Chapter 1

Introduction

" The true laboratory is the mind, where behind illusions we uncover the laws of truth."

-Acharya Jagadish Chandra Bose

'Medhya Rasayana' is a notion rooted in Ayurveda, the traditional Indian medicine system. It consolidates two Sanskrit terms: "Medhya," which refers to cognition, intellect, or mental functions, and "Rasayana," which mentions rejuvenation or revitalization. The concept of 'Medhya Rasayana', therefore, pertains to a kind of Ayurvedic remedies and implementations that are focused on promoting and enhancing mental clarity, cognitive-proper functions, and overall brain health (Kulatunga *et al.,* 2012).

In Ayurvedic system of medicine, the body and mind are interconnected, and maintaining a good balance between them is necessary for overall well-being. Medhya Rasayana is a branch of Ayurveda that addresses cognitive function, mental health, and emotional well-being. These implementations aim to promote brain function, improve memory, increase concentration, and support mental resilience (Nadh *et al.,* 2022).

Medhya Rasayana practices and treatments can involve a combination

of numerous approaches, including:

1. Herbal Formulations: Ayurvedic practitioners use particular herbal formulations that are regarded to have a positive impact on human brain health. These specific herbs are selected based on their resources, such as being antioxidant, adaptogenic, and neuroprotective activity.

2. Diet and Nutrition: Ayurveda emphasizes the significance of diet in maintaining overall health. Medhya Rasayana suggests foods that are nourishing for the overall health , such as those rich in nutrients, omega-3 fatty acids, and antioxidants that support cognitive functions.

3. Lifestyle Recommendations: Lifestyle practices that assist mental well-being are part of Medhya Rasayana also. This may include practices like meditation, yoga, pranayama (breathing exercises), and adequate sleep.

4. Mental Exercises: Mental practices and exercises that stimulate the brain, such as mindfulness, meditation, and engaging in activities that challenge cognitive potentialities, are considered beneficial.

5. Behavioral Guidelines: Ayurveda highlights cultivating positive emotions and behaviors for mental balance. Managing stress, practicing gratitude, and fostering positive social activities are important aspects (Navyashree and Agida, 2019).

Herbal medicines are continuously emerging in developing countries like Asia, Africa, and Latin America with quickly appearing markets in Europe and North America. Traditional usage of herbal medicines implies their substantial historical use to meet the requirements of the health care system. In the report based on the

WHO (World Health Organization), a variety of drugs have been acquired from different herbal sources and about 80% of the world's developing inhabitants depend on traditional medicine systems for their primary healthcare services (Vadhana *et al.,* 2015). In 2001, it was estimated that approximately 25% of formulated drugs originated from natural sources of which 121 active compounds were then in use. In addition, of the 252 medicines considered basic and essential by the WHO, of which 11% emerged exclusively from medicinal plants and a remarkable number were synthesized from naturally occurring precursors. A significant number of plant species are used as a main constituent for the concoction of modern phytomedicines which has been investigated in the last few years e.g. digitoxin obtained from *Digitalis* spp., vincristine, and vinblastine from *Catharanthus roseus*, quinine obtained from *Cinchona* sp., atropine from *Atropa belladonna*, morphine and codeine from *Papaver somniferum*, etc. (Upadhyay, 2023). Plants with medicinal usefulness have several therapeutic perspectives, as they possess various biologically active components. Due to the exceptional diversity in chemical structure and bioactivities of these naturally occurring phyto-compounds, there has been an emerging interest in the area of research related to natural products.

However, the problem with the use of medicinal plants is that they are usually applied without any standardization. This forged it difficult to document and established a system of assessment for the effectiveness of the treatment. Many plant compounds are efficient to treat diseases such as infections, inflammatory conditions, cardiovascular diseases, and so on but

the right scientific information on most of these medicinal plants is still lacking. Therefore, as part of the attempt to encourage the use of medicinal plants either as an alternative or a supplement to traditional medicine, scientists need to carry out further investigations into herbal medicines (Mahishi *et al.,* 2005).

India is well known for its traditional medicinal systems like Ayurveda, Siddha, and Unani. This traditional medical system is found mentioned in the ancient Vedas and other scriptures. These concepts appeared and emerged between 2500 and 500 BC in India (Subhose *et al.,* 2005). It has been well appreciated that positive health means metabolically well-balanced human beings. Ayurveda or alternative medicine is also called the "science of longevity" because it offers a complete system to live a long healthy life. The Indian subcontinent is a massive source of medicinal, aromatic, and ornamental plants. Medicinal plants are used in traditional medical treatments. The alternative medicines in the conventional systems are procured from herbs, and organic matter, while for the formulation of herbal drugs only medicinal plants have been used. The use of medicinal plants as a primary source of medicine has been an ancient practice and is a very important component of the health care system in India. In our country, about 20,000 medicinal plants have been documented; nevertheless, traditional practitioners use only 6,500 -7,500 plants for therapeutic applications (Modak *et al.,* 2007).

Medicinal plants are well-cataloged and have long been employed as the basis of many traditional medicines employing protective effects against

several diseases in the human system due to their antioxidative properties (Barkat *et al.,* 2021). Medicinal plants are rich in secondary metabolites like polyphenols which have the redox and antioxidant properties that act against reactive oxygen species i.e. ROS and reactive nitrogen species i.e. RNS including hydroxyl radical ($OH^{\bullet -}$), superoxide anions ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), nitric oxide (NO), peroxynitrite radicals ($ONOO^-$), etc. (Lala *et al.,* 2020).

Oxygen (O2) is a remarkably influential element for all aerobic living beings. However, at towering concentrations, oxygen could be a toxic substance to aerobes (Taverne *et al.,* 2018). ROS, which contains O2, are mainly responsive molecules due to the presence of unpaired electrons and this is the major cause of oxidative stress. Most of the vandalizing outcomes of O2 are due to ROS, which includes $O2^{\bullet -}$ (superoxide anion radicals), OH• (hydroxyl radicals), and non-free radical species such as O3 (ozone), H2O2 (hydrogen peroxide), and 1O2 (singlet oxygen) (Balaydin *et al.,* 2010). If one electron is added to molecular oxygen, a superoxide ion is produced (Bayr, 2005). Hydrogen peroxide is produced by the enzymatic oxidases group e.g. xanthine and NADPH oxidase as well as other metabolic reactions. The most reactive species hydroxyl radical, among the various oxygen species, can oxidize polyunsaturated fatty acids (lipid peroxidation) causing damage to carbohydrates, lipids, proteins, etc. Being highly reactive, HOCl can alter DNA bases. It is effectively established that free radicals play an important role in the progression of tissue attrition which is subjected to

cell damage leading to inflammatory events (Bursal *et al.,* 2013). ROS in low concentration is crucial for our physiological functions e.g. cellular growth, gene expression, and providing defense against different infections. However, the incapacity to detoxify excess amounts of ROS by our body may cause oxidative stress. This oxidative stress indeed increases the risk of various diseases like diabetes, cancer, obesity, rheumatoid arthritis, cognitive disorders, and to a great extent involved in aging (Poyton *et al.,* 2009). Besides, the mitochondrial respiratory chain under hypoxic conditions produces nitric oxide (NO), which can produce reactive nitrogen species (RNS) (Durackova, 2010). RNS induces immoderate lipid peroxidation which may guide the production of other reactive species like reactive aldehydes and malondialdehyde. The reaction between the nitric oxide radical and superoxide radical is the result of peroxynitrate, a very reactive oxidant that can attack a wide array of biological functions. This 'oxyradical overload' may lead to numerous diseases incorporating cellular inflammation and cancer through DNA damage, protein modification, and changing transcriptional parameters. Assembling of ROS by soluble mediators like arachidonic acid, cytokines, and chemokines excreted by inflammatory cells, activate several signal transduction cascades including changes in transcription factors such as AP-1, NF -κβ, Nrf2, SP1,p53, STAT3HIF-1α, and PPARγ. The activation of these transcription factors by ROS and RNS may lead to sustained inflammation/ oxidative environment which in turn leads to damage of fine neighboring epithelial and stomatal cells over a

prolonged period of exposure and it might lead to cancer (Perwez Hussain and Harris, 2007). The structure and function of DNA are interrupted by the continuous attack of ROS. The hydroxyl radical abstracts the H2 atom from the C-4 position of deoxyribose sugar leading to the wreckage of DNA strands (Sharma *et al.,* 2012). This structural tampering may cause mutation and result in various major afflictions.

Plants are rich in a broad variety of secondary metabolites, like tannins, terpenoids, flavonoids, and alkaloids which have been found in vitro to have medicinal properties. The emergence of science to explore antibiotics largely depends on some of these medicinal plants as raw materials. From time immemorial, medicine had depended exclusively on leaves, barks, and flowers of plants; only currently have synthetic drugs come into use. At present more than 30% of the modern pharmacological drugs have been procured directly or indirectly from herbal sources and in ayurvedic or homeopathic medicines, medicinal plants, their parts, and extracts (Hassan *et al.,* 2013).

Inflammation is a very complex process that is important for the defence of the host. Extensive production of inflammatory mediators may lead the way to chronic disease (Sharma *et al.,* 2012). Phytocompounds from herbal sources can demonstrate anti-inflammatory activities influencing different stages of the process which is involved in inflammation, obstructing the formation of cytokines and eicosanoids; demolition of inflammatory reaction cascade from commencing to reduce itching, flare, and excessive exfoliation (Holtmann and Talley, 2015). Promising research

on plants with inflammatory activities is one of the most interesting and developing areas in modern bio-medicine. Further research on plants in this field is needed since a great percentage of many unexplored plants have not been researched. Inflammation remains a complex process that is important for the defence of the host. Excessive production of inflammatory mediators may lead to chronic disease. The inflammatory process also involves changes in blood flow, surged vascular permeability, destroys tissues via the activation and migration of leucocytes with the synthesis of ROS (oxidative burst), and the union of local inflammatory mediators, such as prostaglandins (PGs), platelet-activating factors, and leukotrienes induced by phospholipase A2, cyclooxygenases (COX1 and COX2), and lipoxygenases. Anti-inflammatory representatives like non-steroidal anti-inflammatory drugs (NSAIDs), immuno-suppressants, and steroids often are used for the betterment of inflammatory situations. But often their use is associated with serious side effects like peptic ulcers, gastrointestinal bleeding, and so on (Lala *et al.,* 2022).

NSAIDs are commonly used as analgesics. NSAIDs have also beneficial effects in treating cancer, Alzheimer's, and arthritis disease (Sharma *et al.,* 2020). They are the most commonly prescribed category of drugs and are used in a large number of countries. Some NSAIDs may be acquired without a prescription. The machinery involved in the therapeutic actions and side effects of NSAIDs has been probed by different methods in the last couple of decades. The principal therapeutic action of NSAIDs via the inhibition of

the cyclooxygenase (COX) enzyme activity to distort the synthesis of prostaglandin which is a principal mediator of pain and inflammation (Sharma *et al.,* 2020). There are many works of literature have been flooded in different scientific journals on the adverse effects of this class of drug. It is a proven fact that they can adversely affect the cardiovascular, renal, and gastrointestinal systems. Some minor effects usually do not require hospitalization but they still consume healthcare assets from outpatient investigation and treatment. The severe adverse effects indeed require hospitalization and medical intervention, thus consuming a considerable amount of money and time (MacDonald and Sheen, 2002).

To reduce the harmful effects of synthetic drugs (e.g. NSAIDs), the use of antioxidants directly procured from herbal sources is very dependable because of their low cost, fewer side effects, and ready availability. Interestingly, herbal sources have been proven to be very effective to combat the recent happening pandemic SARS CoV-2. So, nowadays the main focus of researchers is how to combat these post covid complications with herbal ways to reduce the side effects of Non -steroidal anti-inflammatory medicines because several phytochemicals have been reported as probable therapeutic targets against Post COVID inflammatory conditions. There is a lot of literature to date that has documented the use of *Clerodendrum, Ocimum tenuiflorum*, and *Justicia adhatoda*-derived compounds as potential candidates against COVID-19 (Kar *et al*., 2021). Besides, there are several other phytocompounds, derived from medicinal plants which have shown beneficial effects against Covid 19 e.g.

Phyllanthus amarus, Tinospora cordifolia, Emblica officinalis, Andrographis paniculata, Curcuma longa, Glycyrrhiza glabra, Phyllanthus urinaria, Withania somnifera, etc. (Gangal *et al.,* 2020).

Deregulated inflammatory response plays a crucial role in the commencement, development, and progression of cancers. The possible molecular mechanism that leads to the establishment of an inflammatory-tumor micro-environment is not properly understood due to the complex cross-talk between pro-inflammatory and tumorigenic mediators such as chemokines, cytokines, enzymes, oncogenes, transcription factors, immune cells, etc. These molecular mediators are crucial linchpins between cancer and inflammation (Chai *et al.,* 2015). Utilization of conventional medicines in inflammation and cancer is quite costly as well as obstructive to most patients. Moreover, these drugs exert adverse health effects due to persistent use. Incorporation of herbal remedies into the conventional healthcare system may substantially improve the overall healthcare system. Nowadays, the Screening of medicinal plants for anticancer activity contributed to a huge space for further development of robust anticancer agents (Roy *et al.,* 2017). The supremacy of herbal remedies over modern medicines is that most of the herbal medicines are plant-derived phytocompound-based and comparatively cheaper, possessing fewer side effects due to their easy acceptability. Therefore, the investigation on medicinal plants firmly supports the idea that plant constituents e.g. phenolics, tannins, flavonoids, proanthocyanidins, etc. along with antioxidant properties can exert protective consequences against

oxidative stress, inflammation, cancer, and so on. *Citrus* showed its activity against H22 hepatoma cells (Zhao *et al.,* 2017). *Citrus* belongs to the Rutaceae family, found all over the world. There are 4 important species in the *citrus* plant with a vast variety of hybrids as well. Several kinds of the literature showed citrus as a vigorous anti-tumor agent. Besides, the efficacy of citrus peels against skin cancer has been considered in the skin carcinogenesis model (Rawson *et al*., 2014).

Roughly, 3,000 plant species of more than 200 plant families have been identified to have medicinal properties in the region of Western Ghats, Eastern Himalayas, and Andaman & Nicobar Island of India (Vijayan *et al.,* 2017). Among them, some species have superior medicinal properties. During the literature survey, it was found that the genus *Citrus* has exceptional medicinal values as well as noteworthy traditional knowledge. It is a well-established fact that *Citrus* flavonoids (e.g. Hesperidium, naringenin, and so on) extracted from its peel are effective in various aspects like cardiovascular diseases, lipid metabolism, cancer, *etc*. However, working with their leaves is still elusive. So, an attempt has been taken to explore this as a studied plant material.

In 1753, Linnaeus first described the genus *Citrus*. "Tributte of Yu." is the oldest Chinese reference to *Citrus*. In 1179 A.D., Han Yen-Chih described and named some 27 varieties of the sweet-sour orange-mandarin group in his Chu Lu, the oldest familiar monograph of *Citrus*. Fossil records urged that the *Citrus* Genus dates back to 7 mya (millions of years ago), and

emerging in the Himalayas before spreading throughout Southeast Asia (Wen *et al.,* 2014). There are five main ancient species of *citrus* from which all modern hybrid varieties were created, these are the citron (*Citrus medica*), small flower papeda (*Citrus micrantha*), mandarin (*Citrus reticulata*), pomelo (*Citrus maxima*), and kumquat (*Citrus japonica*) (Barrett and Rhodes 1976). The most fascinating fact is that these five varieties are all unique in their consistency and taste, but have alike enough genomes that permit them to easily crossbreed. Moreover, these hybrids are fertile, admitting them to continue to populate, unlike the seedless bananas or watermelons (Rouseff *et al.,* 2009).

Citrus is a genus of flowering plants in the rue family, Rutaceae. Rutaceae, widely distributed in warm temperate regions. The genus *Citrus* is being considered as Medhya Rasayana as it is said that in Indian traditional Ayurvedic medicinal system which is more than 5000 years old, those medicinal plants are being classified as "medhya rasayana", coming from sanskrit words "medhya" meaning intellect/cognition and "rasayana" which means rejuvenation (Navyashree and Agida 2019).

This plant also found in tropical regions. In our country, the family is represented by several genera like *Citrus*, *Aegle, Murraya, Zanthoxylum, Glycosmis*, etc. Among them, *Citrus* contained many phytochemicals including essential oils, flavonoids, alkaloids, coumarins, carotenoids, and psoralens with a wide range of nutritional components including minerals, vitamins, and trace elements. *Citrus* is a very diverse genus with 170 species recognized. The earliest center of origin of citrus species has

been a subject of discussion and speculation for some time. The most current research indicates an origin in New Caledonia, Australia, and New Guinea. Few researchers believe that *citrus* origin is in the part of Southeast Asia bordered by Northeast India, Burma, and the Yunnan province of China (Dugo and Giacomo, 2002).

The genus *citrus* is chiefly characterized by its erect, woody, branched, glabrous, and solid stem. Leaves are petiolate (winged or flattened), stipulate, coriaceous, glabrous with unicostate reticulate venetion. The flowers of this genus are pedicellate, hermaphrodite, actinomorphic, complete, and hypogynous. Trees produced from seed are inclined to have more thorns and upright branching patterns of growth than the trees produced from grafting. Flowers are approximately 2 –4 cm (0.8–1.6 in) in diameter, solitary axillary or cymose, and often perfect i.e. having both functional stamens and pistils or staminate. Calyx is 4–5 lobed and there are usually five petals having oil glands. Stamen's number varies between 20 and 40. The color of Petal ranges from white to pinkish in Kafir lime; pinkish to purplish externally in citron; reddish in lemon varieties. The sub-globose ovary is superior, with 8–18 locules i.e. cavities having 4–8 ovules per locule in two rows. The fruit is a Berry (hesperidium) that ranges widely in color, size, shape, and juice quality. Fruits are globose to ovoid in shape. The fleshy endocarp is divided into 10–14 sections containing the stalked pulp and is separated by thin septa. Each section accommodates pulp i.e. juice vesicles that contain a sweetish or sour watery juice. Seeds are pale white, fattened, and angular. Their seeds are usually polyembryonic

which means they have multiple embryos that have the potential to germinate. The embryos are either nucellar or zygotic. The zygotic embryos are acquired from pollination of the ovary i.e. sexual reproduction and the nucellar embryos are derived fully from the mother plant and have displayed very similar features to the parent plant (Snafi, 2016).

There are several species from this genus have been reported to have numerous medicinal properties including anti-diabetic, anti-hypertensive, anti-inflammatory, anti-depressive, anti-cancer, and so on (Lala *et al.,* 2020). Many species of this genus are characteristically used by ethnic people as a potent source of poly-herbal formulations in the management of various diseases in different parts of India (Lee *et al.,* 2019). Botanically, *Citrus* is classified as follows (Singh, 2019):

Kingdom: Plantae

Clade: Eudicot

Phylum: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Rutaceae

Sub-family: Aurantioideae

Tribe: Citreae

Sub-tribe: Citrinae

Genus: *Citrus* L.

Citrus plant and its different parts are used vigorously and people in different parts of the world are still utilizing its products. Researchers already established that leaves of *C. aurantifolia* are used for headaches in Malay and leaf extract is used as a tranquilizer in Yucatan. Methanolic leaf extract of *C. aurantium* is a good remedy for anxiety, epilepsy, and nervous disturbance in the folk medicine of Iran and is used

vigorously as a sedative by Afro-brazilians. *C. limetta* leaves are used to enhance mood and sadness by Latin American tribes (Modak *et al.,* 2017). The leaves of *C. maxima* are used as a sedative for the nervous system as well as an antiepileptic in southeast Asia and the Philippines. Boiled leaf extracts of *C. paradisi* are also utilized as a sedative in the Caribbean. *C. sinensis* leaf extracts are heavily used for neurological disorders by tribes of Easter Island (South Pacific) while leaf infusion is utilized as a sedative by several Brazilian tribes like Cabaclos and Quilombolas (Sohi and Shri, 2018). *C. macroptera* fruit peel is used to treat depression and anxiety in Assam (Lala *et al.,* 2020). *C. reticulata* peel is used in many formulations used to treat psychological disorders in Chinese-Japanese traditional medicine. *C. aurantifolia* flowers are utilized for anxiety and nervousness in Mexican Traditional Medicine (Sohi and Shri, 2018). An extract of flowers of *C. aurantium* is used as a nerve sedative in Africa and Latin America while in nervous affections in Oriental Medicine, an aqueous extract of flowers is used. Aromatic waters of *C. reticulata* and *C. maxima* are used to relieve stress and insomnia. *C. sinensis* decoction is used as a sedative in Italy, China, France, and Mexican Traditional Medicine (Gutierrez and Navarrete, 2009). Citrus oils are used for relieving anxiety, and depression and improving cognition, reducing stress and pain, relaxing, sedating, or stimulating, and restoring both emotional and physical well-being (Sapkota *et al.,* 2022).

Shreds of evidence recommended that a few preliminary works including antioxidant activity, anti-diarrhea, anti -asthmatic, hypoglycaemic, anti-

tumor, etc. of fruits and peels have been done with selective species of Citrus. However, there are many lesser -known *citrus* species like *citrus reshni,* and *citrus unshiu* whose activity on different disease models is yet to be explored. Even though an in-depth medicinal property of leaves showing comparative and comprehensive information of *Citrus* is still missing because there is a lot of extensive work has been done only on the fruits and peels of the plant. Furthermore, the wild orange variety of citrus i.e. *C. macroptera* has been given primary focus because very less work has been documented in literature. There is very less information about the activity of *citrus* leaves in terminating the chain reaction generated by ROS in cancer cell lines. Another interesting fact is that, there is no documentation in literature which tells that how phytocompounds derived from *citrus* leaves are capable of suppressing post covid complications generated by cytokine storm.

It has been further observed that there has no sufficient information illustrating the comprehensive genetic variation among the different medicinal species of *Citrus* e.g. *C. maxima, C. macroptera, C. limon, C. medica, C. sinensis, C. reticulata, C. reshni, and C. unshiu.* Hence, further study was carried out to explore the genetic variations among those aforesaid species of *Citrus* through DNA fingerprinting techniques. Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) (microsatellite DNA marker) were recently shown to be sensitive for evaluating variations among individuals within and between species, varieties, and cultivars. Afterward, a new modified molecular

technique i.e. DNA barcoding was progressed to explore the genetic relatedness, evolution, and identification of unknown plants and also animal species resolving various anomalies in the taxonomic levels by utilizing a short stretch of DNA sequence (Hebert *et al.,* 2003).

Hence, based on the therapeutic potential as mentioned above, 8 different species of *Citrus* i.e. *C. maxima* (Merr.), *C. macroptera* (Mont.), *C. limon* (L.) Osbeck, *C. medica* (L.), C. sinensis((L.) Osbeck, *C. reticulata* (Blanco), *C. reshni* (Merr.), *C. unshiu* (Yu. Tanaka ex Swingle) and two other Rutaceae members i.e. *Zanthoxylum budrunga* Wall (Syn: *Zanthoxylum rhesta* Roxb.), and *Glycosmis pentaphylla* (Retz.) DC found in different parts of North Bengal were employed in the present study. Therefore, keeping all these in mind an initiative was undertaken to investigate the leaves of these 8 species with the following objectives:

- Plant selection and maintenance of germplasms.
- Qualitative and quantitative assays of different phytochemicals present in selected species of *Citrus* leaves.
- Assessment of antioxidant profiling i.e. phytochemical screening of different *Citrus* leaf extracts prepared from different extracting solvents ranging from non-polar to polar determines the best solvent.
- Carried out further experiments in that solvent which gave the best result in phytochemical screening.
- Determination of active constituents by GC-MS analysis.
- Determination of cytotoxicity of selected extracts.

- Assessment of antimicrobial activity of selected species of *citrus* leaves against several bacterial strains.
- Anti-inflammatory effect of selected species of *Citrus* leaves.
- Anticovid activity of *Citrus macroptera*.
- Characterization and physiochemical activity evaluation of nanoparticles from selected Rutaceae members.
- Molecular documentation

To study the genetic diversity by barcode primer (matK) of the chloroplast genome, and Random amplified polymorphic DNA analysis of different species of *Citrus* leaves.