# Chapter –1

# Introduction

Plants are the foremost valued entity of living beings in day-to-day life. They provide all needs of human civilization in terms of shelter, clothing and food along with the varied forms of the basic medicines and medicinal systems (Gurib-Fakim 2006). The upgradation of civilization has made human beings progressively curious about the secrets of nature including various medicinal plants (Cohen 1992). The keen observations of people on nature is a significant age old practice. These experiences of people through the ages have lead to discovery of numerous valuable medicine as well as other economically important plants (Lewis and Elvin-Lewis 2003). It is a constant endeavor since time immemorial for identification and application of various medicinal plants for various diseases and disorders The medicinal properties of the plants are due to some chemicals that produce certain physiological actions on the living bodies. These are the chemicals which have protective or preventive actions against the diseases (Krishnaiah et al. 2009). World Health Organization supports the use of traditional medicines which are proven as efficacious and safe to use. According to the WHO, about 65 -80% of the world population in developing countries depend on the plants and plant based compounds for their primary health care (WHO 1985).

The topography and the climatic conditions play an important role in the availability of the specific important floras (Smit and Pilifosova 2003). The territory of Kokrajhar district, Assam, India has a vast tract of land builds up in the zone of Himalayan sub-mountain area. The foothill area covered by dense evergreen and semi-evergreen forest has rich flora and fauna. Forest of the district has perennial sources of various wild medicinal as well as vegetable plants. The dominant tribal community ‘Bodo’ in the region is mainly agrarian. They are mostly the inhabitants of the villages of nearby jungles (Paul et al. 2011). With their venerable experiences, tribal people

possess important medicinal knowledge whether it is an individual or combined formulations from different medicinal plants. For the time being, many village folk medicine practitioners (Bodo Oja) use different therapeutic procedures for various diseases and disorders (Kalita et al. 2013). The traditional uses of plants or the plant parts against the varied diseases and disorders have been an inherent practical knowledge in the culture and livelihood of the people (Mao et al. 2009).

Sushruta Samhita defined that the health is 'the equilibrium of the three biological humors (doshas); the seven body tissues (dhatus), proper digestion and a state of pleasure or happiness of the soul, senses and mind' (Torwane et al. 2014). A balance among the three doshas is necessary for health. Together, the three doshas govern all metabolic activities. When their actions in our mind and body constitution are balance, we experience psychological and physical wellness. When they go slightly out of balance, we may feel uneasy. When they are unbalanced, symptoms of sickness can be observed or experienced (Sharma and Dash 1981).

Our body is comprised of billions of cellular molecules that are held together by electronic bonds. The disturbance in the pro-oxidants and antioxidant balance may lead to potential damage to tissues which is known as oxidative stress. It results in an imbalance between free radical production and antioxidant defense (Kelly 2003). Due to the different factors like - metabolism, poor diet, pollution, drugs, radiation, stress, injury, aging, infection, cigarette smoke, herbicides, lack of exercise, lack of sleep, etc., the weak bonds of cellular molecules split apart which resolve to the unpaired and unstable molecules known as free radicals. These unpaired unstable free radical molecules attack healthy nearby molecules of the cell and capture an electron from the normal healthy paired bonding molecules. The healthy molecule looses an electron from the paired bonding and becomes other unstable unpaired free radicals. It leads to a chain reaction and results in destruction of millions of cellular molecules which leads to the damage of surrounding cells and tissues of the living body. Oxidative stress is accompanied by different disease as diabetes mellitus, cancer, heart

disease, arthritis, autoimmune disease, damage of DNA and several other diseases (Baynes 1991).

Diabetes mellitus or hyperglycemia is a condition in which a disproportionate amount of glucose circulates in the blood plasma. Hyperglycemia is a metabolic disorder with instability of carbohydrate, fat and protein metabolism resulting from defects in the secretion of insulin or the function of insulin or both. Type 1 diabetes mellitus (T1DM) results if pancreas does not produce enough insulin and when the body cannot effectively utilize the insulin it produces type 2 diabetes mellitus (T2DM) (Costa et al. 2017). It is well known that the secretion of insulin occurs when pancreatic β-cell utilize glucose to generate adenosine triphosphate (ATP) from adenosine diphosphate (ADP) (Affourtit and Brand 2006). The resulting increase in cytoplasmic ATP/ADP ratio closes ATP-sensitive potassium channels, causing depolarization of the plasma membrane, which activates voltage – dependant $Ca^{2+}$ channels. This results in elevation of the intracellular $Ca^{2+}$ concentration which triggers insulin secretion (Ashcroft and Rorsman 2004). It is associated with the damage to a wide range of molecular species including lipids, proteins and nucleic acids. The short term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins and excessive exercise. These injured tissues enhance free radical generating enzymes (xanthine oxidase, lipoge-nase, cyclooxygenase), activation of phagocytes, release of iron, copper ions or a disruption of the electron transport chains of oxidative phosphorylation, excess ROS implicates the induction and complications of diabetes mellitus (Lobo et al. 2010). The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs (DCCTRG 1993). The metabolic syndrome of a unifying link between diabetes mellitus and its complication includes nephropathy, liver dysfunction, retinopathy, neuropathy and loss of immune potency and ultimately death (Hutchenson and Rocic 2012).

Epidemiology of 2015 record showed that among the 7.3 billion total populations of worldwide, an estimate of 415 million people had diabetes

which has globally 8.8% prevalence with uncertainty interval. From 2012 to 2015 approximately, 1.5 to 5.0 million deaths each year resulted from diabetes mellitus. The global economic cost of diabetes mellitus in 2015 was estimated to be U$ 673 billion (Ogurtsova et al. 2017). Importantly, the current epidemiology profile of diabetes mellitus reflects globally escalating tendency. The rate of mortality due to diabetes mellitus occupies $8^{th}$ position among the causes of the death (Afifi et al. 2017). Globally, an estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980 which has nearly doubled from 4.7% to 8.5 % in the adult population. The prevalence of diabetes mellitus has risen faster in low and middle income countries than in high income countries (WHO 2017).

Many scientific findings have revealed a link between pathogenecity of long term complications of diabetes mellitus due to free radical mediated oxidative stress. Most free radicals in biological system are oxygen derivatives, i.e. reactive oxygen species (ROS) but there also exist nitrogen derivatives i.e. reactive nitrogen species (RNS). Among ROS, superoxide anion ($O_2^-$) and hydroxyl ions ($OH^-$) are the predominant free radicals. Hydroxyl ions, the most powerful free radical generate *invivo* during the degradation reduction of superoxide and hydrogen peroxide catalyzed by transition metal ion (Valko et al. 2006). Hydroxyl ions ($OH^-$) also produced by Haber–weiss reaction where superoxide and hydrogen peroxide reacts directly. The reactive intermediates like ferryl and perferryl may form other than $OH^-$ ions because *invivo* transition metals (such as iron and copper) do not exist freely and remain bound with other molecues. In pathological conditions like inflammation, ischemia reperfusion there is a breakdown of iron binding protein, hampering the protective iron chelating effect by releasing iron that participate in the production of reactive $OH^-$ radicals which in turn contribute to tissue damage (Halliwell et al. 2000). Several exogenous environmental factors also promote free radical formation in the biological system. Xenobiotics like paracetamol or degradation catalyzed by flavoproteins like cytochrome $P_{450}$ (Karapanayiotides et al. 2004) often

results the formation of superoxide anions which induce hepatic injury. Atmospheric pollutants like ozone, nitrogen dioxide cause a damage to alveolar lining through free radicals generation which leads to the respiratory diseases. A healthy diet, regular physical exercise and maintaining a normal body weight in addition to medical treatment seem important management practices for diabetes mellitus (Georgsson and Staggers 2016).

Antioxidants are noteworthy key to defend against the free radicals. The body constantly needs a reservoir of antioxidant molecules to prevent or reverse the destructive stresses of free radicals. Antioxidant molecules have extra electrons that can donate an electron to the unstable free radical to neutralize by stabilizing the chemical bond. By giving way of an electron, the antioxidant molecules itself do not become an unstable free radical. Antioxidant travels through the blood vessels to reach the damaged cells (Pham-Huy et al. 2008). Healthy dose of antioxidants assures to minimize the destructive oxidative processes as cancer, heart disease, autoimmune disease, diabetes and many other complications (Bhusal 2016).

Many medicinal plants have been extensively screened for various bioactive compounds that lead to the discovery of the new drugs which are effective in treating various diseases (Lachance et al. 2012). Many plants are good sources of natural antioxidants which have lesser side effects and low cost of collection. Therefore, the natural products of plant origin have been proposed as a potential source of nutraceuticals (Shahidi et al. 1992). Traditional medicines obtained from medicinal plants are exercised by about 60% of the world population. The popularity of herbal treatments are increasing world wide as the complementary and alternative medicine (CAM) and traditional medicine (TM) due to the less side effects and low cost in comparison to modern medicines (Furnham and Vincent 2000). The use of traditional medicine remains widespread in the developing countries and the complementary and alternative medicines are increasingly used in the developed countries (WHO 2002).

To reduce the ill effects of diabetes mellitus and its secondary

complications, various formulations are already prepared (Modak et al. 2007). The various herbal ingredients are frequently given for treatment of diabetes mellitus (Al-Rowais 2002). Many herbs are mainly useful for antidiabetic formulations (Jarald et al. 2008) which are comprised of huge antioxidative properties that captivate the complications of diabetes mellitus (Modak et al 2007). The hypoglycemic action of herb has reported to stimulate or regenerate the effect on beta cells of pancreas to enhance insulin secretion (Weisberg et al. 2016).

The Cucurbitaceae is one of the most important family among the vascular plants which is employed as a vegetable as well as medicine (Lira et al. 2002). Cucurbitaceae is a moderately large family of about 130 genera and 900 species. All members of cucurbitaceae are frost sensitive and the family is confined to the warmer parts of the world. The family is most abundantly represented within the tropics, especially in tropical Africa and the neotropics (Jeffrey 1980). Most of the plants of cucurbitaceae family are annual vines, some are woody lianes, thorny shrubs, or tree (Dendrosicyos). Many species of the family bears the hairy and pentangular stem. Tendrils are present with the leaf petioles at the stem nodes. Leaves are exstipulate, alternate, simple, palmately lobed or palmately compound. The flowers are large, yellow or white in colour, unisexual (dioecious) or bisexual (monoeciuos). The female flowers have inferior ovaries. The fruit is a kind of modified berry called pepo (Zienkiewicz et al. 2012).

***Hodgsonia heteroclita*** (Roxb.) Hook.f. & Thomson is an extensive fruit bearing vine belonging to the family Cucurbitaceae. It is a semi woody, dioecious, perennial, climber and grows up to 25-30m height. Leaves are simple, 3-5 lobed and coriaceous. Flowers are reddish-yellow outside while whitish within the corolla, conspicuously veined, pendulous, female flower are solitary while male flower raceme. Fruits are pomiform, compressed globose and reddish brown (on maturity). Seeds are usually 6 in number, ellipsoid covered with woody wrappings embedded in spongy and juicy pulp. Its flowering starts from March; however the ripe fruits are available

during October to January (Hu shiu-ying 1946; Semwal et al. 2014). Male plant blossoms flower only for single night and are recorded to be pollinated mainly through *Herse convolvuli*. Natural pollination efficiency seems very low, only few flowers are pollinated and most flowers are aborted (Schreitera et al. 2007).

*Hodgsonia heteroclita,* popularly known as Chinese lard plant, is locally addressed as Hagrani jwgwnar by the Bodos of Kokrajhar district of BTC, Assam, India (Swargiary et al. 2013). It is closely associated with the life of the tribe of Northeast India. The seeds are consumed by the tribal people in different forms as either wholly or with other accessories like meat, fish or along with other edible vegetables. This plant is not only valued as food but also have significant medicinal values. The crushed seeds are used for the treatment of intestinal worms and the fruit pulp is used to prevent bacterial infections. The leaf juice is used on fresh cuts and wounds for rapid healing. The seed oil is much valued for the base formulation of many eastern medicines (Semwal et al. 2015). Apart from these, the fruit pulp is used as a remedy against diabetes since a time long (Swargiary et al. 2013).

The indigenous medicinal systems are receiving an increased attention in medical research. Human diseases like diabetes mellitus, arteriosclerosis, ischemic injury, cancer, neurodegenerative diseases and the processes like inflammation and ageing are global problems that are known to be caused

***Hodgsonia heteroclita*** is classified as the following taxonomic units-

**Kingdom**: Plantae

**Unranked**: Angiosperms

**Order**: Cucurbitales

**Family**: Cucurbitaceae

**Sub- family**: Cucurbitoideae

**Tribe**: Trichosantheae

**Subtribe**: Hodgsoniinae C.Jeffrey

**Genus**: *Hodgsonia* Hook.f.& Thomson

**Species**: *H. heteroclita* (Roxb.) Hook.f. & Thomson.

or enhanced by free radicals (Finkel and Holbrook 2000). Inhibition of the release of free radical is a potential strategy to control the damage of tissues and organs. The rich antioxidant plants are gaining importance in treating diseases due to the free radicals scavengers. Numerous herbs are availed for the treatment of varied ailments, but unfortunately little is known about their antioxidant properties. The supplements of dietary intake of natural antioxidants are suggested to people that can help to scavenge the free radicals to protect the body against various diseases (Young and Woodside 2001). In India, although the medicinal plants have been extensively used, the traditional medicines have never been investigated in detail for their free radical scavenging antioxidant properties. A systematic study in these lines signifies to understand the sum mechanism of action of plants and plant formulations.

The objectives of this research has been based on the following heads.

- To elucidate phytochemical and *invitro* antioxidant properties of substractive extracts.
- To evaluate anti-hyperglycemic effect of *Hodgsonia heteroclita* fruit pulp on diabetes mellitus induced male Wistar rats.
- To determine antioxidant enhancing capacity of herbal extracts of *Hodgsonia heteroclita* fruit pulp in Alloxan induced diabetic rats by measuring the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx).
- To assess the antiperoxidative and antioxidative efficacy of *Hodgsonia heteroclita* fruit pulp by estimating the concentration of malondialdehyde (MDA) and protein carbonyl content, a biomarker of lipid peroxidation and protein oxidation.
- To understand the effect of *Hodgsonia heteroclita* fruit pulp by Histopathological studies of pancreases on diabetes by comparing *Hodgsonia* treated diabetic rats with the insulin and Glibenclamide treated rats.

Although some inventory tests have been conducted to record the traditional uses, till date only a few

scientific evidences have been substantiated. Thus, *Hodgsonia heteroclita* virtually remains unexplored on scientific grounds. Thus, this study has attempted to estimate the biochemical constituents of *Hodgsonia* fruit pulp. It has validated the effective potential for the antioxidative and anti-hyperglycemic effects in the living organisms.

Since this plant is virtually unexplored, it also validates the inventory works for the new bioconstituents or the formulations for the modern pharmacological science.