Chapter 1
Introduction

"Look deep into nature, and then you will understand everything better"
-Albert Einstein

Man has been dependent on nature for their survival since time immemorial. This dependency led the indigenous people living in harmony with nature to evolve a unique system of medicinal plant practices (Teron and Borthakur, 2014). This new branch of science is known as "*Ethno-botany*". The traditional knowledge of medicinal plants in India has been accumulated in course of many centuries based on several ancient therapeutic systems, including Ayurveda, Unani and Siddha (Lone and Bhardwaj, 2013). According to the survey report of World Health Organization (WHO) (World Health Organization, 2002), 80% people of the developing world use plant remedies for several therapeutic purposes.

India, one of the richest floristic regions of the world, has diverse socioeconomic, ethnic, linguistic and cultural areas. There are about 54 million indigenous people of different ethnic groups colonizing various regions of the country. The aboriginal groups have their own distinctive culture, religious rites, food habit and a rich knowledge of plant utilization (Mahishi *et al.*, 2005; Boro and Sarma, 2013) which pass orally generation to generation. Therefore, the traditional knowledge of medicinal plants and their use in treating several ailments might reasonably be expected in India due to its rich floristic vegetation (Shil *et al.*, 2014). Chandel *et al.* (1996) have reported that nearly 70% of tribal and rural inhabitants of India are to a large extent depended on medicinal plants for their primary healthcare management due to either insufficient or inaccessible or less availability of modern healthcare

system. Virtually, ethnobotanical survey may be regarded as one of the most reliable approaches towards new drug discovery and it is a prerequisite for any developmental planning concerned with the welfare of tribal and their environment (Lokho and Narasimhan, 2013). Nonetheless, in recent times medicinal plants became the backbone of herbal drugs being used over the world wide.

Medicinal plants are also well-documented for their eminent curative properties that prevent alterations in human body due to presence of rich polyphenols (Justesen and Knuthsen, 2001). The polyphenols are the antioxidants with redox properties that act against reactive oxygen species (ROS) and reactive nitrogen species (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. In addition, polyphenols play an important role against oxidative stress which is related to the pathogenesis of various important diseases (Finkel and Holbrook, 2000). However, human body has its own inherent antioxidative mechanism by which it exerts several biological functions such as the anti-mutagenic, anti-carcinogenic, anti-aging, anti-immunosuppression and anti-neurodegeneration responses (Gocer and Gulcin, 2011). An imbalance between ROS and the inherent antioxidant capacity of the human body leads to the use of dietary and/or medicinal supplements particularly during the disease attack. This ROS are mainly originated from molecular oxygen as a result of normal cellular metabolism and also from the mitochondrial electron transport chain (ETC). Besides, FasL, a type II membrane protein belonging to the tumor necrosis factor (TNF) family, has also been reported to be associated with ROS generation causing necrotic cell death (Medan *et al.*, 2005; Vercammen *et al.*, 1998) while NADPH oxidase, a membrane bound enzyme complex was also involved to yield different ROS (Han *et al.*, 1998; Pham-Huy *et al.*, 2008). Simultaneously, toll like receptors (TLR) can also induce the ROS production (Marcato *et al.*, 2008) which trigger the signals for cell apoptosis.

A plethora of evidences suggested that herbal plants, vegetables, and fruits own antioxidative compounds such as phenolics, flavonoids, tannins, and proanthocyanidins which alleviate or neutralize the free radicals. Moreover, the intake of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Gulcin, 2012). However, several synthetic antioxidants namely, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) etc. available in the market that have been reported to show adverse health effects (Shahidi and Zhong, 2005). Hence, there is an emerging tendency to shift towards naturally occurring antioxidants for the prevention

and treatment of diseases as well as maintenance of human health (Halliwell and Gutteridge, 1981).

Cognitive disorders (CDs), one kind of abnormalities that affect brain's capability to remember and process information (Trivedi, 2006) at late age, could be balanced by the antioxidative defense system. CDs include dementia, amnesia, Alzheimer's disease (AD), Parkinson's disease (PD), trauma, seizures, and other neurodegenerative disorders (NDs) (Chattipakorn *et al.*, 2007). Dysfunction of dopaminergic neurons, cholinergic abnormalities, mitochondrial dysfunction and extensive neuronal loss in brain are the main factors of occurring NDs. In addition, ROS generates continuously in brain leading to a progressive accumulation of cellular damage which is correlated with the onset of AD and PD (Gandhi and Abramov, 2012). AD is believed to be linked to a deficiency in the brain neurotransmitter, acetylcholine (ACh). Inhibition of acetylcholinesterase enzyme (AChE) is a rational pathway for the systemic treatment of AD (Prince *et al.*, 2013). PD, the second most common neurodegenerative disorder after AD, is characterized by resting tremor, bradykinesia, muscular rigidity, and postural imbalance occurring due to progressive death of substantia nigral cells leading to dysfunction of dopaminergic neurons (Chattipakorn *et al.*, 2007). A recent survey reflected that about 35.6 million people lived with dementia worldwide in 2010 which is likely to be doubled by 2030 (Prince *et al.*, 2013). Hence, neurodegenerative disorder has emerged as a great public health concern, thereby demand intervention to ameliorate oxidative stress. Several approved drugs including donepezil, tacrine, rivastigmine, galanthamine etc., to some extent, alleviate the symptoms of cognitive impairments. However, their chronic use is often associated with exerting side effects (Chattipakorn *et al.*, 2007). Herbal formulations on the other hand have been documented effective against several cognitive disorders so far (Mathew and Subramanian, 2014).

Correspondingly, diabetes, a metabolic disorder, is progressively affecting a large number of populations and fatally reducing their quality of life. Utilization of conventional medicines in diabetes management is quite expensive as well as unreasonable to most of the patients. Furthermore, these drugs exert adverse side-effects due to chronic use. Inclusion of herbal remedy into conventional healthcare system may considerably improve the overall healthcare system. The advantage of herbal remedy over modern medicines is that most of the herbal medicines are plant-based and comparatively cheaper, possess fewer or negligible side-effects owing easy acceptability.

Therefore, the study on medicinal plants

and vegetables strongly supports the idea that plant constituents (i.e. phenolics, flavonoids, tannins, proanthocyanidins etc.) along with antioxidant properties are capable of exerting protective effects against oxidative stress, diabetes, cancer, neurodegeneration, aging etc. Approximately 3,000 plants species of more than 200 families were identified to have medicinal properties in the region of Eastern Himalayas, Western Ghats and Andaman & Nicobar Island of India (Prakasha *et al.*, 2010). Out of several medicinal families, Mimosoids contributes a major position in the management of several diseases and/or disorders (Joy *et al.*, 1998). In addition, recent surveys made by (Saha *et al.*, 2014a; Saha *et al.*, 2014b), exhibited their medicinal importance not only as a remedial for human being but also in the management of domesticated animals and birds.

According to Cronquist (1981), Mimosoids have been usually recognized either as the family Mimosaceae or as the subfamily Mimosoideae within the family Fabaceae (Leguminosae) of the order Fabales. Mimosoideae or Mimosaceae consists of about 80 genera and 3,370 species of trees, shrubs, and lianas found mainly in tropical, subtropical, and warm temperate regions of the world (Luckow *et al.*, 2003). The members of Mimosoideae are chiefly characterized by their valvate aestivation of petals, bi-pinnate leaves, regular flowers grouped into spicate or capitate inflorescence with flat brown pods. Economically, they have been acknowledged as food and fodder (e.g. *Pisum sativum*, *Glycine max* etc.), oil (e.g. *Arachis hypogea*, *Glycene soya* etc.), fruits (*Tamarindus indica*, *Phaseolus coccineus* etc.) and timber (e.g. *Dalbargia sisso*, *Acacia auriculiformis* etc.). Apart from these, most of members of Mimosoideae have been distinguished to comprise several medicinal properties. The genera like *Mimosa*, *Acacia* and *Albizia* under Mimosaceae were extensively reported to have several medicinal properties including anti-diuretic, anti-dysenteric, anti-diarrhoerial, anti-asthmatic, aphrodisiac, analgesic, antidepressant and antioxidant properties (Ahmad *et al.*, 2012; Ali *et al.*, 2012; Anonymous, 2001; Saha *et al.*, 2016). In fact, these species are characteristically used by the ethnic people as a source of polyherbal formulations in the management of several diseases in different parts of India (Ali *et al.*, 2012; Anonymous, 2001). The traditional classification of these three genera is as follows:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Fabales
Family : Fabaceae
Sub-family : Mimosoideae/ Mimosaceae
Genus : ***Mimosa*** L.
Acacia Mill
Albizia Durazz

Mimosa, (Mimosoideae; Fabaceae) the large monophyletic genus of flowering plants, consists of >500 species, mainly native to the New World (Simon and Proenca, 2000). It is native to South America and Central America and regarded as an invasive species in Tanzania, South Asia, South East Asia and many Pacific Islands (Shelef, 1984). The species vary in habit from tall trees and shrubs to vines and herbs and they are found in a wide variety of habitats from wet to dry, growing on many different soils. Amongst several species, *M. pudica, M. invisa* (Syn: *M. diplotricha*), *M. pigr*a and *M. tenuiflora* are the most familiar species that have been found to possess several medicinal properties in India (Ahmad *et al*., 2012; Joseph *et al*., 2013; Joy *et al*., 1998). *M. pudica* is locally known as Lajjaboti (লজ্জাবতী) or touch-me-not whereas *M. invisa* is called as Swet-lajjaboti (শ্বেত লজ্জাবতী) in Bengali. They are usually found alongside the road of entire Bengal. On the other hand, hardly a report was found regarding the molecular documentation of *Mimosa*.

Acacia, one of most economically important genera under Mimosoideae, is consisting of about 980 species of trees and shrubs that can be found in Australia, and in the tropical and subtropical areas of America, Asia, Africa and Europe (Lewis, 2005). Different Australian species, like *A. mangium*, *A. mearnsii* and *A. saligna* are economically important and are widely planted for wood products, tannin, firewood and fodder. Another African species, *A. senegal*, is the chief source of gum arabic while the Afro-Indian species *A. nilotica* and *A. catechu* are planted in India and elsewhere, as a source of wood, khair and stock fodder. Moreover, gum arabic, catechu or kath or khair, tannins from bark, essential oils from flowers and seeds are mainly used in the industry of pharmaceuticals, preservatives, beverages, confectionery, adhesive, dye, perfumes, cosmetics etc. In addition, acacia woods are mostly used for the manufacture of floorings, furniture, toys, jewellery and tools. Besides, acacias are well-known agents of agro-forestry by increasing nitrogen content of the soil through their interaction with symbiotic bacteria. Despite of immense economic worth, many of *Acacia* species show numerous medicinal properties. In India, acacias, most notably *A. nilotica* (বাবলা), *A. catechu* (খয়েড়), *A. concinna* (শিকাকাই), *A. auriculiformis* (আকাশমণি) etc. are being used in the management of several ailments such as conjunctivitis, haemaptysis, catarrh, cough, pruritus, leprosy, leucoderma, skin diseases, helminthiasis, anorexia, diarrhea, dysentery, ulcers and wounds, eczema, haemoptysis, haematemesis, hemorrhages, fever, anemia, and diabetes (Asolkar *et al.*, 1992; Jain, 1994; Khare, 2008). However, there are some controversies over the past decade generated on the scientific use of

the generic name *Acacia* due to its broad systematic position as well as similar kind of characteristics with different species and still it is a confusing one after the XVIII-International Botanical Congress in Melbourne (Smith and Figueiredo, 2011).

Albizia is a large fast-growing genus comprising approximately 150 species that are widely distributed in Asia, Africa, Madagascar, America and Australia. Albizias are basically known for production of fuelwood and timber. However, some species like *A. lebbeck*, *A. amara*, *A. saman*, *A. ferrugine*a etc. are some medicinally important species used to treat rheumatism, stomachache, cough, diarrhea, wounds, and as an anthelmintic (Singab *et al.*, 2015).

Evidences suggested that a few preliminary works including antioxidant activity, anti-aging, anti-diarrhea, anti-asthmatic, hypoglycemic, anti-tumour etc. have been done with selective members of *Mimosa*, *Acacia* and *Albizia*. However, no attempt was made to support the ethno-pharmacological claims. Even though, in-depth medicinal properties showing a comparative and comprehensive information of Mimosoids is still missing.

More surprisingly, it has further been observed that there has no sufficient information illustrating the comprehensive genetic variation among the medicinal members of Mimosaceae within the order Fabales. Since the morphological variation between two closed species is difficult to distinguish; an appropriate knowledge of molecular documentation would be a rational way to understand the genetic relationship among different families. Hence, an initiative step was carried out to explore the genetic variations of some medicinal Mimosoids through DNA fingerprinting techniques. Of the various DNA fingerprinting techniques developed for plant research, random amplified polymorphic DNA (RAPD) analysis has become increasingly popular which are being used to evaluate the genetic relationship among species, cultivars and varieties (Williams *et al*., 1990). Besides, restriction fragment length polymorphism (RFLP) analysis is also used to investigate the phylogenetic relationships among species. Subsequently, a new modified molecular technique i.e. DNA barcoding was developed recently to explore the evolution, identification and genetic relatedness of unknown plants and animal species resolving various anomalies in the taxonomic levels by using a short stretch of DNA sequence (Hebert and Gregory, 2005).

Hence, based on the therapeutic appraisal as mentioned above, 9 different medicinal species under Mimosoideae including, *Mimosa pudica* L., *M. invisa* Mart. Ex Colla, *Acacia nilotica* (L.) Delile, *A. nilitica* var. *indica* (Benth.) A.F. Hill., *A. catechu* (L.f.) Willd, *A. concinna* (Willd.)

Fig. 1.1. Map of the study area. The spots (*) represent the places of plant collection sites in the northern parts of Bengal province (consisting of three districts) in West Bengal, India.

DC., *Albizia lebbeck* (L.) Willd., *A. chinensis* (Osbeck) Merr. and *Samanea saman* (Jacq.) Merr. (Syn: *Albizia saman*), found in different territories of West Bengal in India (Fig. 1.1), were employed in the present study. Hence, an effectual initiative was undertaken to explore the varied medicinal properties of these 9 species with the following objectives:

- Selection and collection of medicinal members of the family Mimosaceae from different parts of North Bengal.
- Documentation and analysis of ethnic knowledge of Mimosoids.
- Profiling of medicinal and bio-chemical properties of selected Mimosoid extracts.

  I. Contouring of antioxidant activities and other medicinal properties.

II. Screening of correlation patterns between different antioxidant traits used.

- In-depth study of medicinal properties of selected plant including anti-diabetic activity.
- Evaluation of plant extract against cognitive impairment including behavioral trial on mice.
- High throughout phytochemical analysis using FT-IR, GC-MS and NMR analysis.
- Selection of target compounds for novel drug discovery targeting using *in-silico* approach.
- Determination of cytotoxicity of extracts.
- Isolation, characterization and molecular documentation of selected Mimosoids and its micro-symbionts (*Rhizobium* sp.).