishing LLC. https://doi.org/10.1063/5.0018035.
15. Adeyemi, O. S., & Elebiyo, T. C. (2014). Moringa oleifera-supplemented diets prevented nickel-induced nephrotoxicity in Wistar rats. *Journal of nutrition and metabolism*. https://doi.org/10.1155/2014/958621.
16. Adji, A. S., Atika, N., Kusbijantoro, Y. B., Billah, A., Putri, A. and Handajani, F. (2022). A review of Leaves and Seeds Moringa oleifera Extract: The potential Moringa oleifera as Antibacterial, Anti-Inflammatory, Antidiarrhoeal, And Antiulcer Approaches to Bacterial Gastroenteritis. *Open Access Macedonian Journal of Medical Sciences*. 10:305-313. https://doi.org/10.3889/oamjms.2022.8894.
17. Aekthammarat, D., Pannangpetch, P., & Tangsucharit, P. (2019). Moringa oleifera leaf extract lowers high blood pressure by alleviating vascular dysfunction and decreasing oxidative stress in L-NAME hypertensive rats. *Phytomedicine*, *54*, 9-16. https://doi.org/10.1016/j.phymed.2018.10.023.

18. Aghara ID (2014) A comparative study of the effect of aqueous leaf extracts of *Moringa oleifera* and *Telfairia occidentalis* on some biochemical and hematological parameters in wistar rat. Pp.85. Retrieved at http//: www.unn.edu.ng/publications on 3/4/2015
19. Ahmed, K. S., Jahan, I. A., Jahan, F. and Hossain, H. (2021). Antioxidant activities and simultaneous HPLC-DAD profiling of polyphenolic compounds from Moringa oleifera Lam. Leaves grown in Bangladesh. *Food Res*. 5:401-8. https://doi.org/10.26656/fr.2017.5(1).410
20. Airouyuwa, J. O., Mostafa, H., Riaz, A., & Maqsood, S. (2022). Utilization of natural deep eutectic solvents and ultrasound-assisted extraction as green extraction technique for the recovery of bioactive compounds from date palm (Phoenix dactylifera L.) seeds: An investigation into optimization of process parameters. *Ultrasonics Sonochemistry*, *91*, 106233. https://doi.org/10.1016/j.ultsonch.2022.106233.
21. Akajiaku, L. O., Kabuo, N. O., Omeire, G. C., Odimegwu, E. N., & Ogbonna, V. G. (2018). Production and evaluation of Moringa oleifera leaves powder enriched yogurt. *Nutr. F Toxicol*, *2*, 459-466.
22. Al Bratty, M., Makeen, H.A., Alhazmi, H.A., Syame, S.M., Abdalla, A.N., Homeida, H.E., Sultana, S., Ahsan, W. and Khalid, A. (2020). Phytochemical, cytotoxic, and antimicrobial evaluation of the fruits of miswak plant, Salvadora persica L. *Journal of Chemistry*, pp.1-11. https://doi.org/10.1155/2020/4521951.
23. Al Dhaberi SM (2016) *In vitro* re Generation and Marker Assisted Evaluation of Genetic Fidelity in Endangered Tree Species *Moringa peregrina* (Forsk) Fiori
24. Al Juhaimi, F., Özcan, M. M., Uslu, N., & Ghafoor, K. (2018). The effect of drying temperatures on antioxidant activity, phenolic compounds, fatty acid composition, and tocopherol contents in citrus seed and oils. *Journal of food science and technology*, *55*, 190-197. https://doi.org/10.1007/s13197-017-2895-y.
25. Al-Ahwal, R. I. H., Saleh, A. E., & Moussa, M. A. M. (2017). The Importance of Using Moringa Oleifera Extract on the Quality and Nutritive Value of Yoghurt. *Journal of Food and Dairy Sciences*, *8*(6), 237-241.
26. Al-Ghanayem, A. A., Alhussaini, M. S., Asad, M., & Joseph, B. (2022). Moringa oleifera Leaf Extract Promotes Healing of Infected Wounds in Diabetic Rats: Evidence of Antimicrobial, Antioxidant and Proliferative Properties. *Pharmaceuticals*, *15*(5), 528. https://doi.org/10.3390/ph15050528.

27. Alhakmani, F., Kumar, S., & Khan, S. A. (2013). Estimation of total phenolic content, in–vitro antioxidant and anti-inflammatory activity of flowers of Moringa oleifera. *Asian Pacific journal of tropical biomedicine*, 3(8), 623-627. https://doi.org/10.1016/S2221-1691(13)60126-4.
28. Ali, G. W., Abd Ellatif, M. A., & Abdel-Fattah, W. I. (2021). Extraction of Natural Cellulose and Zein Protein from Corn Silk: Physico-Chemical and Biological Characterization. Biointerface *Res. Appl. Chem*, 11, 10614-10619. https://doi.org/10.33263/BRIAC113.1061410619.
29. Alia F, Putri M, Anggraeni N, Syamsunarno MR. The potency of *Moringa oleifera* Lam. as protective agent in cardiac damage and vascular dysfunction. Frontiers in Pharmacology 24:3911
30. Al-Juhaimi, F. Y., Alsawmahi, O. N., Abdoun, K. A., Ghafoor, K., & Babiker, E. E. (2020). Antioxidant potential of Moringa leaves for improvement of milk and serum quality of Aardi goats. *South African Journal of Botany*, *129*, 134-137. doi: 10.1016/j.sajb.2019.03.022.
31. Al-Reza, S. M., Khan, S., Nahar, K., Alauddin, J., & Islam, S. (2022). Estimation of phytoconstituents and screening of antibacterial and antioxidant activity of different extracts from Moringa oleifera. https://doi.org/10.53771/ijbpsa.2022.4.2.0085.
32. Amaglo, N. K., Bennett, R. N., Curto, R. B. L., Rosa, E. A., Turco, V. L., Giuffrida, A., & Timpo, G. M. (2010). Profiling selected phytochemicals and nutrients in different tissues of the multipurpose tree Moringa oleifera L., grown in Ghana. *Food Chemistry*, *122*(4), 1047-1054.
33. Amrutia JN, Lala M, Srinivasa U, Shabaraya AR, & Moses RS (2011) Anticonvulsant activity of *Moringa oleifera* leaf. Int Res J Pharm 2(7): 160-162.
34. Anand, K., Tiloke, C., Phulukdaree, A., Ranjan, B., Chuturgoon, A., Singh, S., & Gengan, R. M. (2016). Biosynthesis of palladium nanoparticles by using Moringa oleifera flower extract and their catalytic and biological properties. *Journal of Photochemistry and Photobiology*, 165, 87-95. https://doi.org/10.1016/j.jphotobiol.2016.09.039.
35. Ani, E., Amove, J., & Igbabul, B. (2018). Physicochemical, microbiological, sensory properties and storage stability of plant-based yoghurt produced from bambaranut, soybean and Moringa oleifera seed milks. *American Journal of Food and Nutrition*, *6*(4), 115-125. DOI: 10.12691/ajfn-6-4-4.

36. Ansari, F., Singh, A., Rana, G. K., & Baidya, K. (2020). Formulation, development, and shelf-life assessment of instant soup mix using Moringa oleifera flower powder. *Journal of Pharmacognosy and Phytochemistry*, 9(6), 301-305.
37. Anthanont, P., Lumlerdkij, N., Akarasereenont, P., Vannasaeng, S., & Sriwijitkamol, A. (2016). Moringa oleifera leaf increases insulin secretion after single dose administration: a preliminary study in healthy subjects. *J. Med. Assoc. Thail*, *99*, 308-313.
38. Antony, A., & Farid, M. (2022). Effect of temperatures on polyphenols during extraction. *Applied Sciences*, *12*(4), 2107. https://doi.org/10.3390/app12042107.
39. Anwar, F., Latif, S., Ashraf, M., & Gilani, A. H. (2007). Moringa oleifera: a food plant with multiple medicinal uses. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *21*(1), 17-25. https://doi.org/10.1002/ptr.2023.
40. Ao, B., Lv, J., Yang, H., He, F., Hu, Y., Hu, B.,& Xia, X. (2022). Moringa oleifera extract mediated the synthesis of Bio-SeNPs with antibacterial activity against Listeria monocytogenes and Corynebacterium diphtheriae. *LWT*, *165*, 113751. https://doi.org/10.1016/j.lwt.2022.113751.
41. AOAC 2000. Official methods of analysis (17th ed.). 17 ed. Rockville Maryland: Association of Official Analytical Chemists.
42. AOAC. Official methods of analysis. 18th ed. (2010). p. 920e5. Washington, DC.
43. Aparna, V., Dileep, K.V., Mandal, P.K., Karthe, P., Sadasivan, C. and Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical biology & drug design*, 80(3), pp.434-439. DOI: 10.1111/j.1747-02852.012.01418.x.
44. Appel, K.E., Abraham, K., Berger-Preiss, E., Hansen, T., Apel, E., Schuchardt, S., Vogt, C., Bakhiya, N., Creutzenberg, O. and Lampen, A. (2013). Relative oral bioavailability of glycidol from glycidyl fatty acid esters in rats. *Archives of toxicology*, 87, pp.1649-1659.
45. Araújo, L. C. C., Aguiar, J. S., Napoleão, T. H., Mota, F. V. B., Barros, A. L. S., Moura, M. C., ... & Paiva, P. M. G. (2013). Evaluation of cytotoxic and anti-inflammatory activities of extracts and lectins from Moringa oleifera seeds. *PloS one*, *8*(12), e81973. https://doi.org/10.1371/journal.pone.0081973.
46. Arise, A. K., Arise, R. O., Sanusi, M. O., Esan, O. T., & Oyeyinka, S. A. (2014). Effect of Moringa oleifera flower fortification on the nutritional quality and sensory

properties of weaning food. *Croatian journal of food science and technology*, 6(2), 65-71.

47. Armenta, S., Garrigues, S., Esteve-Turrillas, F. A., & de la Guardia, M. (2019). Green extraction techniques in green analytical chemistry. *TrAC Trends in Analytical Chemistry*, *116*, 248-253. https://doi.org/10.1016/j.trac.2019.03.016.
48. Atawodi, S. E., Atawodi, J. C., Idakwo, G. A., Pfundstein, B., Haubner, R., Wurtele, G., ... & Owen, R. W. (2010). Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of Moringa oleifera Lam. *Journal of medicinal food*, *13*(3), 710-716. https://doi.org/10.1089/jmf.2009.0057.
49. Auwal, M. S., Tijjani, A. N., Sadiq, M. A., Saka, S., Mairiga, I. A., Shuaibu, A., ... & Gulani, I. A. (2013). Antibacterial and haematological activity of Moringa oleifera aqueous seed extract in Wistar albino rats. *Sokoto Journal of Veterinary Sciences*, *11*(1), 28-37. DOI: 10.4314/sokjvs.v11i1.5.
50. Aviara, N. A., Power, P. P., & Abbas, T. (2013). Moisture-dependent physical properties of Moringa oleifera seed relevant in bulk handling and mechanical processing. *Industrial Crops and Products*, *42*, 96-104. https://doi.org/10.1016/j.indcrop.2012.05.001.
51. Awasthi, M., Pokhrel, C., You, Y. H., Balami, S., Kunwar, R., Thapa, S. & Kim, Y. S. (2023). Comparative assessment of ethnobotany and antibacterial activity of Moringa oleifera Lam. in Nepal. *Ethnobotany Research and Applications*, *25*, 1-13. http://dx.doi.org/10.32859/era.25.14.1-13
52. Awuchi, C. G., Igwe, V. S., & Echeta, C. K. (2019). The functional properties of foods and flours. *International Journal of Advanced Academic Research*, *5*(11), 139-160.
53. Aznury, M., Margerty, E., Yuniar, Y., & AWWALIYAH, S. (2020). Effect of Fermentation Time and Percentage of Moringa (Moringa oleifera) Flour Variations on Vitamin C of Yogurt. In *Forum In Research, Science and Technology (FIRST)-Atlantis Highlights in Engineerin* (Vol. 7, p. 115). POLITEKNIK NEGERI SRIWIJAYA.
54. Bajkacz, S., & Adamek, J. (2017). Evaluation of new natural deep eutectic solvents for the extraction of isoflavones from soy products. *Talanta*, *168*, 329-335. https://doi.org/10.1016/j.talanta.2017.02.065.

55. Bala, M., Handa, S., Mridula, D., & Singh, R. K. (2020). Physicochemical, functional, and rheological properties of grass pea (Lathyrus sativus L.) flour as influenced by particle size. *Heliyon*, 6(11), e05471. https://doi.org/10.1016/j.heliyon.2020.e05471.
56. Balogun, A. O., Adeleke, A. A., Ikubanni, P. P., Adegoke, S. O., Alayat, A. M., & McDonald, A. G. (2021). Kinetics modeling, thermodynamics and thermal performance assessments of pyrolytic decomposition of Moringa oleifera husk and Delonix regia pod. *Scientific Reports*, 11(1), 13862.
57. Barakat, H., & Ghazal, G. A. (2016). Physicochemical properties of Moringa oleifera seeds and their edible oil cultivated at different regions in Egypt. *Food and Nutrition Sciences*, *7*(06), 472.
58. Bhattacharya, A., Tiwari, P., Sahu, P. K. and Kumar, S. (2018). A review of the phytochemical and pharmacological characteristics of Moringa oleifera. *Journal of pharmacy & bioallied sciences*. 10:181.
59. Bikheet, M. M., Yasien, E. E., & Galal, S. M. (2021). Preparation of Functional Yoghrt Drink Fortified with Moringa olifera Leaves. *Journal of Food and Dairy Sciences, 12*(9), 217-223. DOI: 10.21608/JFDS.2021.201813.
60. Biswas, S. K., Chowdhury, A., Das, J., Roy, A., & Hosen, S. Z. (2012). Pharmacological potentials of Moringa oleifera Lam.: a review. *International Journal of Pharmaceutical Sciences and Research*, *3*(2), 305.
61. Brunelli, D., Tavecchio, M., Falcioni, C., Frapolli, R., Erba, E., Iori, R., & D'Incalci, M. (2010). The isothiocyanate produced from glucomoringin inhibits NF-kB and reduces myeloma growth in nude mice in vivo. *Biochemical pharmacology*, *79*(8), 1141-1148.
62. Bulut, M., Tunçtürk, Y., & Alwazeer, D. (2021). Effect of fortification of set-type yoghurt with different plant extracts on its physicochemical, rheological, textural and sensory properties during storage. *International Journal of Dairy Technology*, *74*(4), 723-736.https://doi.org/10.1111/1471-0307.12803.
63. Chandran, D., Khalid, M., Walvekar, R., Mubarak, N. M., Dharaskar, S., Wong, W. Y., & Gupta, T. C. S. M. (2019). Deep eutectic solvents for extraction-desulphurization: A review. *Journal of Molecular Liquids, 275*, 312-322. DOI: 10.1016/j.molliq.2018.11.051.
64. Chauhan, A. P., Patel, D. M., Pate, J. D., & Singh, N. K. (2021). Extraction, Screening, and Characterization of Bioactive Compounds from Moringa oleifera: Extends Lifespan of Caenorhabditis elegans. *Current Trends in Biotechnology and*

Pharmacy, 15(3), 282-298. https://doi.org/10.5530/ctbp.2021.3.30. https://doi.org/10.1016/j.indcrop.2017.01.042.

65. Chavhan, A. (2017). Comparative studies on ascorbic acid content in various fruits, vegetables and leafy vegetables. *Int. J. of Life Sciences*, *5*(4), 667-671.
66. Chen, C., Zhang, B., Huang, Q., Fu, X., & Liu, R. H. (2017). Microwave-assisted extraction of polysaccharides from Moringa oleifera Lam. leaves: Characterization and hypoglycemic activity. *Industrial Crops and Products*, *100*, 1-11.
67. Chen, K. H., Chen, Y. J., Yang, C. H., Liu, K. W., Chang, J. L., Pan, S. F., & Chen, M. J. (2012). Attenuation of the extract from Moringa oleifera on monocrotaline-induced pulmonary hypertension in rats. *Chin J Physiol*, *55*(1), 22-30. DOI: 10.4077/CJP.2012.AMM104.
68. Cheng, W.W., Liu, G.Q., Wang, L.Q. and Liu, Z.S. (2017). Glycidyl fatty acid esters in refined edible oils: A review on formation, occurrence, analysis, and elimination methods. *Comprehensive Reviews in Food Science and Food Safety*, 16(2), pp.263-281. https://doi.org/10.1111/1541-4337.12251.
69. Chipurura, B. (2021). Nutritional content, phenolic composition and antioxidant activity of yoghurt enriched with baobab pulp (Adansonia digitata). *International Journal on Nutraceuticals, Functional Foods and Novel Foods*. DOI 10.17470/NF-016-1035-2.
70. Cousminer, J. J. (2017). *Culinology: The intersection of culinary art and food science*. Wiley.
71. Cui, H., Lu, T., Wang, M., Zou, X., Zhang, Y., Yang, X., ... & Zhou, H. (2019). Flavonoids from Morus alba L. leaves: Optimization of extraction by response surface methodology and comprehensive evaluation of their antioxidant, antimicrobial, and inhibition of α-amylase activities through analytical hierarchy process. *Molecules*, *24*(13), 2398. https://doi.org/10.3390/molecules24132398.
72. Čvorović, J., Ziberna, L., Fornasaro, S., Tramer, F., & Passamonti, S. (2018). Bioavailability of flavonoids: the role of cell membrane transporters. In *Polyphenols: Mechanisms of action in human health and disease* (pp. 295-320). Academic Press. https://doi.org/10.1016/B978-0-12-813006-3.00022-2.
73. Daba, M. (2016). Miracle tree: A review on multi-purposes of Moringa oleifera and its implication for climate change mitigation. *J. Earth Sci. Clim. Change*, *7*(4), 1-5.
74. Dai, Y., Rozema, E., Verpoorte, R., & Choi, Y. H. (2016). Application of natural deep eutectic solvents to the extraction of anthocyanins from Catharanthus roseus with high

extractability and stability replacing conventional organic solvents. *Journal of Chromatography A, 1434*, 50-56. https://doi.org/10.1016/j.chroma.2016.01.037.

75. Dai, Y., Spronsen, J. V., Witkamp, G. J., Verpoorte, R., Choi, Y. H. (2015). Tailoring properties of natural deep eutectic solvents with water to facilitate their applications. *Food Chemistry*, 187, 14-19.
76. Dai, Y., Witkamp, G. J., Verpoorte, R., & Choi, Y. H. (2015). Tailoring properties of natural deep eutectic solvents with water to facilitate their applications. *Food chemistry*, *187*, 14-19. https://doi.org/10.1016/j.foodchem.2015.03.123.
77. Das, K., Choudhary, R., & Thompson-Witrick, K. A. (2019). Effects of new technology on the current manufacturing process of yogurt-to increase the overall marketability of yogurt. *Lwt*, *108*, 69-80. DOI: 10.1016/j.lwt.2019.03.058
78. Das, P. E., Abu-Yousef, I. A., Majdalawieh, A. F., Narasimhan, S. and Poltronieri, P. (2020). Green synthesis of encapsulated copper nanoparticles using a hydroalcoholic extract of Moringa oleifera leaves and assessment of their antioxidant and antimicrobial activities. *Molecules*. 25:555.
79. Deetlefs, M., & Seddon, K. R. (2010). Assessing the greenness of some typical laboratory ionic liquid preparations. *Green Chemistry*, *12*(1), 17-30.
80. Devkota, S., & Bhusal, K. K. (2020). Moringa oleifera: A miracle multipurpose tree for agroforestry and climate change mitigation from the Himalayas–A review. *Cogent Food & Agriculture*, *6*(1), 1805951.
81. Dhakad, A. K., Oberoi, H. K., & Singh, K (2023). Assessment of a Highly Nutritive Medicinal Plant, Moringa Oleifera Germplasm for Nutritional Adaptability under Subtropical Climatic Conditions. *Biodiversity and Bioprospecting*.
82. Dhawi, F., El-Beltagi, H. S., Aly, E., & Hamed, A. M. (2020). Antioxidant, antibacterial activities and mineral content of buffalo yoghurt fortified with fenugreek and Moringa oleifera seed flours. *Foods*, *9*(9), 1157. https://doi.org/10.3390/foods9091157.
83. Diby, N. A.S., Deffan, K. P., Libra, M. A., Biego, G.H. M., (2020). Techno-functional properties of three species of Cucurbitaceae consumed in Côte d'Ivoire. *International Journal of Scientific & Engineering Research*, 11(1), 100-108.
84. Divya, K. G., Rubeena, M., Andalil, R., Erni, B., Sundaramoorthy, B., Kumar, K. N., & Shakila, R. (2019). Identity profile of Moringa oleifera Lam. flower. *Int J Bot Studies*, *4*(4).

85. Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. *Journal of food and drug analysis*, *22*(3), 296-302. https://doi.org/10.1016/j.jfda.2013.11.001.
86. Donovan, S. M., & Rao, G. (2019). Health benefits of yogurt among infants and toddlers aged 4 to 24 months: a systematic review. *Nutrition reviews*, *77*(7), 478-486. https://doi.org/10.1093/nutrit/nuz009.
87. Dubey, D. K., Dora, J., Kumar, A., & Gulsan, R. K. (2013). A multipurpose tree-Moringa oleifera. *International Journal of Pharmaceutical and Chemical Sciences*, 2(1), 415-423.
88. Duy, N. Q., Pham, T. N., Binh, M. L. T., Thuan, M., Van, N. T. T., Lam, T. D., & Nguyen, P. T. N. (2020). Effects of extraction conditions on antioxidant activities of Roselle (Hibiscus sabdariffa L.) extracts. In *Materials Science Forum* (Vol. 977, pp. 201-206). Trans Tech Publications Ltd. DOI: 10.4028/www.scientific.net/MSF.977.201.
89. EFSA Scientific Committee, 2021. Guidance on risk assessment of nanomaterials to be applied in the food and feed chain: Human and animal health. https://doi.org/10.2903/j.efsa.2021.6768.
90. El Achkar, T., Greige-Gerges, H., & Fourmentin, S. (2021). Basics and properties of deep eutectic solvents: a review. *Environmental chemistry letters*, *19*, 3397-3408. DOI: 10.1007/s10311-021-01225-8.
91. Elabd, E. M. Y., Morsy, S. M., & Elmalt, H. A. (2018). Investigating of Moringa oleifera role on gut microbiota composition and inflammation associated with obesity following high fat diet feeding. *Open access macedonian journal of medical sciences*, *6*(8), 1359. doi: 10.3889/oamjms.2018.313.
92. Elboughdiri, N., Ghernaout, D., Kriaa, K., & Jamoussi, B. (2020). Enhancing the extraction of phenolic compounds from Juniper Berries using the box-behnken design. *ACS omega*, *5*(43), 27990-28000. https://doi.org/10.1021/acsomega.0c03396.
93. Erol, I., Cigerci, I. H., Özkara, A., Akyıl, D., & Aksu, M. (2022). Synthesis of Moringa oleifera coated silver-containing nanocomposites of a new methacrylate polymer having pendant fluoroarylketone by hydrothermal technique and investigation of thermal, optical, dielectric and biological properties. *Journal of Biomaterials Science, Polymer Edition*, *33*(10), 1231-1255. https://doi.org/10.1080/09205063.2022.2046986.

94. Escalante, J., Chen, W. H., Tabatabaei, M., Hoang, A. T., Kwon, E. E., Lin, K. Y. A., & Saravanakumar, A. (2022). Pyrolysis of lignocellulosic, algal, plastic, and other biomass wastes for biofuel production and circular bioeconomy: A review of thermogravimetric analysis (TGA) approach. *Renewable and Sustainable Energy Reviews*, *169*, 112914. https://doi.org/10.1016/j.rser.2022.112914.

95. Espino, M., los, D. M., Fernandez, A., Gomez, F. J. V., Silva, M. F.2016). Natural designer solvents for greening analytical chemistry, *Trends in Analytical Chemistry* 76, 126-136.

96. Fahad, J. F., Kumar, M. S., Kodancha, G. P., Adarsh, B., Udupa, A. L., & Rathnakar, U. P. (2010). Antiurolithiatic activity of aqueous extract of bark of Moringa oleifera (Lam.) in rats. *Health*, *2*(4), 352-355. doi:10.4236/health.2010.24053.

97. Faizi, S., Siddiqui, B. S., Saleem, R., Aftab, K., & Shaheen, F. (1998). Hypotensive constituents from the pods of Moringa oleifera. *Planta medica*, *64*(03), 225-228. DOI: 10.1055/s-2006-957414.

98. Falowo, A. B., Mukumbo, F. E., Idamokoro, E. M., Lorenzo, J. M., Afolayan, A. J., & Muchenje, V. (2018). Multi-functional application of Moringa oleifera Lam. in nutrition and animal food products: A review. *Food research international*, *106*, 317-334. https://doi.org/10.1016/j.foodres.2017.12.079.

99. Fapetu, A. P., Karigidi, K. O., Akintimehin, E. S., Olawuwo, T., & Adetuyi, F. O. (2022). Effect of partial substitution of wheat flour with Moringa oleifera leaf powder on physical, nutritional, antioxidant, and antidiabetic properties of cookies. *Bulletin of the National Research Centre*, *46*(1), 1-9. https://doi.org/10.1186/s42269-022-00746-8.

100. Farhan, S. R., AL-Azawi, A. H., Salih, W. Y. and Abdulhassan, A. A. (2021). The antibacterial and antioxidant activity of Moringa oleifera seed oil extract against some foodborne pathogens. *Indian Journal of Forensic Medicine & Toxicology*. 15:2529-2538.

101. Fernandes, E. E., Pulwale, A. V., Patil, G. A., & Moghe, A. S. (2016). Probing regenerative potential of Moringa oleifera aqueous extracts using in vitro cellular assays. *Pharmacognosy research*, *8*(4), 231. doi: 10.4103/0974-8490.188877.

102. Ferreiro-González, M., Amores-Arrocha, A., Espada-Bellido, E., Aliaño-Gonzalez, M. J., Vázquez-Espinosa, M., González-de-Peredo, A. V., ... & Cejudo-Bastante, C. (2019). Escape classroom: Can you solve a crime using the analytical process?. *Journal of Chemical Education*, *96*(2), 267-273.

103. Foidl, N., Makkar, H. P. S., & Becker, K. (2001). The potenti al of moringa oleifera for agricultural and industrial uses.

104. Ford, T. J., Yii, E., Sidik, N., Good, R., Rocchiccioli, P., McEntegart, M., ... & Berry, C. (2019). Ischemia and no obstructive coronary artery disease: prevalence and correlates of coronary vasomotion disorders. *Circulation: Cardiovascular Interventions*, *12*(12), e008126. https://doi.org/10.1161/CIRCINTERVENTIONS.119.008126.

105. Francis, J. A., Jayaprakasam, B., Olson, L. K., & Nair, M. G. (2004). Insulin secretagogues from Moringa oleifera with cyclooxygenase enzyme and lipid peroxidation inhibitory activities. *Helvetica chimica acta*, *87*(2), 317-326. https://doi.org/10.1002/hlca.200490029.

106. Freire, J. E., Vasconcelos, I. M., Moreno, F. B., Batista, A. B., Lobo, M. D., Pereira, M. L., ... & Grangeiro, T. B. (2015). Mo-CBP3, an antifungal chitin-binding protein from Moringa oleifera seeds, is a member of the 2S albumin family. *PLoS One*, *10*(3), e0119871. https://doi.org/10.1371/journal.pone.0119871.

107. Ge, D., Wang, Y., Jiang, Q., & Dai, E. (2019). A deep eutectic solvent as an extraction solvent to separate and preconcentrate parabens in water samples using in situ liquid-liquid microextraction. *Journal of the Brazilian Chemical Society*, *30*, 1203-1210. DOI: 10.21577/0103-5053.20190014.

108. George, T. T., Oyenihi, A. B., Rautenbach, F., & Obilana, A. O. (2021). Characterization of Moringa oleifera leaf powder extract encapsulated in maltodextrin and/or gum arabic coatings. *Foods*, *10*(12), 3044. https://doi.org/10.3390/foods10123044.

109. Getachew, M., & Admassu, H. (2022). Evaluation of functional and rheological properties of the composite flour from oat, wheat and moringa tree leaves. *Cogent Food & Agriculture*, *8*(1), 2120009. https://doi.org/10.1080/23311932.2022.2120009.

110. Gheibi, S., Kashfi, K., & Ghasemi, A. (2017). A practical guide for induction of type-2 diabetes in rat: Incorporating a high-fat diet and streptozotocin. *Biomedicine & pharmacotherapy*, *95*, 605-613. https://doi.org/10.1016/j.biopha.2017.08.098.

111. Gilbert, A., & Turgeon, S. L. (2021). Studying stirred yogurt microstructure and its correlation to physical properties: A review. *Food Hydrocolloids*, *121*, 106970. DOI: 10.1016/j.foodhyd.2021.106970.

112. Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food science and human wellness*, 5(2), 49-56. https://doi.org/10.1016/j.fshw.2016.04.001.

113. Gourai, M., Nayak, A. K., Nial, P. S., Satpathy, B., Bhuyan, R., Singh, S. K., & Subudhi, U. (2023). Thermal plasma processing of Moringa oleifera biochars: adsorbents for fluoride removal from water. *RSC advances*, 13(7), 4340-4350. DOI: 10.1039/D2RA07514H.

114. Guevara, A. P., Vargas, C., Sakurai, H., Fujiwara, Y., Hashimoto, K., Maoka, T., & Nishino, H. (1999). An antitumor promoter from Moringa oleifera Lam. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *440*(2), 181-188. https://doi.org/10.1016/S1383-5718(99)00025-X.

115. Guo, Q., Ma, Q., Xue, Z., Gao, X., & Chen, H. (2018). Studies on the binding characteristics of three polysaccharides with different molecular weight and flavonoids from corn silk (Maydis stigma). *Carbohydrate Polymers*, 198, 581-588. https://doi.org/10.1016/j.carbpol.2018.06.120.

116. Guzmán-Maldonado, S. H., López-Manzano, M. J., Madera-Santana, T. J., Núñez-Colín, C. A., Grijalva-Verdugo, C. P., Villa-Lerma, A. G., & Rodríguez-Núñez, J. R. (2020). Nutritional characterization of Moringa oleifera leaves, seeds, husks and flowers from two regions of Mexico. *Agronomía Colombiana*, *38*(2), 287-297. https://doi.org/10.15446/agron.colomb.v38n2.82644.

117. Hadi, M.Y., Mohammed, G.J. and Hameed, I.H. (2016). Analysis of bioactive chemical compounds of Nigella sativa using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, 8(2), pp.8-24. https://doi.org/10.5897/JPP2015.0364.

118. Hani, N., Azarian, M.H., Torkamani, A.E. and Kamil Mahmood, W.A., (2016). Characterisation of gelatin nanoparticles encapsulated with Moringa oleifera bioactive extract. *International Journal of Food Science & Technology*, 51(11), pp.2327-2337. https:// doi:10.1111/ijfs.13211.

119. Hayyan, M., Mbous, Y. P., Looi, C. Y., Wong, W. F., Hayyan, A., Salleh, Z., & Mohd-Ali, O. (2016). Natural deep eutectic solvents: cytotoxic profile. *SpringerPlus*, *5*, 1-12. DOI 10.1186/s40064-016-2575-9.

120. He, B. S., Wang, X., Zhang, Y., Gao, C., Wu, C. K., Guo, S. R., & Wang, J. H. (2022). Anti-oxidant, anti-inflammatory, and anti-fibrotic effects of Moringa oleifera

seeds on renal injury diabetic induced by streptozotocin. *Journal of Functional Foods*, *95*, 105168. https://doi.org/10.1016/j.jff.2022.105168.

121. He, C., Zheng, J., Liu, F., Woo, M. W., Xiong, H., & Zhao, Q. (2020). Fabrication and characterization of oat flour processed by different methods. *Journal of Cereal Science*, *96*, 103123. https://doi.org/10.1016/j.jcs.2020.103123.

122. Herlina, H., & Sinaga, R. B. (2022). Sago Starch Bagea Cookies with Moringa Leaf Powder Composite as a Fiber and Antioxidant Enhancer. *Food ScienTech Journal*, *4*(1), 47-61.

123. Horn, L., Shakela, N., Mutorwa, M. K., Naomab, E., & Kwaambwa, H. M. (2022). Moringa oleifera as a sustainable climate-smart solution to nutrition, disease prevention, and water treatment challenges: a review. *Journal of Agriculture and Food Research*, 100397.

124. Hossain, M. A., Disha, N. K., Shourove, J. H., & Dey, P. (2020). Determination of antioxidant activity and total tannin from drumstick (Moringa oleifera lam.) leaves using different solvent extraction methods. *Turkish Journal of Agriculture-Food Science and Technology*, *8*(12), 2749-2755. https://doi.org/10.24925/turjaf.v8i12.2749-2755.4038.

125. Huang, G. J., Deng, J. S., Huang, S. S., Shao, Y. Y., Chen, C. C., & Kuo, Y. H. (2012). Protective effect of antrosterol from Antrodia camphorata submerged whole broth against carbon tetrachloride-induced acute liver injury in mice. *Food Chemistry*, *132*(2), 709-716.

126. Ibrahim, M. E. E. D., Alqurashi, R. M., & Alfaraj, F. Y. (2022). Antioxidant activity of moringa oleifera and olive Olea europaea L. leaf powders and extracts on quality and oxidation stability of chicken burgers. *Antioxidants*, *11*(3). doi: 10.3390/antiox11030496.

127. Ijarotimi, O. S., Adeoti, O. A., & Ariyo, O. (2013). Comparative study on nutrient composition, phytochemical, and functional characteristics of raw, germinated, and fermented Moringa oleifera seed flour. *Food science & nutrition*, *1*(6), 452-463. https://doi.org/10.1002/fsn3.70.

128. Iqbal, S., & Bhanger, M. I. (2006). Effect of season and production location on antioxidant activity of Moringa oleifera leaves grown in Pakistan. *Journal of food Composition and Analysis*, *19*(6-7), 544-551. https://doi.org/10.1016/j.jfca.2005.05.001.

129. Iriondo-DeHond, M., Miguel, E., & Del Castillo, M. D. (2018). Food byproducts as sustainable ingredients for innovative and healthy dairy foods. *Nutrients*, *10*(10), 1358.

130. Islam, Z., Islam, S. M., Hossen, F., Mahtab-ul-Islam, K., Hasan, M., & Karim, R. (2021). Moringa oleifera is a prominent source of nutrients with potential health benefits. *International Journal of Food Science*. https://doi.org/10.1155/2021/6627265.

131. Ivanović, M., Islamčević Razboršek, M., & Kolar, M. (2020). Innovative extraction techniques using deep eutectic solvents and analytical methods for the isolation and characterization of natural bioactive compounds from plant material. *Plants*, *9*(11), 1428. 8. DOI: 10.3390/plants9111428.

132. Izadi, Z., Nasirpour, A., Garoosi, G. A., & Tamjidi, F. (2015). Rheological and physical properties of yogurt enriched with phytosterol during storage. *Journal of Food Science and Technology*, *52*(8), 5341-5346. DOI 10.1007/s13197-014-1593-2.

133. Jadhav, V., Bhagare, A., Wahab, S., Lokhande, D., Vaidya, C., Dhayagude, A., & Dutta, M. (2022). Green Synthesized Calcium Oxide Nanoparticles (CaO NPs) Using Leaves Aqueous Extract of Moringa Oleifera and Evaluation of Their Antibacterial Activities. *Journal of Nanomaterials*, *2022*. https://doi.org/10.1155/2022/9047507.

134. Jain, A., Jain, P. and Parihar, D. K. (2019). Comparative study of in-vitro antidiabetic and antibacterial activity of non-conventional curcuma species. *Journal of Biologically Active Products from Nature*. 9:457-464.

135. Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. (2017). Medicinal plants: Past history and future perspective. *Journal of herbmed pharmacology*, *7*(1), 1-7.

136. Janabi, A. H. W., Kamboh, A. A., Saeed, M., Xiaoyu, L., BiBi, J., Majeed, F., ... & Lv, H. (2020). Flavonoid-rich foods (FRF): A promising nutraceutical approach against lifespan-shortening diseases. *Iranian Journal of Basic Medical Sciences*, *23*(2), 140. doi: 10.22038/IJBMS.2019.35125.8353.

137. Jha, P., Meghwal, M., Prabhakar, P. K., & Singh, A. (2021). Exploring effects of different pre-treatments on drying kinetics, moisture diffusion, Physico-functional, and flow properties of banana flower powder. *Journal of Food Processing and Preservation*, 45(4), e15356. https://doi.org/10.1111/jfpp.15356.

138. Joy, A. E., Kunhikatta, S. B., & Manikkoth, S. (2013). Anti-convulsant activity of ethanolic extract of Moringa concanensis leaves in Swiss albino mice. *Archives of Medicine and Health sciences*, *1*(1), 6.

139. Kandeepan, C., Sabitha, M., Parvathi, K., Senthilkumar, N., Ramya, S., Boopathi, N. M., & Jayakumararaj, R. (2022). Phytochemical screening, GCMS profile, and In-silico properties of bioactive compounds in methanolic leaf extracts of Moringa oleifera. *Journal of Drug Delivery and Therapeutics*, *12*(2), 87-99. *DOI: http://dx.doi.org/10.22270/jddt.v12i2.5250*.

140. Kar, A., Choudhary, B. K., & Bandyopadhyay, N. G. (2003). Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *Journal of ethnopharmacology*, *84*(1), 105-108. https://doi.org/10.1016/S0378-8741(02)00144-7.

141. Karray, A., Alonazi, M., Jallouli, R., Alanazi, H., & Ben Bacha, A. (2022). A proteinaceous alpha-amylase inhibitor from Moringa oleifera leaf extract: purification, characterization, and insecticide effects against C. maculates insect larvae. *Molecules*, *27*(13), 4222. https://doi.org/10.3390/molecules27134222.

142. Kashyap, P., Kumar, S., Riar, C. S., Jindal, N., Baniwal, P., Guiné, R. P. and Kumar, H. (2022). Recent advances in Drumstick (Moringa oleifera) leaves bioactive compounds: Composition, health benefits, bioaccessibility, and dietary applications. *Antioxidants*.11:402.

143. Kasolo, J. N., Bimenya, G. S., Ojok, L., Ochieng, J., & Ogwal-Okeng, J. W. (2010). Phytochemicals and uses of Moringa oleifera leaves in Ugandan rural communities.

144. Katke, S. D., & Deshpande, H. W. (2022). Studies on the development of fibre-rich probiotic frozen yogurt. *Asian Journal of Dairy and Food Research*, *41*(2), 142-149. DOI: 10.18805/ajdfr.DR-1753.

145. Kaur, S., Chauhan, P. N., Harwansh, R. K., Chakma, M. and Kaur, S. (2023). Nutraceutical Potential and Processing Aspects of Moringa oleifera as a Superfood. *Current Nutrition & Food Science*.19:357-376.

146. Kc, Y., Rai, R., Katuwal, N., Shiwakoti, L. D., Pant, B. R., Bajgai, T. R., ... & Upadhyaya, J. (2021). Phytochemicals, nutritional, antioxidant activity, and sensory analyses of Moringa oleifera Lam. collected from the mid-hill region of Nepal. *Natural Product Research*, *36*(1), 470-473. https://doi.org/10.1080/14786419.2020.1781113.

147. Khalid, S., Arshad, M., Mahmood, S., Siddique, F., Roobab, U., Ranjha, M. M. A. N., & Lorenzo, J. M. (2023). Extraction and Quantification of Moringa oleifera Leaf Powder Extracts by HPLC and FTIR. *Food Analytical Methods*, 1-11. https://doi.org/10.1007/s12161-023-02470-z

148. Khan, M. S. A., & Ahmad, I. (2019). Herbal medicine: current trends and future prospects. In *New look to phytomedicine* (pp. 3-13). Academic Press.

149. Khan, W., Parveen, R., Chester, K., Parveen, S., & Ahmad, S. (2017). Hypoglycemic potential of aqueous extract of Moringa oleifera leaf and in vivo GC-MS metabolomics. *Frontiers in pharmacology*, *8*, 577. https://doi.org/10.3389/fphar.2017.00577.

150. Kheir, S. M., Kafi, S. K., & Elbir, H. (2014). The antimicrobial activity and phytochemical characteristic of moringa oleifera seeds, leaves, and flowers. WJPR, 3, 4.

151. Khorshidian, N., Yousefi Asli, M., Arab, M., Adeli Mirzaie, A., & Mortazavian, A. M. (2016). Fenugreek: potential applications as a functional food and nutraceutical. *Nutrition and Food Sciences Research*, *3*(1), 5-16.

152. Koley, R., Ghorai, U. K., Chattopadhyay, S., & Bhowmick, A. K. (2023). An investigation on Moringa oleifera fruit (drumstick)-derived cellulose micro and nanofiber reinforced styrene butadiene rubber composites. *Polymer Composites*, *44*(5), 2778-2793.

153. Kolo, U. B., Madu, S. J., & Muazu, J. (2018). Formulation and Evaluation of Oral Reconstitutable Suspension of Aqueous Moringa oleifera Lam Root Extract. *Nigerian Journal of Pharmaceutical Research*, *14*(1), 81-89.

154. Koul, B., & Chase, N. (2015). Moringa oleifera Lam.: Panacea to several maladies. *J. Chem. Pharm. Res*, *7*(6), 687-707.

155. Krishnamurthy, P. T., Vardarajalu, A., Wadhwani, A., & Patel, V. (2015). Identification and characterization of a potent anticancer fraction from the leaf extracts of Moringa oleifera L.

156. Kumar, B., Kumar, S., & Kumar-Singh, A. (2015). Ayurvedic Medicine: A review on medicinal importance of shigru (Moringa oleifera Lam.) in Samhitas. *Indian Journal of Agriculture and allied Science*, *1*(3), 127-137.

157. Kumar, M., Guleria, S., Chawla, P., Khan, A., Modi, V. K., Kumar, N., & Kaushik, R. (2020). Anti-obesity efficacy of the selected high altitude Himalayan herbs: In vitro studies. *Journal of Food Science and Technology*, *57*, 3081-3090. https://doi.org/10.1007/s13197-020-04341-5.

158. Kumar, T., Dholpuria, S., Kumar, A., Kumar, P., & Mehara andPooja, M (2024). Effect of moringa (Moringa oleifera) leaves extract administration on testosterone profile in Marwari pubertal rams.

159. Leone, A., Spada, A., Battezzati, A., Schiraldi, A., Aristil, J., & Bertoli, S. (2015). Cultivation, genetic, ethnopharmacology, phytochemistry and pharmacology of Moringa oleifera leaves: An overview. *International journal of molecular sciences*, *16*(6), 12791-12835. https://doi.org/10.3390/ijms160612791.

160. Li, H., Deng, Z., Wu, T., Liu, R., Loewen, S., & Tsao, R. (2012). Microwave-assisted extraction of phenolics with maximal antioxidant activities in tomatoes. *Food Chemistry*, *130*(4), 928-936. https://doi.org/10.1016/j.foodchem.2011.08.019.

161. Li, S., Ye, A., & Singh, H. (2021). Effects of seasonal variations on the quality of set yogurt, stirred yogurt, and Greek-style yogurt. *Journal of Dairy Science*, *104*(2), 1424-1432. DOI: 10.3168/jds.2020-19071.

162. Lisak Jakopović, K., Repajić, M., Rumora Samarin, I., Božanić, R., Blažić, M., & Barukčić Jurina, I. (2022). Fortification of Cow Milk with Moringa oleifera Extract: Influence on Physicochemical Characteristics, Antioxidant Capacity and Mineral Content of Yoghurt. *Fermentation*, *8*(10), 545. https://doi.org/10.3390/fermentation8100545.

163. Liu, R., Liu, J., Huang, Q., Liu, S., & Jiang, Y. (2022). Moringa oleifera: a systematic review of its botany, traditional uses, phytochemistry, pharmacology and toxicity. *Journal of Pharmacy and Pharmacology*, *74*(3), 296-320.

164. Liu, Y., Wang, X. Y., Wei, X. M., Gao, Z. T., & Han, J. P. (2018). Values, properties and utility of different parts of Moringa oleifera: An overview. *Chinese Herbal Medicines*, 10(4), 371-378. https://doi.org/10.1016/j.chmed.2018.09.002.

165. Llorent-Martínez, E. J., Gordo-Moreno, A. I., Fernández-de Córdova, M. L., & Ruiz-Medina, A. (2023). Preliminary Phytochemical Screening and Antioxidant Activity of Commercial Moringa oleifera Food Supplements. *Antioxidants*, *12*(1), 110. https://doi.org/10.3390/antiox12010110.

166. Ma, Z. F., Ahmad, J., Zhang, H., Khan, I., & Muhammad, S. (2020). Evaluation of phytochemical and medicinal properties of Moringa (Moringa oleifera) as a potential functional food. *South African Journal of Botany*, *129*, 40-46. https://doi.org/10.1016/j.sajb.2018.12.002.

167. Madane, P., Das, A. K., Pateiro, M., Nanda, P. K., Bandyopadhyay, S., Jagtap, P., & Lorenzo, J. M. (2019). Drumstick (Moringa oleifera) flower as an antioxidant dietary fiber in chicken meat nuggets. *Foods*, *8*(8), 307. https://doi.org/10.3390/foods8080307.

168. Madhu, C., Krishna, K. M., Reddy, K. R., Lakshmi, P. J., & Kelari, E. K. (2017). Estimation of crude fiber content from natural foodstuffs and its laxative activity induced in rats. *Int J Pharma Res Health Sci*, 5(3), 1703-1706. DOI:10.21276/ijprhs.2017.03.04.

169. Madi, N., Dany, M., Abdoun, S., & Usta, J. (2016). Moringa oleifera's nutritious aqueous leaf extract has anticancerous effects by compromising mitochondrial viability in an ROS-dependent manner. *Journal of the American College of Nutrition*, *35*(7), 604-613. https://doi.org/10.1080/07315724.2015.1080128.

170. Madkhali, H. A., Alharthy, K. M., Asiri, M. A., Ganaie, M. A., Ansari, M. N., Rehman, N. U., & Hamad, A. M. (2019). Moringa oleifera Lam (family Moringaceae) leaf extract attenuates high-fat diet-induced dyslipidemia and vascular endothelium dysfunction in Wistar albino rats. *Tropical Journal of Pharmaceutical Research*, *18*(12), 2597-2604.

171. Mahajan, R., & More, D. (2012). Evaluation of anticoagulant activity of aqueous and ethanolic extract and their isolated phytochemicals of some medicinal plants. *Int J Pharm Pharm Sci*, *4*(4), 498-500.

172. Mahajan, S. G., & Mehta, A. A. (2010). Immunosuppressive activity of ethanolic extract of seeds of Moringa oleifera Lam. in experimental immune inflammation. *Journal of ethnopharmacology*, *130*(1), 183-186. https://doi.org/10.1016/j.jep.2010.04.024.

173. Mahmood, K. T., Mugal, T., & Haq, I. U. (2010). Moringa oleifera: a natural gift-A review. *Journal of Pharmaceutical Sciences and Research*, *2*(11), 775.

174. Mahmoud, M. H., Abou-Arab, A. A., & Abu-Salem, F. M. (2017). Research Article Quality Characteristics of Beef Burger as Influenced by Different Levels of Orange Peel Powder. DOI: 10.3923/ajft.2017.262.270.

175. Maisto, M., Iannuzzo, F., Schiano, E., Ciampaglia, R., Labanca, A., Montesano, D. and Tenore, G. C. (2022). Effects of Fortified Laying Hen Diet with Moringa Oleifera Leaves and Goji Berries on Cholesterol and Carotenoid Egg Content. *Foods*.11: 3156.

176. Majd, M. H., Rajaei, A., Bashi, D. S., Mortazavi, S. A., & Bolourian, S. (2014). Optimization of ultrasonic-assisted extraction of phenolic compounds from bovine pennyroyal (Phlomidoschema parviflorum) leaves using response surface methodology. *Industrial Crops and Products*, 57, 195-202. https://doi.org/10.1016/j.indcrop.2014.03.031.

177. Mani-López, E., Palou, E., & López-Malo, A. (2014). Probiotic viability and storage stability of yogurts and fermented milks prepared with several mixtures of lactic acid bacteria. *Journal of Dairy Science*, *97*(5), 2578-2590. https://doi.org/10.3168/jds.2013-7551.

178. Manjari, G., Saran, S., Arun, T., Rao, A. V. B., & Devipriya, S. P. (2017). Catalytic and recyclability properties of phytogenic copper oxide nanoparticles derived from Aglaia elaeagnoidea flower extract. *Journal of Saudi Chemical Society*, *21*(5), 610-618. https://doi.org/10.1016/j.jscs.2017.02.004.

179. Manurung, R., Simanjuntak, G. C., Perez, R. N., Syahputra, A., Alhamdi, M. A., Siregar, H. D., & Zuhri, R. R. S. (2019, May). Production of choline chloride-based deep eutectic solvent with hydrogen bond donor D-glucose and ethylene glycol. In *IOP conference series: Materials Science and Engineering* (Vol. 505, No. 1, p. 012134). IOP Publishing. DOI: 10.1088/1757-899X/505/1/012134.

180. Marand, M. A., Amjadi, S., Marand, M. A., Roufegarinejad, L., & Jafari, S. M. (2020). Fortification of yogurt with flaxseed powder and evaluation of its fatty acid profile, physicochemical, antioxidant, and sensory properties. *Powder Technology*, *359*, 76-84. DOI: 10.1016/j.powtec.2019.09.082

181. Masurekar, T. S., Kadam, V., & Jadhav, V. (2015). Roles of Moringa oleifera in medicine-a review. *World Journal of Pharmacy and Pharmaceutical Sciences*, *5*(1), 375-385.

182. Matabura, V. V., & Rweyemamu, L. M. (2022). Formulation of plant-based food and characterisation of the nutritional composition: A case study on soy-moringa beverage. *Journal of Food Science and Technology*, *59*(10), 3794-3805. https://doi.org/10.1007/s13197-022-05397-1

183. Mbikay, M. (2012). Therapeutic potential of Moringa oleifera leaves in chronic hyperglycemia and dyslipidemia: a review. *Frontiers in pharmacology*, *3*, 24. https://doi.org/10.3389/fphar.2012.00024.

184. Mbous, Y. P., Hayyan, M., Hayyan, A., Wong, W. F., Hashim, M. A., & Looi, C. Y. (2017). Applications of deep eutectic solvents in biotechnology and bioengineering—Promises and challenges. *Biotechnology advances*, *35*(2), 105-134. https://doi.org/10.1016/j.biotechadv.2016.11.006.

185. Mehmood, A., Naveed, K., Khan, S. U., Haq, N. U., Shokat, M. F., Iqbal, M., ... & Ali, S. (2022). Phytochemical screening, antioxidants properties and antibacterial

efficacy of moringa leaves. *Journal of Xi'an Shiyou University, Natural Sciences Edition*, *18*(10), 59-70.

186. Mehra, N., Tamta, G., & Nand, V. (2022). Phytochemical screening and in vitro antioxidant assays in Foeniculum vulgare Mill.(Fennel) seeds collected from Tarai region in the Uttarakhand. *Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR)]*, *13*(2), 213-222. DOI: 10.56042/ijnpr.v13i2.51347.

187. Mendoza-Taco, M. M., Cruz-Hernández, A., Ochoa-Flores, A. A., Hernández-Becerra, J. A., Gómez-Vázquez, A., Moo-Huchin, V. M., ... & Vargas-Bello-Pérez, E. (2022). Physicochemical Characteristics of Yogurt from Sheep Fed with Moringa oleifera Leaf Extracts. *Animals*, *12*(1), 110. doi: 10.3390/ani12010110.

188. Mohammed, A. A., Mohamed, A., El-Naggar, N. E. A., Mahrous, H., Nasr, G. M., Abdella, A. and Ali, A. S. (2022). Antioxidant and antibacterial activities of silver nanoparticles biosynthesized by Moringa Oleifera through response surface methodology. *Journal of Nanomaterials*. *pp* 1-15.

189. Montemurro, M., Pontonio, E., Coda, R., & Rizzello, C. G. (2021). Plant-based alternatives to yogurt: State-of-the-art and perspectives of new biotechnological challenges. *Foods*, *10*(2), 316. https://doi.org/10.3390/foods10020316.

190. Motallebi, M., Mishra, S. K., & Panda, P. K. A Comparison Study between Orlistat and Moringa Oleifera as an Appetite Suppressant in Case of Obese Patients. DOI: 10.47191/ijcsrr/V6-i1-32.

191. Mousavi, M., Heshmati, A., Daraei Garmakhany, A., Vahidinia, A., & Taheri, M. (2019). Texture and sensory characterization of functional yogurt supplemented with flaxseed during cold storage. *Food science & nutrition*, *7*(3), 907-917. DOI: 10.1002/fsn3.805.

192. Moyo, B., Masika, P. J., Hugo, A., & Muchenje, V. (2011). Nutritional characterization of Moringa (Moringa oleifera Lam.) leaves. African Journal of Biotechnology, 10(60), 12925-12933. DOI: 10.5897/AJB10.1599.

193. Muhl, Q. E., du Toit, E. S., Steyn, J. M., & Apostolides, Z. (2013). Bud development, flowering and fruit set of Moringa oleifera Lam. (Horseradish Tree) as affected by various irrigation levels.

194. Mulat, M., Khan, F., Muluneh, G., & Pandita, A. (2020). Phytochemical profile and antimicrobial effects of different medicinal plant: current knowledge and future

perspectives. *Current Traditional Medicine*, *6*(1), 24-42. https://doi.org/10.2174/2215083805666190730151118.

195. Müller, L., Fröhlich, K., & Böhm, V. (2011). Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. *Food Chemistry*, *129*(1), 139-148. https://doi.org/10.1016/j.foodchem.2011.04.045.
196. Mulvihill, E. E., Burke, A. C., and Huff M. W. (2016). Citrus Flavonoids as Regulators of Lipoprotein Metabolism and Atherosclerosis. *Annual Review of Nutrition*, 36:13.1–13.25.
197. Munevar, N. X., & Zambrano Orozco, E. P. (2019). Evaluación del efecto antimicrobiano del polvo y extracto acuoso liofilizado de hojas de Moringa Oleífera en un derivado cárnico (chorizo crudo).
198. Nadeem, M., Abdullah, M., Khalique, A., Hussain, I., Mahmud, A., & Inayat, S. (2013). The effect of Moringa oleifera leaf extract as antioxidant on stabilization of butter oil with modified fatty acid profile.
199. Nakajima, A., Ohizumi, Y. (2019). Potential Benefits of Nobiletin, A Citrus Flavonoid, against Alzheimer's Disease and Parkinson's Disease. *International Journal of Molecular Science, 20(14)*, 3380.
200. Naklong, K., Therdtatha, P., Sumonsiri, N., Leksawasdi, N., Techapun, C., Rachtanapun, P., & Khemacheewakul, J. (2023). Microencapsulation of Bifidobacterium breve to Enhance Microbial Cell Viability in Green Soybean Yogurt. *Fermentation*, *9*(3), 296. https://doi.org/10.3390/fermentation9030296.
201. Nawash, O. S., & Ahmad Al-S, H. (2011). The most important medicinal plants in Wadi Araba desert in South West Jordan: a review article. *Advances in Environmental Biology*, 418-426.
202. Nimyel, C. D., & Lori, J. A. (2022). Phytochemistry, Proximate Analysis and Acute Toxicity Study of Ethanolic Moringa Oleifera Root Bark Extract. *Nigerian Journal of Chemical Research, 27*(1), 025-034.
203. Ningsih, P., Rahmawati, S., Hamzah, B., Santoso, T., Nurbaya, N., Hardani, M. F., & Hardani, R. (2021). Histology of hematoxylin-eosin and immunohistochemical diabetes rat pancreas after giving a combination of moringa leaves (Moringa oleifera) and clove flower (Syzygium aromaticum) extracts. *Open Access Macedonian Journal of Medical Sciences*, *9*(A), 257-262. https://doi.org/10.3889/oamjms.2021.5928.

204. Noh, H. J., Seo, H. M., Lee, J. H., & Chang, Y. H. (2013). Physicochemical and sensory properties of yogurt supplemented with corni fructus during storage. *Preventive nutrition and food Science*, *18*(1), 45. doi: 10.3746/pnf.2013.18.1.045.

205. Ntshambiwa, K. T., Seifu, E., & Mokhawa, G. (2023). Nutritional composition, bioactive components and antioxidant activity of Moringa stenopetala and Moringa oleifera leaves grown in Gaborone, Botswana. *Food Production, Processing and Nutrition*, *5*(1), 1-13. https://doi.org/10.1186/s43014-022-00124-x.

206. Nwidu, L. L., Elmorsy, E., & Carter, W. G. (2016). In-vitro Anticholinesterases Screening and antioxidant activity of three standardized herbal products used in ethnomedicine of South-East Nigeria. *The FASEB Journal*, *30*, 1191-2.

207. Oboh, G., Oyeleye, S. I., Akintemi, O. A., & Olasehinde, T. A. (2018). Moringa oleifera supplemented diet modulates nootropic-related biomolecules in the brain of STZ-induced diabetic rats treated with acarbose. *Metabolic brain disease*, *33*, 457-466. https://doi.org/10.1007/s11011-018-0198-2.

208. Ogundipe, A., Adetuyi, B., Iheagwam, F., Adefoyeke, K., Olugbuyiro, J., Ogunlana, O., & Ogunlana, O. (2022). In Vitro Experimental Assessment of Ethanolic Extract of Moringa oleifera Leaves as an α-Amylase and α-Lipase Inhibitor. *Biochemistry Research International*, *2022*. https://doi.org/10.1155/2022/4613109.

209. Ojewumi, M. E., Obanla, O. R., & Atauba, D. M. (2021). A review on the efficacy of Ocimum gratissimum, Mentha spicata, and Moringa oleifera leaf extracts in repelling mosquito. *Beni-Suef University Journal of Basic and Applied Sciences*, *10*, 1-12.

210. Oladele, I. O., Ogunwande, G. S., Taiwo, A. S., & Lephuthing, S. S. (2022). Development and characterization of moringa oleifera fruit waste pod derived particulate cellulosic reinforced epoxy bio-composites for structural applications. *Heliyon*, *8*(6), e09755. https://doi.org/10.1016/j.heliyon.2022.e09755.

211. Omar, K. A., & Sadeghi, R. (2022). Physicochemical properties of deep eutectic solvents: A review. *Journal of Molecular Liquids*, 119524. https://doi.org/10.1016/j.molliq.2022.119524.

212. Omodanisi, E. I., Aboua, Y. G., Chegou, N. N., & Oguntibeju, O. O. (2017). Hepatoprotective, antihyperlipidemic, and anti-inflammatory activity of Moringa oleifera in diabetic-induced damage in male wistar rats. *Pharmacognosy research*, *9*(2), 182. doi: 10.4103/0974-8490.204651.

213. Oyeyinka, A. T., & Oyeyinka, S. A. (2018). Moringa oleifera as a food fortificant: Recent trends and prospects. *Journal of the Saudi Society of Agricultural Sciences*, *17*(2), 127-136. https://doi.org/10.1016/j.jssas.2016.02.002.

214. Padayachee, B., & Baijnath, H. (2020). An updated comprehensive review of the medicinal, phytochemical, and pharmacological properties of Moringa oleifera. *South African Journal of Botany*, 129, 304-316. https://doi.org/10.1016/j.sajb.2019.08.021.

215. Paikra, B. K., & Gidwani, B. (2017). Phytochemistry and pharmacology of Moringa oleifera Lam. *Journal of pharmacopuncture*, *20*(3), 194. doi: 10.3831/KPI.2017.20.022.

216. Parvathy, M. V. S., & Umamaheshwari, A. (2007). Cytotoxic effect of Moringa oleifera leaf extracts on human multiple myeloma cell lines. *Trends in Medical Research*, *2*(1), 44-50. 10.3923/tmr.2007.44.50.

217. Passos, H., Tavares, D. J., Ferreira, A. M., Freire, M. G., & Coutinho, J. A. (2016). Are aqueous biphasic systems composed of deep eutectic solvents ternary or quaternary systems?. *ACS Sustainable Chemistry & Engineering*, *4*(5), 2881-2886. https://doi.org/10.1021/acssuschemeng.6b00485.

218. Patel, V. K., Acharya, R., & Patel, B. R. (2019). A Comprehensive Review of Karavīra, A Familiar Plant as Depicted in Classical Texts of Āyurveda. *J. Drug Res. Ayurvedic Sci.*, *4*(3), 138-156.

219. Patil, M. M., Anand, T., Ilaiyaraja, N., & Khanum, F. (2017). In-vitroantioxidant and anti-obesity properties of Bauhinia variegata. *Defence Life Science Journal*, *2*(2), 128-132. DOI : 10.14429/dlsj.2.11355.

220. Patwardhan, B. (2000). Ayurveda: The designer medicine. *Indian drugs*, *37*(5), 213-227.

221. Peerkhan, N., & Nair, S. (2021). Optimization of wheat dextrin yogurt formulation using response surface methodology. *Journal of Food Science and Technology*, *58*(5), 1740-1749. https://doi.org/10.1007/s13197-020-04683-0.

222. Peixoto, J. R. O., Silva, G. C., Costa, R. A., Vieira, G. H. F., Fonteles Filho, A. A., & dos Fernandes Vieira, R. H. S. (2011). In vitro antibacterial effect of aqueous and ethanolic Moringa leaf extracts. *Asian Pacific journal of tropical medicine*, *4*(3), 201-204. https://doi.org/10.1016/S1995-7645(11)60069-2.

223. Peñalver, R., Martínez-Zamora, L., Lorenzo, J. M., Ros, G. and Nieto, G. (2022). Nutritional and antioxidant properties of Moringa oleifera leaves in functional foods. *Foods*. 11:1107.

224. Pereira, F. P., Kloskowski, A., Namiesnik, J. (2015). Perspectives on the replacement of harmful organic solvents in analytical methodologies: a framework toward the implementation of a generation of eco-friendly alternatives, *Green Chemistry*, 17, 3687-3705.

225. Poornima, K. R., Kumargouda, V., Dronachari Manvi, D., Jayashree, G. C., PN, E. K., & Vimala, M. (2023). Studies on engineering properties of moringa (Moringa oleifera L.) pods.

226. Pop, O. L., Kerezsi, A. D., & Ciont, C. (2022). A Comprehensive Review of Moringa oleifera Bioactive Compounds—Cytotoxicity Evaluation and Their Encapsulation. *Foods*, *11*(23), 3787. https://doi.org/10.3390/foods11233787.

227. Premi, M., & Sharma, H. K. (2017). Effect of different combinations of maltodextrin, gum arabic and whey protein concentrate on the encapsulation behavior and oxidative stability of spray dried drumstick (Moringa oleifera) oil. *International journal of biological macromolecules*, *105*, 1232-1240. https://doi.org/10.1016/j.ijbiomac.2017.07.160.

228. Quintanilha, G. E. O., Baptista, A. T. A., Gomes, R. G., & Vieira, A. M. S. (2021). Yogurt production added ultrafiltered seed extract of Moringa oleifera Lam. *Biocatalysis and Agricultural Biotechnology*, *37*, 102159. https://doi.org/10.1016/j.bcab.2021.102159.

229. Rai, R. R. G., Mehta, S. D., Pandey, S. M. V., & Shukla, R. (2022). Design, development and evaluation of nutraceutical tablet Moringa oleifera. *Advance Pharmaceutical Journal.*

230. Rai, S. K., Pal, A. K., Pandey, J., & Dayal, A. (2023). Moringa. *Indian Farming*, *73*(09), 21-24.

231. Rajalakshmi, R., Rajalakshmi, S., & Parida, A. (2017). Evaluation of the genetic diversity and population structure in drumstick (Moringa oleifera L.) using SSR markers. *Current Science*, 1250-1256.

232. Reddy, S. H., Al-Neeri, I. S., Al-Issaei, H. K., & Al-Jabri, S. M. (2015). Effect of selective medicinal plant extract on blood glucose, sperm shape and various physiological parameters. *American Journal of Plant Sciences*, *6*(08), 1109.

233. Rockwood, J. L., Anderson, B. G., & Casamatta, D. A. (2013). Potential uses of Moringa oleifera and an examination of antibiotic efficacy conferred by Moringa oleifera seed and leaf extracts using crude extraction techniques available to

underserved indigenous populations. *International Journal of Phytotherapy Research*, *3*(2), 61-71.

234. Rongpipi, S., Ye, D., Gomez, E. D., & Gomez, E. W. (2019). Progress and opportunities in the characterization of cellulose–an important regulator of cell wall growth and mechanics. *Frontiers in plant science*, *9*, 1894. https://doi.org/10.3389/fpls.2018.01894.

235. Rutz, A., Sorokina, M., Galgonek, J., Mietchen, D., Willighagen, E., Gaudry, A. and Allard, P. M. (2021). The LOTUS initiative for open natural products research: knowledge management through Wikidata. *BioRxiv*.

236. Saeed, M. S., Saeed, A., Iqbal, M., & Adnan, M. (2020). Nutritional and Medicinal Importance of Moringa–A Review. *Curr. Rese. Agri. Far*, *1*(4), 31-34. DOI: http://dx.doi.org/10.18782/2582-7146.124.

237. Saeed, M., Ali, S. W., & Ramzan, S. (2021). Physicochemical analysis of mango flavored yogurt supplemented with moringa oleifera leaf powder. *Journal of Food Science and Technology*, *58*(12), 4805-4814. https://doi.org/10.1007/s13197-021-05146-w.

238. Sah, B. N. P., Vasiljevic, T., McKechnie, S., & Donkor, O. N. (2015). Effect of refrigerated storage on probiotic viability and the production and stability of antimutagenic and antioxidant peptides in yogurt supplemented with pineapple peel. *Journal of Dairy Science*, *98*(9), 5905-5916. https://doi.org/10.3168/jds.2015-9450.

239. Sahan, N. U. R. A. Y., Yasar, K., & Hayaloglu, A. A. (2008). Physical, chemical and flavour quality of non-fat yogurt as affected by a β-glucan hydrocolloidal composite during storage. *Food hydrocolloids*, *22*(7), 1291-1297. https://doi.org/10.1016/j.foodhyd.2007.06.010.

240. Sahay, S., Yadav, U., & Srinivasamurthy, S. (2017). Potential of Moringa oleifera as a functional food ingredient: A review. *Magnesium (g/kg)*, *8*(9.06), 4-90.

241. Salem, M. Z., Ali, H. M., & Akrami, M. (2021). Moringa oleifera seeds-removed ripened pods as alternative for papersheet production: Antimicrobial activity and their phytoconstituents profile using HPLC. *Scientific Reports*, *11*(1), 19027.

242. Salman, T. M., Olayaki, L. A., & Oyeyemi, W. A. (2008). Aqueous extract of Telfairia occidentalis leaves reduces blood sugar and increases haematological and reproductive indices in male rats. *African Journal of Biotechnology*, *7*(14).

243. Sandeep, G., Anitha, T., Vijayalatha, K. R., & Sadasakthi, A. (2019). Moringa for nutritional security (Moringa oleifera Lam.). *International Journal of Botany Studies*, *4*(1), 21-24.

244. Sankhalkar, S., & Vernekar, V. (2016). Quantitative and Qualitative analysis of Phenolic and Flavonoid content in Moringa oleifera Lam and Ocimum tenuiflorum L. *Pharmacognosy research*, 8(1), 16. doi: 10.4103/0974-8490.171095.

245. Santos, M. S., Estevinho, L. M., de Carvalho, C. A. L., da Silva Conceição, A. L., & de Castro Almeida, R. C. (2020). Rheological and sensorial evaluation of yogurt incorporated with red propolis. *Journal of food science and technology*, *57*, 1080-1089. DOI: 10.1007/s13197-019-04142-5.

246. Santoso, A., Retnosari, R., Pramudita, V., Wijaya, A. R., Anisa, T. N., & Sanjaya, E. H. (2021, May). The effect of starter concentration and incubation time on yogurt characteristics. In *AIP Conference Proceedings* (Vol. 2353, No. 1, p. 030080). AIP Publishing LLC. https://doi.org/10.1063/5.0052694.

247. Savaiano, D. A., & Hutkins, R. W. (2021). Yogurt, cultured fermented milk, and health: A systematic review. *Nutrition reviews*, *79*(5), 599-614. DOI: 10.1093/nutrit/nuaa013.

248. Schlick-Hasper, E., Bethke, J., Vogler, N., & Goedecke, T. (2022). Flow properties of powdery or granular filling substances of dangerous goods packagings—Comparison of the measurement of the angle of repose and the determination of the Hausner ratio. *Packaging Technology and Science*, *35*(10), 765-782. https://doi.org/10.1002/pts.2678.

249. Sehrawat, R., Rathee, P., Akkol, E. K., Khatkar, S., Lather, A., Redhu, N. and Khatkar, A. (2022). Phenolic Acids-Versatile Natural Moiety with Numerous Biological Applications. *Current Topics in Medicinal Chemistry*. 22:1472-1484.

250. Seo, M. H., Lee, S. Y., Chang, Y. H., & Kwak, H. S. (2009). Physicochemical, microbial, and sensory properties of yogurt supplemented with nanopowdered chitosan during storage. *Journal of Dairy Science*, *92*(12), 5907-5916. https://doi.org/10.3168/jds.2009-2520.

251. Shady NH, Mostafa NM, Fayez S, Abdel-Rahman IM, Maher SA, Zayed A, Saber EA, Khowdiary MM, Elrehany MA, Alzubaidi MA, Altemani FH (2022) Mechanistic Wound Healing and Antioxidant Potential of *Moringa oleifera* Seeds Extract Supported by Metabolic Profiling, In Silico Network Design, Molecular Docking, and In Vivo Studies. Antioxidants 11(9):1743S

252. Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of functional foods*, *18*, 757-781. https://doi.org/10.1016/j.jff.2015.01.047.

253. Shailaja, B., Mishra, I., Gampala, S., Singh, V. J., & Swathi, K. (2014). Panchagavya-an ecofriendly insecticide and organic growth promoter of plants. *International Journal of Advanced Research*, *2*(11), 22-26.

254. Shanmugavel, G., Prabakaran, K., & George, B. (2018). Evaluation of phytochemical constituents of Moringa oleifera (Lam.) leaves collected from Puducherry region, South India. *Int J Zool Appl Biosci*, 3(1), 1-8. https://doi.org/10.5281/zenodo.1312977.

255. Sharma, K., Kumar, M., Waghmare, R., Suhag, R., Gupta, O. P., Lorenzo, J. M., ... & Kennedy, J. F. (2022). Moringa (Moringa oleifera Lam.) polysaccharides: Extraction, characterization, bioactivities, and industrial application. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2022.04.047.

256. Sharma, M., Mridula, D., Yadav, D. N., & Gupta, R. K. (2015). Physico-chemical characteristics of maize and sorghum as affected by popping. Proceedings of the National Academy of Sciences, India Section B: *Biological Sciences*, 85(3), 787-792.

257. Sharma, V., & Paliwal, R. (2013). Isolation and characterization of saponins from Moringa oleifera (moringaceae) pods. *Int J Pharm Pharm Sci*, *5*(1), 179-183.

258. Shashiraj, K. N., Hugar, A., Kumar, R. S., Rudrappa, M., Bhat, M. P., Almansour, A. I., ... & Nayaka, S. (2023). Exploring the antimicrobial, anticancer, and apoptosis inducing ability of biofabricated silver nanoparticles using Lagerstroemia speciosa flower buds against the Human Osteosarcoma (MG-63) cell line via flow cytometry. *Bioengineering*, *10*(7), 821. https://doi.org/10.3390/bioengineering10070821.

259. Shikwambana, N., & Mahlo, S. M. (2020). A survey of antifungal activity of selected south African plant species used for the treatment of skin infections. *Natural Product Communications*, *15*(5), 1934578X20923181. DOI: 10.1177/1934578X20923181.

260. Shokery, E. S., El-Ziney, M. G., Yossef, A. H., & Mashaly, R. I. (2017). Effect of green tea and Moringa leave extracts fortification on the physicochemical, rheological, sensory and antioxidant properties of set-type yoghurt. *J Adv Dairy Res*, *5*(179), 2. DOI: 10.4172/2329-888X.1000179.

261. Simon, S., Joseph, J., & George, D. (2022). Optimization of extraction parameters of bioactive components from Moringa oleifera leaves using Taguchi method. *Biomass Conversion and Biorefinery*, 1-10.

262. Singh, A. K., Rana, H. K., Tshabalala, T., Kumar, R., Gupta, A., Ndhlala, A. R., & Pandey, A. K. (2020). Phytochemical, nutraceutical and pharmacological attributes of a functional crop Moringa oleifera Lam: An overview. *South African Journal of Botany*, *129*, 209-220. https://doi.org/10.1016/j.sajb.2019.06.017.

263. Singh, B., Singh, J. P., Kaur, A., & Singh, N. (2017). Phenolic compo-sition and antioxidant potential of grain legume seeds: A review. *Food research international*, 101, 1-16. FAO. https://doi.org/10.1016/j.foodres.2017.09.026.

264. Singh, J., Rasane, P., Kaur, S., & Nanda, V. (2022). Comparative analysis of antioxidant potential and techno-functional properties of selected corn silk varieties at different developmental stages. *Journal of Food Measurement and Characterization*, *16*(4), 2685-2698. https://doi.org/10.1007/s11694-022-01382-6.

265. Smith, E. L., Abbott, A. P., & Ryder, K. S. (2014). Deep eutectic solvents (DESs) and their applications. *Chemical reviews*, *114*(21), 11060-11082. DOI: 10.1021/cr300162p.

266. Spinella M (2002) The importance of pharmacological synergy in psychoactive herbal medicines. Alternative Medicine Review 7(2): 130-137

267. Stalikas, C. D. (2007). Extraction, separation, and detection methods for phenolic acids and flavonoids. *Journal of separation science*, *30*(18), 3268-3295. https://doi.org/10.1002/jssc.200700261.

268. Subramaniam, D. N., & Sathiparan, N. (2022). Comparative study of fly ash and rice husk ash as cement replacement in pervious concrete: mechanical characteristics and sustainability analysis. *International Journal of Pavement Engineering*, 1-18. https://doi.org/10.1080/10298436.2022.2075867.

269. Sultana, S., (2020). Nutritional and functional properties of Moringa oleifera. Metabolism open, 8, 100061. https://doi.org/10.1016/j.metop.2020.100061.

270. Sun, J., Chang, C., Su, Y., Gu, L., Yang, Y., & Li, J. (2022). Impact of saccharides on the foam properties of egg white: Correlation between rheological, interfacial properties and foam properties. *Food Hydrocolloids*, *122*, 107088. https://doi.org/10.1016/j.foodhyd.2021.107088.

271. Suryawanshi, V. S., & Umate, S. R. (2018). Phytochemical Screening of Flowers from Moringa oleifera Lam. *Plantae Scientia*, *1*(01), 31-35. https://doi.org/10.32439/ps.v1i01.31-35.

272. Sutakwa, A., Nadia, L. S., & Suharman, S. (2021). Addition of blue pea flower (Clitoria ternatea L.) extract increase antioxidant activity in yogurt from various types of milk. *Jurnal Agercolere*, *3*(1), 31-37. https://doi.org/10.37195/jac.v3i1.123.

273. Sutalangka, C., Wattanathorn, J., Muchimapura, S., & Thukham-mee, W. (2013). Moringa oleifera mitigates memory impairment and neurodegeneration in an animal model of age-related dementia. *Oxidative medicine and cellular longevity*, *2013*. https://doi.org/10.1155/2013/695936.

274. Swamy, G. M., & Meriga, B. (2020). Therapeutic effect of Moringa Oleifera leaf extracts on oxidative stress and key metabolic enzymes related to obesity. *Int J Pharm Sci Res*, *11*, 1949-57. DOI: 10.13040/IJPSR.0975-8232.11(4).1949-57.

275. Talath, S., Wali, A. F., & Menezes, G. A., (2022). Ultra-sound-assisted extraction, anti-oxidant, cytotoxic and antimicrobial studies of Moringa oleifera flowers. *Pakistan Journal of Pharmaceutical Sciences,* 35(4). DOI: 10.36721/PJPS.2022.35.4.REG.1109-1116.1.

276. Tang, Y., Choi, E. J., Han, W. C., Oh, M., Kim, J., Hwang, J. Y., ... & Kim, E. K. (2017). Moringa oleifera from Cambodia ameliorates oxidative stress, hyperglycemia, and kidney dysfunction in type 2 diabetic mice. *Journal of Medicinal Food*, *20*(5), 502-510. https://doi.org/10.1089/jmf.2016.3792.

277. Teixeira, E. M. B., Carvalho, M. R. B., Neves, V. A., Silva, M. A., & Arantes-Pereira, L. (2014). Chemical characteristics and fractionation of proteins from Moringa oleifera Lam. leaves. *Food chemistry*, *147*, 51-54. https://doi.org/10.1016/j.foodchem.2013.09.135.

278. Thirumal, Y., & Laavu, S. (2017). HPLC profile of medicinal plant extracts and its application in aquaculture. *J Aquacult Res Dev*, *8*(484), 2-6. DOI: 10.4172/2155-9546.1000484.

279. Thomas, P. (2016) Export Market for Moringa Products from South India Growing by Leaps and Bounds. The New Indian Express, published: 10th March.

280. Tien Lea, D., Duc Chua, H., & Quynh Lea, N. (2016). Improving the nutritional quality of plant proteins through genetic engineering. *Current genomics*, 17(3), 220-229.

281. Torres-Castillo, J. A., Sinagawa-García, S. R., Martínez-Ávila, G. C. G., López-Flores, A. B., Sánchez-González, E. I., Aguirre-Arzola, V. E., ... & Gutiérrez-Díez, A. (2013). Moringa oleifera: phytochemical detection, antioxidants, enzymes and antifugal properties. *International Journal of Experimental Botany*, *82*, 193-202.

282. Udechukwu, M. C., Abbey, L., Nwodo, U., & Udenigwe, C. C. (2018). Potential of Moringa oleifera seeds and leaves as functional food ingredients for human health promotion. *Journal of Food & Nutrition Research*, *57*(1).

283. Ünlü, A. E., Arıkaya, A., & Takaç, S. (2019). Use of deep eutectic solvents as catalyst: A mini-review. *Green Processing and Synthesis*, *8*(1), 355-372. https://doi.org/10.1515/gps-2019-0003.

284. Valcarcel, M. (2012). Analytical Chemistry Today and Tomorrow, *Analytical Chemistry*, 93-114.

285. Van Tienen, A.; Hullegie, Y.; Hummelen, R.; Hemsworth, J.; Changalucha, J.; Reid, G. Development of a locally sustainable functional food for people living with HIV in Sub-Saharan Africa: Laboratory testing and sensory evaluation. Benef. Microbes 2011, 2, 193–198.

286. Vangoori, Y., Dakshinamoorthi, A., & Kavimani, S. (2019). Prominent pancreatic lipase inhibition and free radical scavenging activity of a myristica fragrans ethanolic extract in vitro. Potential role in obesity treatment. *Maedica*, *14*(3), 254. doi: 10.26574/maedica.2019.14.3.254.

287. Vasanth, K., Minakshi, G. C., Velu, K., Priya, T., Kumar, R. M., Kaliappan, I. and Dubey, G. P. (2022). Anti-adipogenic β-sitosterol and lupeol from Moringa oleifera suppress adipocyte differentiation through regulation of cell cycle progression. *Journal of Food Biochemistry*. 46:e14170.

288. Ventura, S. P., e Silva, F. A., Quental, M. V., Mondal, D., Freire, M. G., & Coutinho, J. A. (2017). Ionic-liquid-mediated extraction and separation processes for bioactive compounds: past, present, and future trends. *Chemical Reviews*, *117*(10), 6984-7052. http://dx.doi.org/10.1021/acs.chemrev.1026b00550.

289. Verma, A., Singh, D., & Mitra, A. (2022). Development of gluten-free energy bar and its proximate analysis. *The Pharma Innovation Journal*, 11(6), 569-576.

290. Verma, V. K., Singh, N., Saxena, P., & Singh, R. (2012). Anti-ulcer and antioxidant activity of Moringa oleifera (Lam) leaves against aspirin and ethanol induced gastric ulcer in rats. *Int Res J Pharm*, *2*(2), 46-57.

291. Vian, M., Breil, C., Vernes, L., Chaabani, E., Chemat, F. (2017). Green solvents for sample preparation in analytical chemistry. *Current Opinion in Green and Sustainable Chemistry*, 5, 44-48.

292. Vijay, L., Karthikeyan, N., Kumaresan, G., & Kathirvelan, C. (2022). Sensory properties of functional Greek yoghurt incorporated with Moringa oleifera leaf powder. *The Pharma Innovation Journal*, *11*(4), 251-254.

293. Villarruel-López, A., López-De La Mora, D. A., Vázquez-Paulino, O. D., Puebla-Mora, A. G., Torres-Vitela, M. R., Guerrero-Quiroz, L. A., & Nuño, K. (2018). Effect of Moringa oleifera consumption on diabetic rats. *BMC complementary and alternative medicine*, *18*(1), 1-10.

294. Virk, P., Awad, M. A., Alsaif, S. S. A. L., Hendi, A. A., Elobeid, M., Ortashi, K., ... & Salama, H. A. (2023). Green synthesis of Moringa oleifera leaf nanoparticles and an assessment of their therapeutic potential. *Journal of King Saud University-Science*, *35*(3), 102576. https://doi.org/10.1016/j.jksus.2023.102576.

295. Vonghirundecha, P., Chusri, S., Meunprasertdee, P., & Kaewmanee, T. (2022). Microencapsulated functional ingredients from a Moringa oleifera leaf polyphenol-rich extract: Characterization, antioxidant properties, in vitro simulated digestion, and storage stability. *LWT*, *154*, 112820. https://doi.org/10.1016/j.lwt.2021.112820.

296. Wali, A. F., Alchamat, H. A. A., Hariri, H. K., Hariri, B. K., Menezes, G. A., Zehra, U. and Ahmad, P. (2020). Antioxidant, antimicrobial, antidiabetic, and cytotoxic activity of Crocus sativus L. petals. *Applied Sciences*. 10:1519.

297. Wali, A. F., Hamad, E. A., Khazandar, A. A., Al-Azzawi, A. M., Sarheed, O. A., Menezes, G. A. and Alam, A. (2019). Antimicrobial and in vitro antioxidant activity of Salvia officinalis L. against various re-emergent multidrug resistance microbial pathogens. *Annals of Phytomedicine*. 8:115-120.

298. Wan Mahmood, W. M. A., Lorwirachsutee, A., Theodoropoulos, C., & Gonzalez-Miquel, M. (2019). Polyol-based deep eutectic solvents for extraction of natural polyphenolic antioxidants from Chlorella vulgaris. *ACS Sustainable Chemistry & Engineering*, *7*(5), 5018-5026. https://doi.org/10.1021/acssuschemeng.8b05642.

299. Wang, F., Bao, Y., Zhang, C., Zhan, L., Khan, W., Siddiqua, S., & Xiao, J. (2022). Bioactive components and anti-diabetic properties of Moringa oleifera Lam. *Critical Reviews in Food Science and Nutrition*, *62*(14), 3873-3897. https://doi.org/10.1080/10408398.2020.1870099.

300. Wang, L., Luo, Y., Wu, Y., Liu, Y., & Wu, Z. (2018). Fermentation and complex enzyme hydrolysis for improving the total soluble phenolic contents, flavonoid aglycones contents and bio-activities of guava leaves tea. *Food Chemistry*, *264*, 189-198. https://doi.org/10.1016/j.foodchem.2018.05.035.

301. Wang, M., Wang, J., Zhang, Y., Xia, Q., Bi, W., Yang, X., & Chen, D. D. Y. (2016). Fast environment-friendly ball mill-assisted deep eutectic solvent-based extraction of natural products. *Journal of Chromatography A*, *1443*, 262-266. https://doi.org/10.1016/j.chroma.2016.03.061.

302. Wang, N., Kuczmanski, A., Dubrovska, G., & Gollasch, M. (2018). Palmitic acid methyl ester and its relation to control of tone of human visceral arteries and rat aortas by perivascular adipose tissue. *Frontiers in physiology*, *9*, 583. https://doi.org/10.3389/fphys.2018.00583.

303. Wang, T., Jiao, J., Gai, Q. Y., Wang, P., Guo, N., Niu, L. L., & Fu, Y. J. (2017). Enhanced and green extraction polyphenols and furanocoumarins from Fig (Ficus carica L.) leaves using deep eutectic solvents. *Journal of pharmaceutical and biomedical analysis*, *145*, 339-345. https://doi.org/10.1016/j.jpba.2017.07.002.

304. Wang, X., Kristo, E., & LaPointe, G. (2020). Adding apple pomace as a functional ingredient in stirred-type yogurt and yogurt drinks. *Food Hydrocolloids*, *100*, 105453. DOI: 10.1016/j.foodhyd.2019.105453.

305. Weerathilake, W. A. D. V., Rasika, D. M. D., Ruwanmali, J. K. U., & Munasinghe, M. A. D. D. (2014). The evolution, processing, varieties and health benefits of yogurt. *International Journal of Scientific and Research Publications*, *4*(4), 1-10.

306. Wei, P., Zhang, Y., Wang, Y. Y., Dong, J. F., Liao, B. N., Su, Z. C., ... & Peng, C. (2023). Efficient extraction, excellent activity, and microencapsulation of flavonoids from Moringa oleifera leaves extracted by deep eutectic solvent. *Biomass Conversion and Biorefinery*, 1-15. https://doi.org/10.1007/s13399-023-03877-8.

307. Weimann, E., Silva, M.B.B., Murata, G.M., Bortolon, J.R., Dermargos, A., Curi, R. and Hatanaka, E. (2018). Topical anti-inflammatory activity of palmitoleic acid improves wound healing. *PLoS One*, 13(10), p.e0205338. doi: 10.1371/journal.pone.0205338.

308. Wong, S. E., Illingworth, K. A., & Siow, L. F. (2024). Moringa Proteins: Nutrition, Functionality, and Applications. In *Sustainable Protein Sources* (pp. 493-513). Academic Press.

309. Wu, L., Li, L., Chen, S., Wang, L., & Lin, X. (2020). Deep eutectic solvent-based ultrasonic-assisted extraction of phenolic compounds from Moringa oleifera L. leaves: Optimization, comparison and antioxidant activity. *Separation and Purification Technology*, *247*, 117014. https://doi.org/10.1016/j.seppur.2020.117014.

310. Yamamoto, N., Ueda-Wakagi, M., Sato, T., Kawasaki, K., Sawada, K., Kawabata, K., & Ashida, H. (2015). Measurement of glucose uptake in cultured cells. *Current protocols in pharmacology*, *71*(1), 12-14. https://doi.org/10.1002/0471141755.ph1214s71.

311. Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B. and Li, M. (2021). Research progress on antibacterial activities and mechanisms of natural alkaloids: A review. *Antibiotics*. 10:318.

312. Yang, S., Yan, D., Zou, Y., Mu, D., Li, X., Shi, H., Lou, X., Yang, M., Yue, X., Wu, R., & Wu, J. (2021). Fermentation temperature affects yogurt quality: A metabolomics study. *Food Bioscience*, *42*, 101104. DOI: 10.1016/j.fbio.2021.101104.

313. Zainal-Abidin, M. H., Hayyan, M., Hayyan, A., & Jayakumar, N. S. (2017). New horizons in the extraction of bioactive compounds using deep eutectic solvents: A review. *Analytica Chimica Acta*, *979*, 1-23. https://doi.org/10.1016/j.aca.2017.05.012.

314. Zaku, S. G., Emmanuel, S., Tukur, A. A., & Kabir, A. (2015). Moringa oleifera: An underutilized tree in Nigeria with amazing versatility: A review. *African Journal of Food Science*, *9*(9), 456-461.

315. Zarrinmehr, M. J., Daneshvar, E., Nigam, S., Gopinath, K. P., Biswas, J. K., Kwon, E. E., & Bhatnagar, A. (2022). The effect of solvents polarity and extraction conditions on the microalgal lipids yield, fatty acids profile, and biodiesel properties. *Bioresource Technology*, *344*, 126303. https://doi.org/10.1016/j.biortech.2021.126303.

316. Zhang, Q., Vigier, K. D. O., Royer, S., & Jérôme, F. (2012). Deep eutectic solvents: syntheses, properties, and applications. *Chemical Society Reviews*, *41*(21), 7108-7146. doi:10.1039/C2CS35178A.

317. Zhang, T., Jeong, C. H., Cheng, W. N., Bae, H., Seo, H. G., Petriello, M. C., & Han, S. G. (2019). Moringa extract enhances the fermentative, textural, and bioactive properties of yogurt. *LWT*, *101*, 276-284. DOI: 10.1016/j.lwt.2018.11.010.

318. Zhao, X., Fan, X., Shao, X., Cheng, M., Wang, C., Jiang, H., ... & Yuan, C. (2022). Modifying the physicochemical properties, solubility and foaming capacity of milk

proteins by ultrasound-assisted alkaline pH-shifting treatment. *Ultrasonics Sonochemistry*, *88*, 106089. https://doi.org/10.1016/j.ultsonch.2022.106089.

319. Zhou, J., Zhang, L., Li, Q., Jin, W., Chen, W., Han, J., & Zhang, Y. (2018). Simultaneous optimization for ultrasound-assisted extraction and antioxidant activity of flavonoids from Sophora flavescens using response surface methodology. *Molecules*, *24*(1), 112. https://doi.org/10.3390/molecules24010112.

APPENDICES

Characteristic IR Absorption Frequencies of Organic Functional Groups

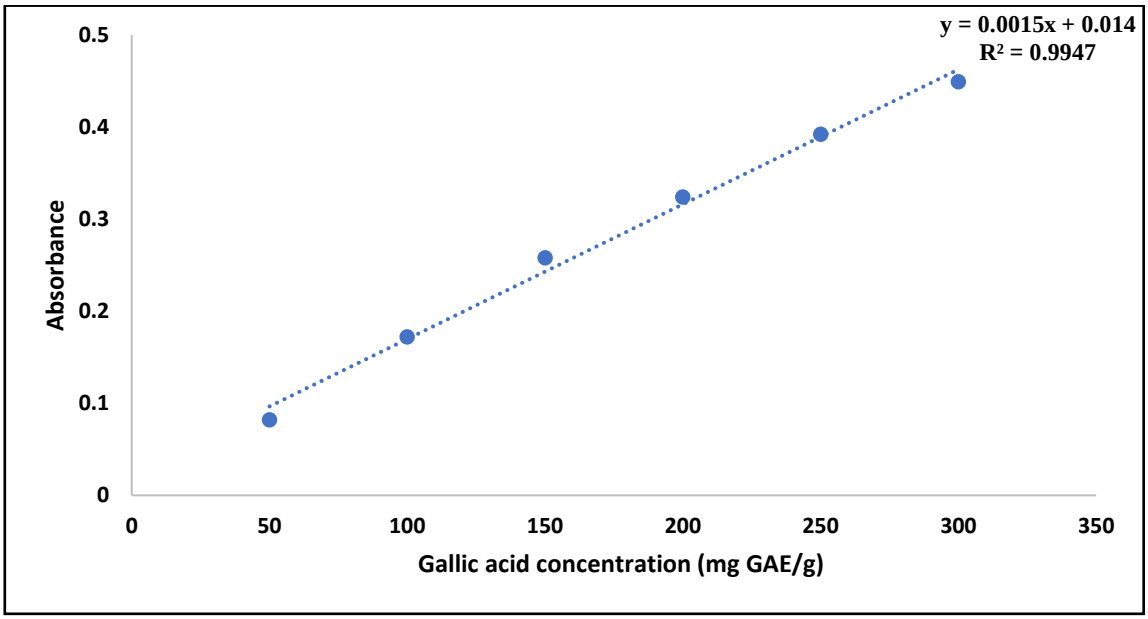
Functional Group	Type of Vibration	Characteristic Absorptions (cm-1)	Intensity
Alcohol			
O-H	(stretch, H-bonded)	3200-3600	strong, broad
O-H	(stretch, free)	3500-3700	strong, sharp
C-O	(stretch)	1050-1150	Strong
Alkane			
C-H	stretch	2850-3000	Strong
-C-H	bending	1350-1480	Variable
Alkene			
=C-H	stretch	3010-3100	Medium
=C-H	bending	675-1000	Strong
C=C	stretch	1620-1680	Variable
Alkyl Halide			
C-F	stretch	1000-1400	Strong
C-Cl	stretch	600-800	Strong
C-Br	stretch	500-600	Strong
C-I	stretch	500	Strong
Alkyne			
C-H	stretch	3300	strong, sharp
–C≡C–	stretch	2100-2260	variable, not present in symmetrical alkynes
Amine			
N-H	stretch	3300-3500	medium (primary amines have two bands; secondary have one band, often

			very weak)
C-N	stretch	1080-1360	medium-weak
N-H	bending	1600	Medium
Aromatic			
C-H	stretch	3000-3100	Medium
C=C	stretch	1400-1600	medium-weak, multiple bands
Analysis of C-H out-of-plane bending can often distinguish substitution patterns			
Carbonyl	Detailed Information on Carbonyl IR		
C=O	stretch	1670-1820	Strong
(Conjugation moves absorptions to lower wave numbers)			
Ether			
C-O	stretch	1000-1300 (1070-1150)	Strong
Nitrile			
CN	stretch	2210-2260	Medium
Nitro			
N-O	stretch	1515-1560 & 1345-1385	strong, two bands

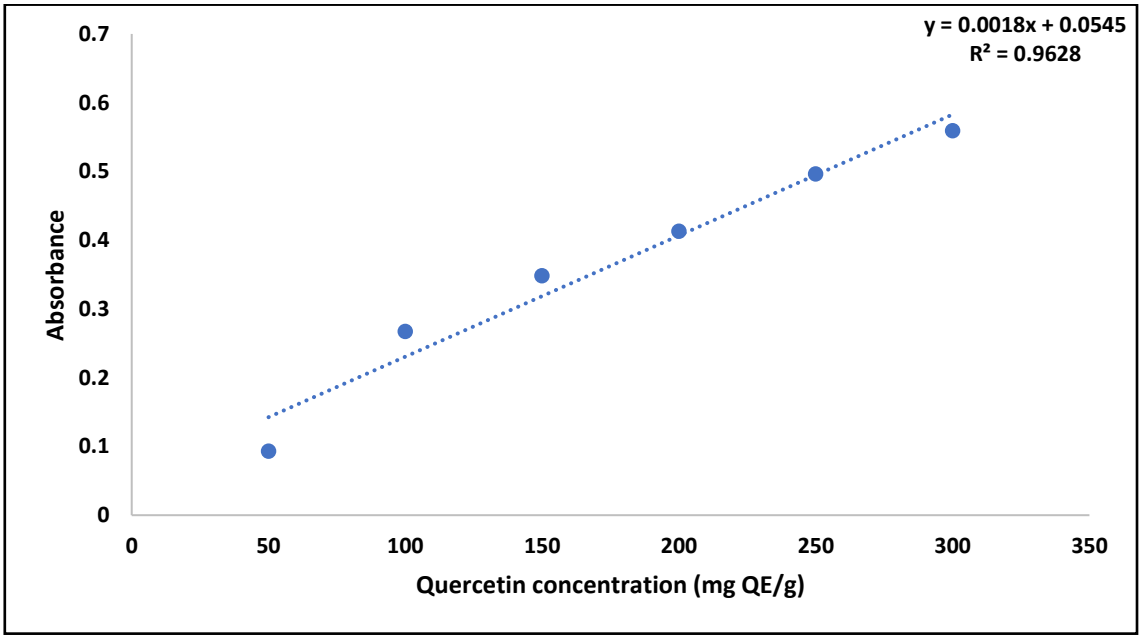
t- Table

cum. prob	$t_{.50}$	$t_{.75}$	$t_{.80}$	$t_{.85}$	$t_{.90}$	$t_{.95}$	$t_{.975}$	$t_{.99}$	$t_{.995}$	$t_{.999}$	$t_{.9995}$
one-tail	0.50	0.25	0.20	0.15	0.10	0.05	0.025	0.01	0.005	0.001	0.0005
two-tails	1.00	0.50	0.40	0.30	0.20	0.10	0.05	0.02	0.01	0.002	0.001
df											
1	0.000	1.000	1.376	1.963	3.078	6.314	12.71	31.82	63.66	318.31	636.62
2	0.000	0.816	1.061	1.386	1.886	2.920	4.303	6.965	9.925	22.327	31.599
3	0.000	0.765	0.978	1.250	1.638	2.353	3.182	4.541	5.841	10.215	12.924
4	0.000	0.741	0.941	1.190	1.533	2.132	2.776	3.747	4.604	7.173	8.610
5	0.000	0.727	0.920	1.156	1.476	2.015	2.571	3.365	4.032	5.893	6.869
6	0.000	0.718	0.906	1.134	1.440	1.943	2.447	3.143	3.707	5.208	5.959
7	0.000	0.711	0.896	1.119	1.415	1.895	2.365	2.998	3.499	4.785	5.408
8	0.000	0.706	0.889	1.108	1.397	1.860	2.306	2.896	3.355	4.501	5.041
9	0.000	0.703	0.883	1.100	1.383	1.833	2.262	2.821	3.250	4.297	4.781
10	0.000	0.700	0.879	1.093	1.372	1.812	2.228	2.764	3.169	4.144	4.587
11	0.000	0.697	0.876	1.088	1.363	1.796	2.201	2.718	3.106	4.025	4.437
12	0.000	0.695	0.873	1.083	1.356	1.782	2.179	2.681	3.055	3.930	4.318
13	0.000	0.694	0.870	1.079	1.350	1.771	2.160	2.650	3.012	3.852	4.221
14	0.000	0.692	0.868	1.076	1.345	1.761	2.145	2.624	2.977	3.787	4.140
15	0.000	0.691	0.866	1.074	1.341	1.753	2.131	2.602	2.947	3.733	4.073
16	0.000	0.690	0.865	1.071	1.337	1.746	2.120	2.583	2.921	3.686	4.015
17	0.000	0.689	0.863	1.069	1.333	1.740	2.110	2.567	2.898	3.646	3.965
18	0.000	0.688	0.862	1.067	1.330	1.734	2.101	2.552	2.878	3.610	3.922
19	0.000	0.688	0.861	1.066	1.328	1.729	2.093	2.539	2.861	3.579	3.883
20	0.000	0.687	0.860	1.064	1.325	1.725	2.086	2.528	2.845	3.552	3.850
21	0.000	0.686	0.859	1.063	1.323	1.721	2.080	2.518	2.831	3.527	3.819
22	0.000	0.686	0.858	1.061	1.321	1.717	2.074	2.508	2.819	3.505	3.792
23	0.000	0.685	0.858	1.060	1.319	1.714	2.069	2.500	2.807	3.485	3.768
24	0.000	0.685	0.857	1.059	1.318	1.711	2.064	2.492	2.797	3.467	3.745
25	0.000	0.684	0.856	1.058	1.316	1.708	2.060	2.485	2.787	3.450	3.725
26	0.000	0.684	0.856	1.058	1.315	1.706	2.056	2.479	2.779	3.435	3.707
27	0.000	0.684	0.855	1.057	1.314	1.703	2.052	2.473	2.771	3.421	3.690
28	0.000	0.683	0.855	1.056	1.313	1.701	2.048	2.467	2.763	3.408	3.674
29	0.000	0.683	0.854	1.055	1.311	1.699	2.045	2.462	2.756	3.396	3.659
30	0.000	0.683	0.854	1.055	1.310	1.697	2.042	2.457	2.750	3.385	3.646
40	0.000	0.681	0.851	1.050	1.303	1.684	2.021	2.423	2.704	3.307	3.551
60	0.000	0.679	0.848	1.045	1.296	1.671	2.000	2.390	2.660	3.232	3.460
80	0.000	0.678	0.846	1.043	1.292	1.664	1.990	2.374	2.639	3.195	3.416
100	0.000	0.677	0.845	1.042	1.290	1.660	1.984	2.364	2.626	3.174	3.390
1000	0.000	0.675	0.842	1.037	1.282	1.646	1.962	2.330	2.581	3.098	3.300
z	0.000	0.674	0.842	1.036	1.282	1.645	1.960	2.326	2.576	3.090	3.291
	0%	50%	60%	70%	80%	90%	95%	98%	99%	99.8%	99.9%
	Confidence Level										

STANDARD CURVE



Phenolic standard graph



Flavonoid standard curve

Descriptive Score Card for Yogurt

Date: **Storage time:**

Name of Penalist:

Objective: To develop and evaluate stirred yoghurt enriched with *Moringa oleifera* flower extract

A) Assign score for each sample for different characteristics

Attribute	Maximum Score	Sample score			
		1	2	3	4
Flavour	**45**				
Body & Texture	**30**				
Acidity	**10**				
Colour & Appearance	**10**				
Container & Closures	**5**				
Total	**100**				

B) Indicate the degree of defects such as the following. Encircle the one applicable and deduct from attributes

Attribute	Defect	Intensity of defect		
		Slight	**Definite**	**Pronounced**
Flavour	Highly acidic, bitter, metallic, yeasty, cheesy	**7** **10**	**9** **13**	**11** **16**
Body and texture	Grainy, thin body, ropy and wheying off	**2** **4**	**5** **8**	**8** **12**
Acidity	Too low, too high	**1**	**3**	**5**
Colour and appearance	Unnatural colour, presence of foreign matter	**1** **2**	**3** **4**	**5** **6**
Container and closure	Soiled, improperly covered	**1**	**2**	**3**

Comments:

Signature:

PLAGIARISM REPORT BY TURNITIN

Thesis

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LIST OF PUBLICATIONS

Sr. No.	Title of paper	Name of journal	Publication date	ISSN no./Vol no., issue no.	Indexing in Scopus/ Web of science/ UGC-CARE list
1.	"Studies on Phytochemical Properties and Antibacterial Activity of Moringa Oleifera Flower Extract"	Annals of Biology	Accepted April 26th, 2023	ISSN: 0970-0153, Volume 36 number 2 of December 2023	Scopus, Web of science, UGC-CARE list
2.	"Structural, thermal, techno-functional and chemical characterization using Fourier Transform Infrared Spectroscopy, Gas-Chromatography-Mass Spectrophotometry, Thermogravimetric Analyser, Field Emission Scanning Electron Microscopy and Energy-Dispersive X-Ray Spectrometer of *Moringa Oleifera* flower powder"	International Journal of Food Science and Technology	Published, September 8th, 2023	ISSN: 09505423, 13652621 Vol 58, Issue 11	Scopus indexed

3.	"A critical review on Moringa oleifera: Current status, physicochemical attributes, and food industrial applications"	Natural Product Research	Corresponded to Journal	-	Scopus indexed
4.	"Optimization of the extraction process of *Moringa oleifera* flower by using Deep Eutectic Solvents (DES)"	Journal of applied research on Medicinal and Aromatic Plants	Corresponded to Journal	-	Scopus indexed
5.	"Development of novel herbal yogurt enriched with Moringa oleifera flower extract and assessment of antioxidant activity, sensory analysis, and storage stability".	Journal of Food Science and Technology	Corresponded to Journal	-	Scopus indexed

LIST OF CONFERENCES

S. No.	Name of the conference	Organized by	Title of the oral presentation	Date of conference
1.	"Global Initiatives in Research, Innovation and Sustainable Development of Agriculture and Allied Sciences"	"Agro Environmental Education and Farmer's Welfare Society (AEEFWS), Punjab, Guru Kashi University, Bathinda"	Optimization of the extraction process of *Moringa oleifera* flower by using Deep Eutectic Solvents (DESs)	June 6-8th, 2022
2.	"Innovative Food System Transformations for Sustainable Development in Agro-Food and Nutrition Sector"	'Vignan's Foundation for Science, Technology and Research (VFSTR), Guntur and University of Malaysia, Kelantan"	Techno-functional Properties of *Moringa oleifera* Flower Powder	November 16th-17th, 2022
3.	"6th INTERNATIONAL CONFERENCE ON ADVANCES IN AGRICULTURE TECHNOLOGY AND ALLIED SCIENCES (ICAATAS 2023)"	"Society of Agriculture Research and Social Development (New Delhi) & Loyola Academy, Telangana"	Optimization of Moringa oleifera Flower Extract Enriched Yogurt Using Response Surface Methodology	April 13th - 14th, 2023

International Journal of Food Science +Technology

Original article

Structural, thermal, techno-functional and chemical characterization using Fourier Transform Infrared Spectroscopy, Gas-Chromatography-Mass Spectrophotometry, Thermogravimetric Analyser, Field Emission Scanning Electron Microscopy and Energy-Dispersive X-Ray Spectrometer of *Moringa Oleifera* flower powder

Poonam Jaglan, Deepika Kaushik, Mukul Kumar, Ashwani Kumar, Jasjit Kaur, Emel Oz, Charles Brennan, Charalampos Proestos, Margaret Brennan, Naushad Ahmad, Tahra Elobeid, Fatih Oz

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Summary

The aim of this research is to characterise the physicochemical and techno-functional properties of the *Moringa oleifera* flower powder, and various techniques were used in this regard, such as FTIR, GC–MS, TGA, FESEM, and EDS. The bulk and tapped density of the *Moringa oleifera* flower powder were found to be 0.44 ± 0.03 (g/cm^3) and 0.51 ± 0.01 (g/cm^3), respectively. The water absorption index and water solubility index were 10.60 ± 0.10 (g/g) and 8.70 ± 0.20 (%), respectively. The foaming capacity was found to be 15.20 ± 0.20 (%), and the foaming stability was 56.34 ± 0.10 (%). The total phenolic content of the *Moringa oleifera* flower extract was 15.97 ± 0.223 mg GAE/g, whereas the flavonoid content was 12.38 ± 0.321 mg QE/g. The antioxidant activity of the flower extract was measured by DPPH assay and was found to be 59.65%. The flower extract showed 11 mm and 8 mm of the zone of inhibition against *Escherichia coli* and *Staphylococcus aureus*, respectively, whereas, with antibiotic streptomycin, the zones of inhibition were found to be 20 and 18 mm, respectively. Based on the research findings, *Moringa oleifera* flower powder and extract can be used as food fortification, antioxidant, and antibacterial agents.

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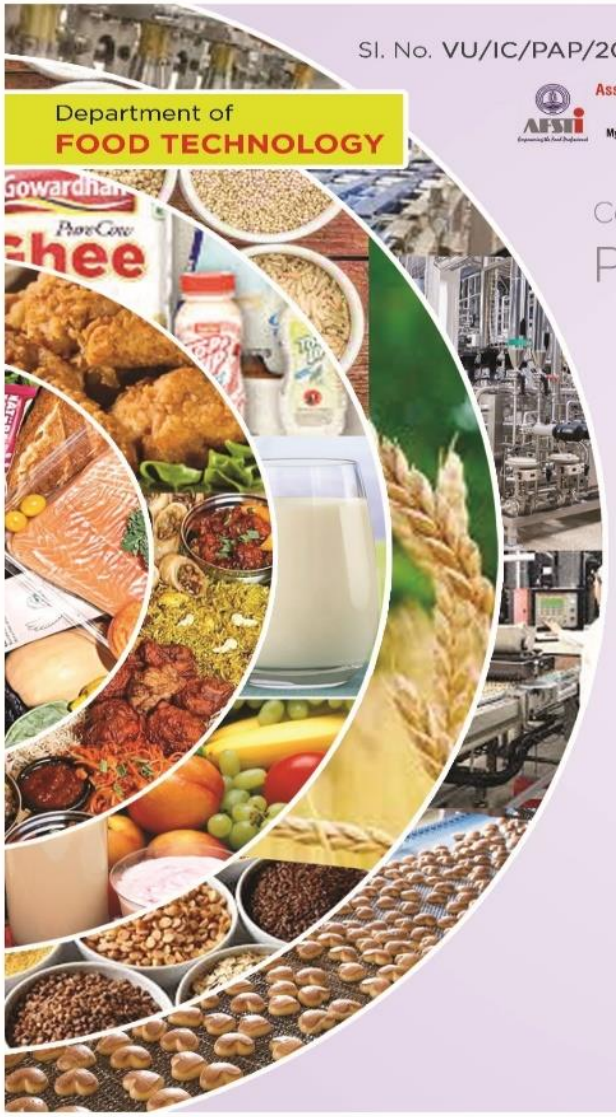

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This is to certify that ~~Mr./~~Ms./~~Dr.~~ Poonam Jaglan has participated as **Delegate/Oral Presenter/Poster Presenter** in 6th AMIFOST 2023 on **Multidisciplinary Approach for Healthy and Sustainable Foods** held at Amity Institutes of Food Technology, Amity University Uttar Pradesh, Noida during 13-14th April 2023.

Title of Abstract "Optimization of Moringa oleifera flower extract enriched yogurt using Response Surface Methodology."

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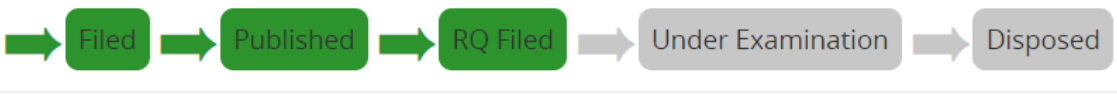
INTELLECTUAL PROPERTY INDIA
PATENTS | DESIGNS | TRADE MARKS | GEOGRAPHICAL INDICATIONS

Application Details	
APPLICATION NUMBER	202211050481
APPLICATION TYPE	ORDINARY APPLICATION
DATE OF FILING	05/09/2022
APPLICANT NAME	**Lovely Professional University**
TITLE OF INVENTION	A NOVEL COMPOSITION OF YOGHURT CONTAINING FLOWER EXTRACT OF MORINGA OLEIFERA AND PROCESS THEREOF
FIELD OF INVENTION	MECHANICAL ENGINEERING
E-MAIL (As Per Record)	dip@lpu.co.in
ADDITIONAL-EMAIL (As Per Record)	dip@lpu.co.in
E-MAIL (UPDATED Online)	
PRIORITY DATE	
REQUEST FOR EXAMINATION DATE	25/03/2023
PUBLICATION DATE (U/S 11A)	09/09/2022

Application Status	
APPLICATION STATUS	**Application Awaiting Examination**

View Documents



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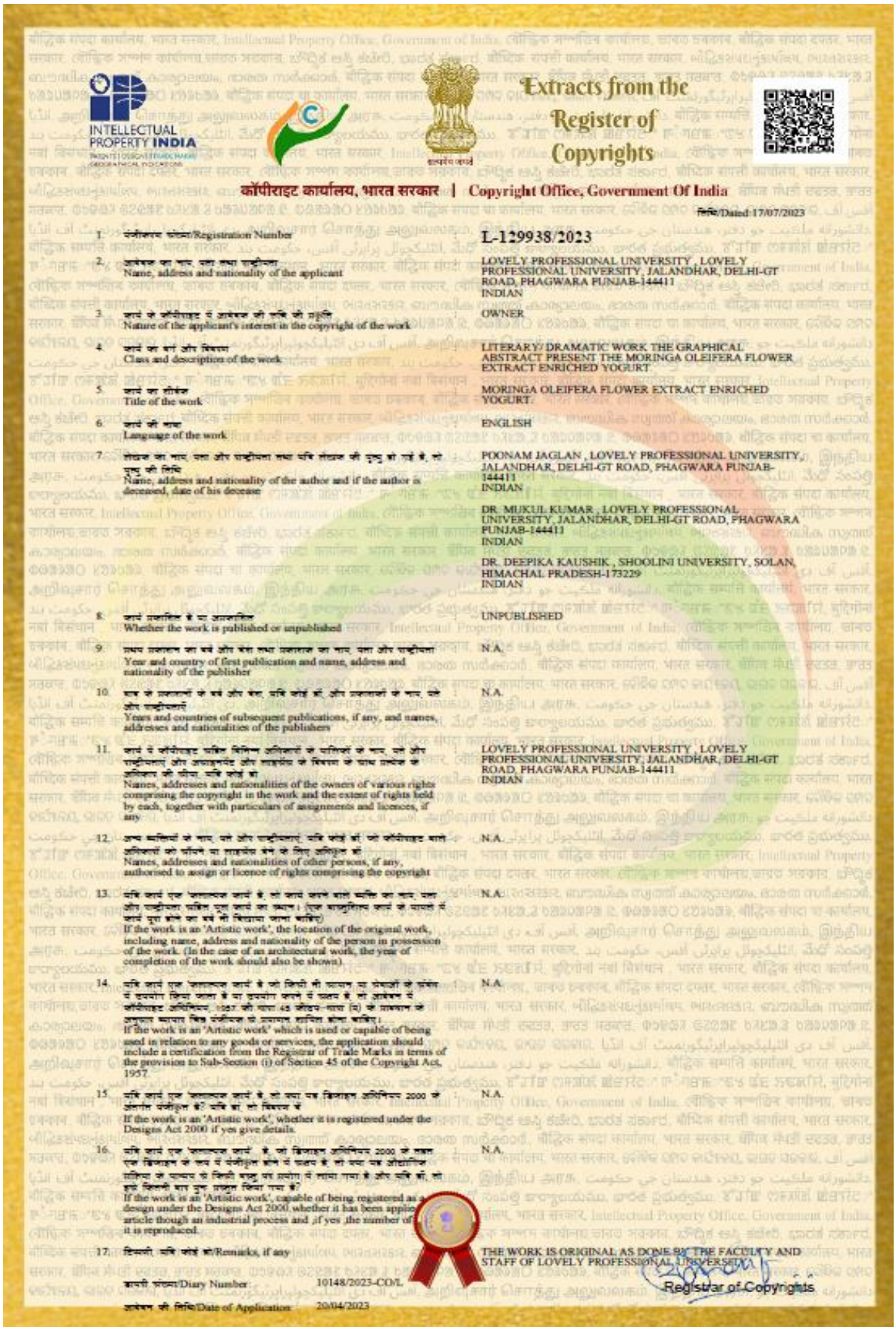

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5.	कार्य का शीर्षक Title of the work	MORINGA OLEIFERA FLOWER EXTRACT ENRICHED YOGURT.
6.	कार्य की भाषा Language of the work	ENGLISH
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आवेदन की तिथि/Date of Application 20/04/2023

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