Chapter 1
Introduction

The true laboratory is the mind, where behind illusions we uncover the laws of truth -Jagadish Chandra Bose

People from many cultures have used plants as their primary source of knowledge for ages, with various uses. This information has been passed down through generations of nomadic travel and is frequently exchanged between friends and tribes. The multidisciplinary study of ethnobotany, a subfield of ethnobiology, studies the interaction between plants and people. It encompasses not just using plants for food, clothing, and shelter but also for religious rituals, decoration, and medical treatment. The study of ethnobotany examines how plants have been utilized, controlled, and seen in human civilizations. This includes how they have been used for rituals, food, medicine, divination, cosmetics, dyes, textiles, construction materials, money, clothes, and music. All our existence is greatly influenced by plants, which control the number of airborne gases and convert sunlight into food energy, which is necessary for all other living forms. Indigenous people continue to be the best source for recovering this knowledge for purposes of application, particularly in modern medicine, because of their wide variety of understanding of medicinal plants.

Traditional herbal remedies have long

been essential to many countries' healthcare systems. Indian natives employ a variety of herbal treatments to cure a wide range of ailments successfully. Each locale uses different plant materials for drug formulation, administration, and utilization. While some traditional herbalists are still effectively utilizing herbal therapies, the knowledge of these treatments is gradually vanishing. Locals frequently use these plants to treat various illnesses. The conventional knowledge, abilities, and practices are shared throughout the communities as their common property. Studies into the traditional use and management of the local flora have revealed a wealth of local knowledge about the physical and chemical properties, phenological patterns, and ecological traits of many plant species, especially domesticated species. Ethnobotany has long been studied in India and is widely known there. Since the 1950s, there has been a rise in ethnobotany research. Numerous studies are being conducted on the botany, pharmacognosy, chemistry, pharmacology, and biotechnology of herbal remedies. Research on household remedies, hallucinogenic plants, and plants used in street drugs is being done because ethnomedicine is essential.

Ethnomedical research has led to the development of essential medicines like vinblastine, reserpine, and podophyllotoxin. Around 25% of prescription medications contain active ingredients from higher plants. Novel anti-inflammatory compounds, like prostratin, have been discovered through high-throughput bioassays and candidate plants (Pandey *et al.*, 2017).

Nutritional shortages affect at least one-third of poor nations' population. Fruits are a rich source of vitamins, minerals, fibre, and phytochemicals which help in fighting illness. Only a select few are well-known, healthy, and traded. Endemismic fruits, wild or semi-wild, can be a significant dietary supplement containing vitamins, minerals, and polyphenols (Barua *et al.*, 2021). Underutilized fruit crops are those not widely traded or economically raised. Locals grow, trade, and consume these crops, offering advantages like natural hardiness, dietary and medical security, and affordability. They are well-known to Indian farmers (Deka and Rymbai, 2014).

Tropic and subtropical fruits have therapeutic properties, but lesser-used, unknown, and unproven fruits are less commonly consumed and have not gained attention as antioxidant sources (Loganayaki and Manian, 2010). Wild fruits are becoming increasingly popular as potential dietary supplements or cheaper alternatives to commercial fruits worldwide (Rawat *et al.*, 2011; Zhang *et al.*, 2017). To promote a diverse and healthy diet and fight against micronutrient deficiencies, the so-called "hidden hunger," and other dietary deficiencies, attention should be paid to neglected and underutilized species. This is especially true for the rural poor and other vulnerable social groups in developing nations. Local people have utilized their plant species for many generations. However, their uses are being lost because of the loss of local knowledge. Many underutilized species can significantly improve the nutrition of nearby populations (Kour *et al.*, 2018).

Brazil boasts an extensive biodiversity hotspot in the Atlantic rainforest, with an estimated 5,000 edible plant species. Despite its 8.5 million km^2 territory, most Brazilian fruit species remain unknown. However, they could be included in human diets as functional foods to improve living conditions and prevent chronic diseases. Some of these lesser-known species include *Eugenia brasiliensis* Lam. (grumixama), *Psidium cattleianum* Sabine (araça), *Eugenia pyriformisCambess* (uvaia), *Byrsonimalancifolia* (muriciguaçú), *Campomanesiaphaea*(cambuci), *Jacaratia spinosa* (jaracatiá), *Solanum alternatopinnatum* (juquirioba), and *Acnistusarborescens* (fruta de sabiá). Consuming fruits and vegetables can induce ROS/ RNS scavenging and prevent chronic non-communicable diseases. Brazil's fruit diversity offers potential for functional meals and nutraceuticals, attracting interest in the food and pharmaceutical sectors for market growth and economic development (Soares *et al.*, 2020).

India's North-Eastern Hill (NEH) area is one of the agrobiodiversity hot spots in India's gene center. It has ethnic and traditional diversity (Dutta *et al.*, 2018). According to the National Bureau of Plant Genetic Resources (NBPGR) of the Indian Council of Agricultural Research (ICAR), the NEH area is rich in wild relatives of agricultural plants. Underutilized fruit crops comprise a sizeable portion of these regions' biodiversity richness. Among the major fruit crops of the country, the NEH region has maximum diversity in citrus, banana, and jack fruit; more over wide variety in other temperate, tropical, and subtropical fruits belonging to the genera *Pyrus, Rubus, Prunus, Garcinia, Phyllanthus, Averrhoa, Persia, Passiflora* etc. are also reported from the region (Deka *et al.*, 2012 & Singh *et al.*, 2014). The research has been studied by some writers about the underutilized and underexploited fruits of North-East India, their ethnomedicinal usage, socioeconomic value, and conservation initiatives. Most research has described many indigenous fruits' geographic distribution and ethnomedical applications. However, few have described the number of bioactive chemicals present in these fruits (Dutta *et al.*, 2018a).

Polyphenols, which have antioxidant and redox characteristics, are abundant in fruits. These substances work against reactive oxygen and nitrogen species (ROS and RNS), necessary for physiological processes, including growth, gene expression, and infection defense. Detoxification of ROS is crucial, as too much of it can lead to oxidative stress, which raises the risk of conditions including diabetes, cancer, obesity, and brain problems. Nitric oxide can produce reactive nitrogen species (RNS) in hypoxic environments, which can cause lipid peroxidation and the generation of reactive aldehydes and malondialdehyde. Nitric oxide and superoxide react, producing an excess oxidative stress that can lead to cancer. Inflammatory cells release soluble mediators that activate transcription factors and signal transduction cascades. This may result in long-lasting inflammation, harm to

Table 1.1 Different Free radicals

Name	Formula	Comments
Hydrogen atom	H'	The most basic kind of radical.
Trichloromethyl	$CCl_3.$	An unpaired electron is found on carbon, making it a radical with a carbon center. A component of the solvent's harmful effects, CCI_3, is created in the liver during the metabolism of CCl4. The quick reaction of carbon-centered radicals with oxygen often produces peroxyl radicals., e.g., $CCI_3 . + O_2 \rightarrow CCI_3O_2$
Superoxide	$^{\cdot}O_2^{-}$	Radical with a center of oxygen. discerningly responsive.
Hydrogen Peroxide	H_2O_2	As an oxidizing agent, hydrogen peroxide (H_2O_2) damages cells at unsuitable amounts and causes cell cycle arrest, resulting in cell death. According to recent research, H_2O_2 oxidizes specific thiol proteins to accelerate the advancement of the cell cycle.
Hydroxyl radical	$^{\cdot}OH$	A highly reactive oxygen-centered radical. It attacks all molecules in the human body.
Nitrogen oxides	$NO^{\cdot}$, $NO_2^{\cdot}$	L-arginine is the amino acid that is converted to nitric oxide in vivo. Nitrogen dioxide is produced when NO2 combines with oxygen and is present in both contaminated air and smoke produced by burning organic compounds, such as tobacco smoke.
Peroxynitrite	ONOO−	Reactive to biomolecules

good cells, and even cancer development (Table 1.1.) (Banerjee *et al*., 2022a; Banerjee *et al*., 2022b; Dutta *et al*., 2018b).

The brain is vulnerable to oxidative stress due to its high O_2^-consumption capacity, lipid-rich makeup, and weak antioxidant defense system. Numerous mental illnesses, including neuro-degenerative diseases and depression, can be brought on by oxidative stress in the brain (Kar *et al*., 2020). In line with this, renal disease fatally lowers the quality of life for many communities as it spreads. Most patients find using standard medications to treat liver and kidney damage illogical and costly. Prolonged use of modern medicines leads to adverse side effects. Natural remedies, like herbal therapy, are plant-based, less expensive, and widely accepted, offering potential improvements in healthcare (Banerjee *et al*., 2022c).

Male infertility is a significant reproductive health concern, accounting for around 50% of human infertility concerns. Research links it to oxidative stress on testicular functioning, as testicular tissues are susceptible to damage due to unsaturated fatty acids and mitochondrial energy demands (Salau *et al*., 2023).

Diabetes mellitus (DM) is the third most serious hazard to life, affecting 5% of the world's population. By 2025, there will be over 300 million individuals worldwide. Oral hypoglycaemic medications like acarbose, glucosidase inhibitors, insulin sensitizers, and biguanides are used to treat DM. However, side effects like liver issues and diarrhoea limit their therapeutic applicability. With their antioxidant and hypoglycemia properties, polysaccharides offer potential diabetic therapy options (Chen *et al*., 2016).

Thus, research on medicinal plants provides substantial evidence that phytochemicals and their antioxidant characteristics might have protective benefits against oxidative stress, renal diseases, cancer, aging, etc.

Polyphenols naturally occurring essential plant metabolites affecting the sensory and nutritional quality of fruits, vegetables, and other plants. In many fruits, they account for most of the antioxidant activity (Li *et al*. 2012). Polyphenols have been linked to preventing obesity, heart disease, colon cancer, and gastrointestinal disorders. They also protect fatty acids from oxidative decay and defend against oxidizing agents and free radicals. (Ignat *et al*. 2011). It is strongly believed that regular consumption of plant-derived phytochemicals may drift the balance towards an adequate antioxidant status in the body (Mahomoodally *et al*. 2012). Thus, interest in natural antioxidants, especially of plant origin, has increased recently. The growing interest in antioxidant-containing substances has led to their inclusion in preservation technology and modern healthcare.

Polyphenols are divided into flavonoids, phenolic acids, and tannins, depending on their number and structural elements. **Flavonoids**, essential antioxidants, are found in citrus fruits, tomatoes, and aromatic plants. They protect plants from UV light, fungal parasites, and oxidative cell injury. Flavonoids can be classified into anthocyanins, flavones, isoflavones, flavanones, and flavanols. **Anthocyanins** are water-soluble vacuolar pigments in plant tissues, including leaves, stems, roots, flowers, and fruits. They act as antioxidants by donating hydrogen to reactive radicals, preventing further formation. Their antioxidant potential depends on the number and arrangement of hydroxyl groups, structural conjugation, and electron-donating and electron-withdrawing substituents. **Isoflavones** have health effects and are suggested for disease prevention or cure. **Phenolic acids**, comprising around one-third of dietary phenols, are found in plants' free and bound forms. They exhibit varying susceptibilities to oxidation and adaptation to extraction conditions. Oxybenzoic acids have a C_6-C_1 structure, while hydroxycinnamic acids have a C_6-C_1 structure. Examples include caffeic, ferulic, p-coumaric, and sinapic acids. **Tannins** have a variety of impacts on biological systems because they can be biological antioxidants, protein precipitators, and metal ion chelators. Fruits with higher phenolic content show more robust antioxidant capacities. These antioxidants prevent degenerative diseases and have diverse effects on biological systems. Resveratrol, produced by plants, and lignans, produced by oxidative dimerization, are growing in interest due to potential applications in cancer chemotherapy and other pharmacological effects. (Ignat *et al*. 2011) (Figure 1.1.).

Berry fruits are also rich in natural phytochemicals like polyphenols and volatiles. Studies show significant health benefits from berry phenolics, including antioxidant, anti-inflammatory, anticancer, anti-obesity, antidiabetic, and cardiovascular disease-preventing effects. (Quesada *et al*., 2020). Volatile compounds in plants are compounds with high vapor pressure and low boiling points, responsible for berries' unique aroma and flavor. These compounds are antioxidants and play a crucial role in sensory perception. Berry consumption can alleviate nutritional deficiency and can increase health-beneficial effects. However, there is limited information on berry volatiles' chemical composition and health-beneficial effects (Gu *et al*., 2020).

Elaeagnus, Myrica, Hippophae, and other edible, fruit-bearing actinorhizal plants are found throughout the entire NEH region, and *Elaeagnus pyriformis*, a deciduous shrub, is particularly famous for its therapeutic properties. *Elaeagnus* is a member of the Order Rosales family Elaeagnaceae, also known as the silverberry or oleaster

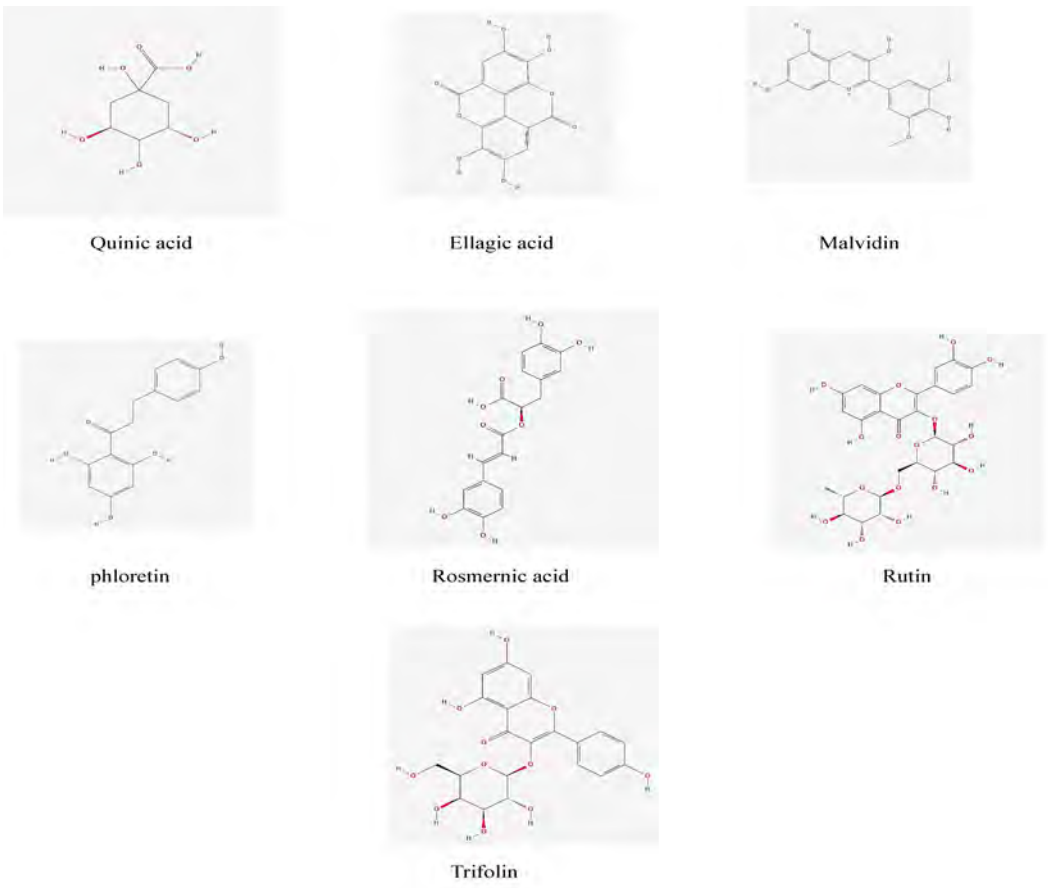

Figure 1.1. Some common polyphenols of Fruits

(Kar *et al*., 2016). According to Sharma and Kumar (2006), only four species of *Elaeagnus* have been recorded in India, namely *E. pyriformis, E. angustifolia*, *E. latifolia,* and *E. umbellata.* Among others, *Myrica esculenta, Berberis asiatica, Rubus ellipticus, Pyracantha crenulate,*and *Morus alba* from the Himalayan region have been reported as fruits of nutraceutical importance and are, therefore, also livelihood sources in IHR (Indian Himalayan Region) (Belwal *et al*., 2018).

Phyllanthus, a phyllanthaceae family with over 700 species, is primarily found in tropical and subtropical regions. *P. acidus* (L.) Skeels, a widely spread plant in southern India, is known for its bioactive compounds and traditional folk remedies for various ailments. Despite its extensive phytochemical diversity, the plant is underutilized in Northeastern Indian states as a source of TMK (Traditional Medicinal Knowledge) (Banerjee *et al.*, 2022c).

*Baccaurea ramiflora*is a popular ethnomedicinal fruit in East and Northeast India, is known for its high nutritive value and ethnomedicinal properties. It belongs to the Phyllanthaceae family and is in sub-Himalayan areas, South Tibet, and the Indo-Burma region. Various names, including Burmese grape, Mafai, Latkan, Bhubi, Lotka, Leteku, and Lerko, know the fruit. The seeds of *B. ramiflora* are effective against indigestion and constipation, and it is also known as an oral antidote against snake bites in Assam. (Banerjee *et al*. 2022b).

Sohiong, an indigenous wild fruit from the Rosaceae family, is grown in Khasi and Jaintia Hills, Meghalaya, India. It is a cherry-like dark purple fruit with unique organoleptic properties and is rich in phenols, vitamin C, flavonoids, and anthocyanin. Despite its potential in the food, pharmaceutical, and textile industries, it faces underutilization in India due to storage facilities and processing (Vivek *et al*., 2018).

Allopathic medicines are now widely available for treating serious chronic diseases, but they may have future side effects that may affect other human organs. For example, prolonged use of GM (gentamicin), a broad-spectrum antibiotic, is not recommended due to its potential nephrotoxicity. Moreover, numerous novel drugs with unknown toxic potential are constantly released for clinical use, making treatment expensive and invasive. Appropriate strategies must be developed to discover lead compounds and develop new drugs with minimal hazards and maximum advantages (Banerjee *et al*., 2022c). Various phytoconstituents are present in plants and fruits; also, those have the ethnomedicinal potential to alleviate chronic disease problems in healthcare systems. For instance, β-sitosterol, a phytosterol in avocados, nuts, and vegetable oils, has anti-inflammatory, antioxidant, and anti-cancer activities. In addition to maintaining normal blood cholesterol levels, it has been shown to lessen liver damage and benign prostatic hyperplasia (BPH). However, the enormous molecular weight and limited absorption of β-sitosterol make it challenging to use in therapeutic settings. With low molecular weight and a high surface area by volume ratio, nanoparticles are chemically inert. Due to these characteristics, a nanoparticle-based drug delivery strategy is established as a promising remedy to this problem in pharmaceutical research (Kar *et al.*, 2022).

Medicinal plants include many biomolecules and phytochemicals that might be employed as green reducing and capping agents to manufacture nanoparticles. For instance, silver nitrate is reduced, and the produced silver nanoparticles are stabilized by phyto-compounds, some long-chain fatty acids, and their derivatives with various chain lengths. *Elaeagnus pyriformis* fruit juice contains several significant fatty acids, including fumaric acid ($C_4H_4O_4$), dodecanoic acid ($C_{12}H_{24}O_2$), and hexadecanoic acid ($C_{16}H_{32}O_2$). The production of aliphatic carboxylic acid silver salt (AgNPs) from the aliphatic carboxylic acid occurs in the presence of silver nitrate. Here is how the reaction happens:

$$2C_nH_{2n+1}COOH + 2AgNO_3 \longrightarrow 2C_nH_{2n+1}COOAg + 2HNO_3$$

The principle is almost like the biogenic synthesis of nanoparticles like ZnONPs and CuONPs. TiONPs etc. The synthesis of biocompatible plant-mediated fusion of metal nanoparticles is a promising technique for commercially viable, non-toxic, and eco-friendly nanomaterials. Chemical reduction methods use more energy, produce larger particles, and produce unfriendly byproducts. Alternative eco -friendly procedures (green/biogenic synthesis) create stable and dispersible nanoparticles with less energy, including bacteria, fungi, and plant extracts. These eco-friendly biological/green synthesis techniques are quicker, cheaper, easier, and more successful than traditional chemical processes. Green synthesis does not require harmful chemicals, making it compatible and safer for biomedical and pharmaceutical applications. The wide-ranging uses of metal nanoparticles in medicine, biotechnology, and electronics have boosted the need for green chemistry (Banerjee *et al*., 2022c). Alarming data indicate that bacterial resistance to various antibiotic substances used to treat various illnesses is increasing. Additionally, the employment of several types of anticancer medications against various cancers justifies the urgent quest for more potent alternative agents for therapy. Silver nanoparticles' broad-spectrum bioactivities suggest that they can partially address several issues with microbial resistance. Silver nanoparticles have lately caught the interest of scientists worldwide due to their antibacterial, antifungal, antiviral, antiprotozoal, acaricidal, larvicidal, and anticancer properties. Their therapeutic potential as intelligent nanomedicine may offer new application horizons, opportunities, and ways to combine them advantageously with other biologically active substances.

Food security is one of the primary demands that no civilization can ever disregard. The unfortunate result is that global food production may soon be insufficient to feed everyone on the planet due to the vast increases in environmental degradation brought on by inappropriate farming practices and the pressure from human population growth. Increased nutrient consumption is also required for increased yields, and because fertilizers are in such high demand, farmers' expenses have gone up globally. The biotic (caused by fungus, bacteria, diseases, herbivores, etc.) and abiotic (caused by environmental stressors including drought, salt, cold, and heavy meals) factors that are present are acknowledged to be among the most significant barriers to agricultural output worldwide.

Research on plant and soil production is crucial to address environmental stressors and feed the growing global population while addressing biotic and abiotic pressures (Etesami *et al*., 2018) Heavy metal contamination is now rising and causing some environmental issues. Heavy metals (HMs) and metalloid pollution pose serious health risks to people worldwide and have accelerated industrialization and urbanization at an unheard-of rate in recent decades. These contaminants affect drinking water, soil, and the atmosphere. Since HM affects the atmosphere, water, and soil, environmental pollution has become a significant problem. The five most hazardous metal/metalloid pollutants are copper (Cu), cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As). Among them, As is now to blame for significant groundwater pollution in several other nations. The World Health Organisation (WHO) warns that 200 million people worldwide are exposed to the risks of As poisoning and that the ideal amount of As in drinking water is ten gL. The primary sources of As in water, soil, and air include extensive mining, burning of fossil fuels, metal smelting, and the careless use of herbicides, insecticides, and pesticides. With the creation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide radicals (O_2•), and hydroxyl radicals, As causes oxidative stress in plants. As (V) is changed to As (III), resulting in the formation of ROS. Plants exposed to arsenic have altered morphology, physiology, and growth patterns. Morphologically, As induces defoliation, which reduces the number of leaves and produces chlorosis, as well as leaf senescence and necrosis. Arsenic has physiological effects on antioxidant activity, photosynthesis suppression, and stomatal conductance that produce ROS, which can harm DNA, induce lipid peroxidation, damage carbohydrates, and impair the chloroplast membrane. Regarding plant growth, it restricts the growth and development of roots and plant biomass and lowers plant production (Emamverdian *et al.*, 2022).

FAO standards state that water with arsenic levels > 0.1 mg L-1 should not be used for irrigation. However, it was discovered that the FAO permitted limit for arsenic has been exceeded in the groundwater of numerous Indian provinces, particularly West Bengal (Mukherjee et al., 2006). Arsenic is quickly absorbed by plant roots after exposure, moving from the source to the shoot and affecting plant growth and development (Banerjee *et al.*, 2023). Additionally, it penetrates fruits and seeds via phloem transport, polluting the entire food chain. Higher levels of DNA damage, such as single-strand breaks (SSBs) and double-strand breaks (DSBs), are caused by this. Endoreduplication, chromosomal abnormalities, reduced genomic stability, and programmed cell death are the final effects since SSBs and DSBs are far more challenging to repair (Dutta *et al.*, 2018c). Stress hurts macromolecules as well as flowering time, pollen viability, and crop output (Chirivi *et al.*, 2023).

By encouraging plant development, carbon nanotubes have been shown to reduce the toxicity caused by Cd. By accelerating photosynthetic rate and growth metrics, Singh et al. discovered that TiO_2 NP treatment decreased the Cd toxicity of the soybean plants. In locations with low zinc levels, ZnO has become a popular crop fertilizer in recent years. ZnO enhanced the physiology and development of plants (Yan *et al*., 2021).

Zinc (Zn) is used as a micronutrient and a metallic cofactor in various defensive enzymes, making ZnONPs the most effective nanomaterial (Ahmad *et al*., 2020). Due to their tiny size, high surface-to-volume ratio, and efficacy at adsorbing heavy metals (HM) or metalloids, nanoparticles (NPs) are very efficient at preventing nutrient absorption and nutrient loss and maintaining crop yield by modifying several metabolic processes. Several crops, including black gram (Banerjee *et al*., 2023), maize (Salam *et al*., 2022), rice (Yan *et al*., 2021), and soybean (Ahmad *et al*., 2020), have shown that the application of ZnONPs is one of the most practical and long-lasting ways to reduce HM or metalloid stress and its translocation. Blackgram as a pulse crop is a rich source of dietary protein and highly susceptible to As-stress, making it imperative to develop strategies/technologies to avoid the toxic effects of As (Saha *et al.,* 2020; Srivastava *et al*., 2017).To address the zinc deficit in agricultural soils, Zinc Oxide Nanoparticles (ZnONPs), a subset of NPs, are frequently utilized as nano fertilizers. In rice seedlings, ZnONPs increase growth and photosynthesis while reducing As-accumulation. Commercial agriculture requires a high emergence rate, consistent germination of seeds, and rapid seedling development. Seed priming is a potential method to rapidly increase seed germination rate and early development by changing physiological factors since arsenic directly inhibits seed germination. (Banerjee *et al*., 2023).

During the literature survey, it was found that the underutilized fruits have excellent medicinal values and prominent traditional knowledge. Therefore, an attempt has been made to explore this as a studied topic.

Objectives of the Study:

- Survey and collection of underutilized fruits from northeast India.

- Assessment of cytotoxicity of selected fruit juices on mice/Rats.
- Evaluation of antioxidant properties of selected fruit samples.
- Evaluation of the antimicrobial activity of selected underutilized fruits consumed in northeast India.
- Polyphenolic characterization and high throughput phytochemical assays using UV-HRLC-MS, FTIR, GC-MS, etc. analysis.
- Biogenic synthesis of nanoparticles (NPs) using selected fruits from northeast India and its thorough characterization by way of bioactivity analysis.
- In-depth study of medicinal properties of selected fruits and NPs, including nephrotoxicity on mice model.
- To study the efficacy of biogenic nanoparticles on selected crop plants against heavy metal stress and its thorough evaluation.