Chapter 2
Review of Literature

"Study the past, if you would define the future."
-Confucius

The recent vogue on plant research has enlightened all over the world and a plethora of evidences have been collected to confirm the immense potentiality of medicinal plants used in traditional healthcare systems. Several medicinal plants have been identified and studied using modern scientific approaches for their phytochemistry. Selected members of Mimosoideae have already been remarked as valuable medicinal plants, which are rigorously used in Ayurvedha and Unani systems of medicine. Previous phytochemical reports on some species of Mimosaceae have already revealed their probable role in medical care.

This chapter, besides representing a general overview, considers selected members of Mimosoideae from the perspectives of Ethnobotany, Parmacological activities, Antioxidant activities, Neuroprotective activity, Phytochemistry, focusing also on Molecular documentation of selected taxa as well as on rhizobial diversity.

2.1. Brief history of Mimosoideae

Mimosoids form a major group within legumes. According to Cronquist (1981), Mimosoids have been usually recognized either as the family Mimosaceae or as the subfamily Mimosoideae of the family Fabaceae (Leguminosae) under the order Fabales. Mimosoideae consist 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 usually characterized by their bipinnately compound, alternate leaves; spicate or capitate inflorescence; bisexual flowers, rarely unisexual, actinomorphic, in tight clusters with numerous stamens and legume fruits. Besides, most of the species under Mimosaceae exhibit an associationship with nitrogen fixing bacteria.

2.2. Ethnomedicinal studies

'Ethnomedicine' may be defined as the sum of knowledge of plants, skills and

practices based on the oral theories, beliefs, and experiences curing diseases and disorders by native people belonging to different culture (Tamuli and Saikia, 2004). Several plants are being used to treat different ailments since time immemorial. Utilization of different plant species of different families like, Asteraceae, Poaceae, Malvaceae, Mimosaceae etc. were mentioned in Indian traditional and ayurvedic medical system to alleviate dysentery, inflammation, burning sensation, asthma, leucoderma, leprosy, cholera, vaginal and uterine complaints, bile, bilious fevers, piles, jaundice, leprosy, bronchitis, cold and cough, fatigue, blood diseases etc (Kirtikar and Basu, 2006). Selected members of Mimosaceae, especially, *Mimosa pudica*, *M. hamata*, *Samanea saman*, *Prosopis cineraria*, *Parkia biglandulosa*, *Albizia procera*, *Acacia senegal*, *A. chundra* etc. were reported to be used as ethnomedicine by local people of the Idar-Vadali forest area of Sabarkantha district of India to treat rheumatoid arthritis, fever, headache, piles, fistula, swellings, diarrhea, diabetes, cataract, hydrocele erysipelas, ulcer, leucorrhoea and also used as anti-venom agent in case of scorpion sting (Patel and Jangid, 2013). An ethnomedicinal report from Araku valley Mandalam, Visakhapatnam district, India claimed the effectiveness of Mimosaceae in local healthcare management (Padal and Sathya vathi, 2013). Besides, ethnomedicinal survey in Caprivi region of Namibia (Chinsembu and Hedimbi, 2010) reflected the beneficial effects 6 Mimosoids treating different HIV/AIDS-related opportunistic infections. Saini *et al.* (2008) mentioned the ethnomedicinal value of five acacias (*Acacia nilotica*, *A. tortilis*, *A. senegal*, *A. catechu* and *A. jacquemontii*) in Rajasthan of India which were regularly used to treat asthma, toothache, stomach complaint, skin infections, caugh, leprosy, indigestion and diarrhea. Simultaneously, selected members of Mimosaceae are also used by the indigenous people of Bargarh district of Orissa, India for their local therapeutic purposes (Sen and Behera, 2008). In continuation, Saha *et al*. (2014a) described the use of *M. pudica* (root and leaf decoction) as a remedy of leucorrhea and breast cancer whereas *A. nilotica* was found to have anti-diabetic property (Saha *et al.*, 2014c) in Malda district of West Bengal, India.

2.3. Medicinal properties of selected Mimosoids

Plants have been exploited for the management of diseases for centuries because of their very limited adverse effects. Therefore, their scientific evaluation is a logical way of searching new drugs. Moreover, 80% of world population depends entirely on herbal medicines prepared almost exclusively from plants. Numerous indigenous medicinal plants have been found to be

successfully used to control different ailments, which can counter the high cost and poor availability of the current synthetic drugs for many rural populations in developing countries like India. Consequently, different medicinal members of Mimosoideae have already been evaluated for their various pharmacological activities followed by isolation of active principles. In this section, an overview of different pharmacological activities of selected Mimosoids is summarized below:

2.3.1. Antimicrobial activity

Antimicrobials are such kind of substances that kill microorganisms or inhibit their growth. Numerous plants are being used as antimicrobial agent since the beginning of human civilization. Likewise, plenty of plants under Mimosaceae were studied for their antimicrobial activity. Genest *et al.* (2008) reported a comparative account of antibacterial and antioxidant activities of dichloromethane (DCM) and methanolic extracts of stems of *Mimosa rubicaulis* and *M. pudica* exhibiting considerable encouraging activity against *Bacillus subtilis*, *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In addition, potent antibacterial activity of *M. pudica* was also tested by Balakrishnan *et al.* (2006). The ethanolic leaf extract of *Acacia nilotica* effectively showed highest zone of inhibition at 70mg/ml extract concentration against the bacteria *Campylobacter coli*, isolated from goats (Solomon-Wisdom and Shittu, 2010). Antibacterial activity of *A. concinna* bark was also observed against *P. aerogenosa* and *S. aureus* (Vergeese and Sivaraj, 2012). Furthermore, the antimicrobial activity of the *A. nilotica* against several bacterial strains was also examined by Khan *et al.* (2009) while ethyl acetate extract of *Albizzia lebbeck* leaves confirmed successive antibacterial activity against gram positive and gram negative bacteria (Rahul *et al.*, 2010). Antibacterial activity and antifungal activity of *Samanea saman* leaves were also noticed against *Pseudomonas aeruginosa*, *Fusarium solani* and *Trichophyton longifusus* (Azhar *et al.*, 2009).

2.3.2. Anti-inflammatory activity

Inflammation may be regarded as a complex pathophysiologic process and can be initiated in response to injury involving the accumulation of cells and exudates in the affected tissues (Markiewski and Lambris, 2007). Several plants were being applied therapeutically for many years leading to the production of major anti-inflammatory drugs. Therefore, natural products with anti-inflammatory activity are a chief concern in the present circumstance. Studies with *M. pudica* leaves revealed significant (p<0.05) inhibitory activity in a dose-dependent manner than the standard drugs, indomethacin when it was tested on carrageenan-induced paw oedema and cotton pellet granuloma in rats (Goli *et al.*,

2011). In addition, similar type of experiment with ethanolic leaf extract of *M. pudica* was executed by Mistry *et al.* (2012) and the result was found to be significant against acute and chronic inflammation. Catechin, isolated from *Acacia catechu* revealed the inhibition of Cyclooxygenase (COX) and 5-Lipoxygenase (LOX) enzyme activity showing its anti-inflammatory potentiality (Altavilla *et al.*, 2009). Further, the butanolic fraction of *Acacia pennata* dried leaves exhibited significant protective effects against chemical stimuli (acetic acid and formalin) as well as an inhibitory effect in carrageenin-induced rat paw oedema in the late phase (Dongmo *et al.*, 2005).

2.3.3. Wound healing activity

Wound healing is an immune-mediated obscure process where the skin or other soft tissue repairs itself after injury. The healing process begins with the clotting of bloods as well as a set of biochemical actions takes place to repair the damage (You and Han, 2014). Numerous plants were explored in the management and treatment of wounds over the years as they promote the repair mechanisms in natural way. The methanolic extract of aerial parts and roots of *M. pudica* was found to be a potent candidate revealing greater wound healing activity in Wistar Albino rats compared to the standard drug Gentamicin (Kannan *et al.*, 2009). In addition, Kokane *et al.* (2009) reported noteworthy wound healing activity of ointment containing methanolic and aqueous extract of *M. pudica* root at 2% level in rat models. Concurrently, similar type of result was also found in bark extract of *Albizia lebbeck* (Gupta and Jain, 2010).

2.3.4. Antinociceptive activity

Pain, a subjective symptom, is affected by psychological factors which could be alleviated by different chemical agents by means of central or peripheral mechanisms. Different plant species have been considered as natural pain-relievers in search of new potent drugs. The methanolic extract of *A. catechu* was proved as a relieving agent from pain when tested in acetic acid-induced gastric pain mice models (Rahmatullah *et al.*, 2013). The aqueous extract of *Mimosa pudica* exhibited significant ($p<0.001$) inhibition of writhing response in acetic acid-induced animal model which might be due to the inhibition or reduction of proinflammatory mediators (Karthikeyan and Deepa, 2010). On the other hand, analgesic activity of a Brazilian native plant, *Abarema cochliacarpos* was evaluated in mice model by Silva *et al.* (2009) exhibiting higher activity of bark extracts than that of standard drug used.

2.3.5. Antidiabetic activity

Diabetes, a metabolic disorder critically affecting the population, is rapidly emerging as a major public health challenge in developing countries.

Although, several drugs and interventions are available to manage diabetes, in most of cases these are either expensive or show adverse effects like hypoglycemia. Therefore, there always remains a need to find an effective and safety drug for the treatment of diabetes. Despite of having decreased or nil adverse effects, natural products play a vital role in this regard. For example, the ethanolic leaf extract of *M. pudica* revealed significant decrease of blood glucose level in alloxan induced diabetic Wistar rats as compared to standard, Metformin whereas pet ether extract did not show any significant decrease in serum glucose level up to 7 days of treatment (Sutar *et al.*, 2009). Likewise, the methanolic extract of the pods and tender leaves of *Acacia nilotica* was found to be very beneficial to treat diabetes (Gilani *et al.*, 1999). Besides, significant reduction of blood glucose level was observed in diabetic albino rats at the doses of 250 and 500mg/kg body weight (p<0.001) by the application of ethyl acetate extract of *Acacia catechu* (Ray *et al.*, 2006). The bark extract of *Albizia odoratissima* also significantly (p< 0.01) reduced the levels of serum cholesterol, triglycerides, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase as well as the extract decreased the level of total proteins in alloxan induced diabetic mice suggesting their potent role as antidiabetic agent (Kumar *et al.*, 2011).

2.3.6. Diuretic activity

Diuretics are the drugs which increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids. Traditionally, several plants were claimed to be possessed diuretic properties, but lack proper clinical validation. However, a few reports are available regarding the diuretic activity of members of Mimosoideae. Sangma *et al.* (2010) revealed that the aqueous extract of *M. pudica* leaves was significant (at 100mg/kg) showing diuretic activity in normally fed albino rats with increased electrolyte excretion (p<0.01 for urine output, p<0.01 for Cl, p<0.05 for K^+, and p<0.01 for Na^+). The methanolic extract of *Albizia lebbeck* was administered to experimental rats at a dose of 200 and 400mg/kg for diuretic activity and obtained significant increase in the volume of urine and urinary concentration of Na^+, K^+ (Sivakumar *et al.*, 2013).

2.3.7. Antidiarrheal activity

Diarrhea is a frequent digestive chaos caused by enterotoxins of several bacteria. Indigenous systems of Indian therapeutic exhibited several numbers of medicinal plants to be used as antidiarrheal activity. For instance, the crude ethyl acetate extract of *A. catechu* at a dose of 250mg/ kg was found to be highly significant (p<0.001) against diarrhea in albino rats in respect of latent period of onset of diarrhea (Ray *et al.*, 2006) whereas the ethanolic

leaf extract of *M. pudica* inhibited castor oil induced diarrhea and PGE2 induced enteropooling in Wistar albino rats and found 200 and 400mg/kg was significant (p<0.001) dose (Khalid *et al.*, 2011). Besides, the bark powder of *A. nilotica* was found to be a potent inhibitor when castor oil and magnesium sulphate induced diarrhea in Swiss albino mice was studied by Misar *et al.* (2006). Similar type of result was also reported by Besra *et al.* (2002), when they tested the antidiarrhoeal activity of *Albizzia lebbeck* seeds employing conventional rodent models.

2.3.8. Antiulcer activity

Ulcers are the lesions on the surface of skin or a mucous membrane caused by superficial loss of tissue. Ulcer healing is a complex process involving the combination of wound retraction and re-epithelialization. Several medicinal plants within Mimosoideae were mentioned in ayurvedic medicine for their antiulcer properties over the years. For example, Vinothapooshan and Sundar (2010) reported the antiulcer activity of different extracts of *M. pudica* in aspirin, alcohol and pyloric ligation induced models of gastric ulcer in albino rats revealing ulcer damage suppression capability of all extracts (p<0.001). The aqueous extract of *A. catechu* heartwood significantly inhibited the formation of ulcers in the pylorus ligated rat models (Patankar *et al.*, 2011). Similar type of activity was also noticed in hydroethanolic extracts of young seedless pods of *A. nilotica* (Bansal and Goel, 2012).

2.3.9. Hypolipidemic activity

Hypolipidemic drugs are the substances that reduce the level of lipids and lipoproteins in the blood. The plant species namely, *M. pudica* exhibited potent hypolipidemic activity (p<0.05) against atherogenic diet in wistar albino rats by lowering the serum levels (Rajendran and Krishnakumar, 2010).

2.3.10. Hepatoprotective activity

Hepatoprotection is such type of capability to prevent the damage of liver. Natural products have been used traditionally for the prevention and treatment of liver disease. Scientific research on hepatoprotective activity has been supported the claims of the medicinal efficacy of several herbal compounds. Ethanolic (50%) extract of *M. pudica* exhibited significant hepatoprotection when the extract was examined on CCl_4-induced liver damage in Wistar albino rats (Kumar and Kumar, 2010). Additionally, methanolic leaf extract of *M. pudica* was also found to be hepatoprotective (p<0.05) by means of lowering of serum levels (Sohil and Sundaram, 2009). Subsequently, ethanolic extract of *Acacia concinna* pods exhibited significant hepatoprotective effect in $CC1_4$ induced liver damage rat model (Maqdoom, 2016). Further, significant (p<0.001) hepatoprotective activity was observed at a

dose of 250 mg/kg p.o. of ethyl acetate extract of *Acacia catechu* on albino rats after the administration of seven days (Ray *et al*., 2006).

2.3.11. Immunomodulatory activity

An immunomodulatory agent is a drug or inhibitor that may be used as an immunosuppressant or an immunostimulator based on its effect on the immune system. The logical design of novel drugs from traditional formulation offers new prospects in modern healthcare management. Ayurveda, one of the oldest traditional medical systems in India, reveals certain plants which strengthen the host immune system. For instance, the hot water extract and butanol fraction of *Albizzia lebbeck* bark successfully proved immunostimulatory effect against macrophage migration model as well as cell mediated arms of the murine immune system (Barua *et al.*, 2000). The aqueous extract of *Acacia catechu* stimulated the murine neutrophil adhesion and the phagocytic index. Additionally, the extract was found to be helpful in protection against cyclophosphamide induced neutropenia in murine system which was evident through its immunoglobulin production (Ismail and Asad, 2009). The ethanolic extract of *Albizia lebbeck* leaves have been reported to be exhibit strong immunomodulatory effect by increasing the swimming or survival time ($P<0.001$) and also decreased the writhing produced by glacial acetic acid ($P<.001$) employing swim endurance test and acetic acid induced writhing test model (Chaudhary *et al.*, 2012). Further, significant immunological adjuvant activities of saponin extracts from the pods of *A. concinna* was examined by Kukhetpitakwong *et al.* (2006).

2.4. Antioxidant activities of selected Mimosoids

Reactive Oxygen Species (ROS) or free radicals such as hydroxyl radical, singlet oxygen, superoxide anion and hydrogen peroxide play a chief role in the development of various diseases including atherosclerosis, heart disease, ageing, immunosuppression, and others (Young and Woodside, 2001). The free radicals in human body are produced by means of aerobic respiration or from exogenous sources and react with various biological molecules namely proteins, lipids and deoxyribonucleic acids resulting in the imbalance between oxidants and antioxidants. Moreover, oxidative stress or excessive oxidation of cellular substrates result in the formation of type II diabetes, neurodegenerative diseases, or even some types of cancer. The most effective pathway to reduce the action of free radicals causing oxidative stress is antioxidative defense mechanism. Antioxidants may be defined as the compounds inhibiting or delaying the oxidation of other molecules by means of reducing the initiation or propagation of

oxidizing chain reactions.

Medicinal plants play a key role in treating various human ailments due to presence of certain components of therapeutic value. The study done so far on medicinal plants and vegetables strongly supports the idea that plant constituents having antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Cao *et al.*, 1996). In fact, plant based drugs are an important resource of therapeutic agents with easier availability, relatively cheaper cost and non-toxic nature as compared to modern medicine. Various methods are used to investigate the antioxidant property of plant samples. In this section, merely compiled descriptions of selected Mimosoids are summarized.

Zhang *et al.* (2011) demonstrated the antioxidant activities of the methanol extracts of *M. pudica* through DPPH and FRAP assays as well as quantified the total phenolic and flavonoid contents. The results demonstrated potent antioxidant activities in the sequence of leaf> whole plant> seed> stem. Among the five flavonoids isolated, 5,7,3′,4′-tetrahydroxy-6-C-[β-D-apiose-(1→4)]-β-D-glycopyranosyl flavone revealed trolox equivalent antioxidant capacities. In a further experiment, ethanolic leaf extract of *M. pudica* exhibited moderate hydrogen peroxide and nitric oxide scavenging activity with IC_{50} value of 449.60±2.55µg/ml and 78.1±1.75µg/ml respectively (Muthukumaran *et al.*, 2011). In addition, reduced lipid peroxidation and superoxide dismutase (SOD) level was observed when leaf extract of *M. pudica* was tested in rat model (Muthukumaran *et al.*, 2010; Nazeema and Brindha, 2009). Kalaivani and Mathew (2010) studied the antioxidant and free radical scavenging activities of *A. nilotica* methanolic leaf extract, demonstrating greater potentiality of the extract to scavenge DPPH, hydroxyl radical, prevent lipid peroxidation and to possess superior reducing power. Crude 70% acetone and 50% ethanolic extracts from the leaf and bark of *Acacia nilotica* were tested for DPPH activity and found that acetone leaf and bark extract was more effective than the ethanolic extract (Gowri *et al.*, 2011). Moreover, a comparative evaluation of the antioxidant activities of 3 acacia species (*A. nilotica*, *A. seyal* and *A. laeta*) demonstrated superior bioactivities of *A. nilotica* (Abdel-Farid *et al.*, 2014). The bark extract of *Acacia catechu* exhibited significant inhibition of DPPH, H_2O_2 and reducing power activity *in-vitro*-cally followed by significant increase level of superoxide dismutase, catalase, glutathione-S-transferase and reduced glutathione at the dose of 100 and 200mg/kg bwt (Alam *et al.*, 2013). Antioxidant activity of *A. concinna* pods was determined by three different assays DPPH, ABTS-radical scavenging assay and linoleic acid peroxidation assay which reflected its

potent inhibitory activity with lower IC_{50} value (Poomanee *et al.*, 2015). Subsequently, bark extract of *Albizia lebbeck* was also proven as potent free radical scavenger (Suruse *et al.*, 2013; Vasanthi *et al.*, 2014). In addition, antioxidant activity of three enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione S transferase (GST) were assessed in diabetic rat model with decline level of damage after the administration of *A. lebbeck* (Resmi *et al.*, 2006).

2.5. Phytochemistry

Plants are the remarkable sources of chemical compounds having therapeutic effect on the body, referred to as phytochemicals. Virtually, these phytochemicals in plants perform a variety of physiological functions including defense mechanism against pathogens like bacteria, fungi as well as insects. Every plant synthesizes different types of phytochemicals which play an important role in pharmaceutical, food and chemical industries. In addition, these bioactive phytocompounds serve as templates for preparing several synthetic drugs. The phytochemicals have been divided into several groups such as, alkaloids, flavonoids, phenols, saponins, coumarins, anthocyanins, essential oils etc. A plethora of evidences revealed that these different types of phytochemicals are the main factors for several pharmacological activities. A glimpse of phytochemicals identified so far from the selected members of Mimosoideae is enlisted in this section.

M. pudica, one of the most well studied members of Mimosaceae, was accounted to have several bioactive components like flavonoids, phenols, alkaloids, terpenoids, glycosides, quinines, tannins, saponins and coumarin (Gandhiraja *et al.*, 2009). A few other phytochemicals including mimosine, tyrosine 3,4-dihydroxypiridine, mimosinamine, mimosinic acid were reported in *M. pudica* by Johnson *et al.* (2014). Further, five compounds namely 5,7,3′,4′-tetrahydroxyl-6-C- β-D-glucopyranosyl flavone, 5,7,3′,4′-tetrahydroxyl-8-C- β-D-glucopyranosyl flavone, succinic acid, β-sitosterol and stigmasterol were isolated and identified from the same plant i.e. *M. pudica* (Yuan *et al.*, 2006). The active metabolites such as gallic acid, protocatechuic acid, pyrocatechol, (+)- catechin, (-) epigallocatechin-7-gallate, (-) epigallocatechin-5,7- digallate, (-) epicatechin, (+) dicatechin, quercetin, (+) leucocyanidin gallate, sucrose and (+) catechin-5-gallate was reported to be found in bark extracts of *A. nilotica* (Anonymous, 2001; Malviya *et al.*, 2011; Singh *et al.*, 2009) followed by protocatechuic acid, ellagic acid, leucocyanidin, m-digallic dimer 3,4,5,7-tetrahydroxy flavan-3-ol, oligomer 3,4,7-

trihydroxy flavan 3,4-diol, 3,4,5,7-tetrahydroxy flavan-3-ol and (-) epicatechol from gum of *A. nilotica* (Malviya *et al*., 2011; Singh *et al*., 2009). Another important Mimosoid namely, *A. catechu* was reported to possesses epicatechin, epigallocatechin, epicatechin gallate, phloroglucin, protocatechuic acid, quercetin, poriferasterol glucosides, lupenone, procyanidin, L-arabinose, D-galactose, D-rhamnose, aldobiuronic acid, and taxifolin (Jain *et al.*, 2007; Sharma *et al.*, 1997). Further, 12 compounds namely 4-hydroxybenzoic acid, kaempferol, quercetin, 3,4',7-trihydroxyl-3', 5-dimethoxyflavone, catechin, epicatechin, afzelechin, epiafzelechin, mesquitol, ophioglonin, aromadendrin, and phenol were isolated from *A. catechu* by Li *et al.* (2010) whereas *A. concinna* was found to be own tartaric acid, oxalic acid, succinic acid, calycotomine, nicotine and rutin (Gupta and Nigam, 1970). The phytoconstituents reported in *Albizzia lebbeck* bark are melacacidin, friedelin, D-catechin, β-sitosterol, albiziahexoside, betulnic acid and echinocystic acid while the leaves contains albigenic, albigenin, kaempferol, quercetin; albizziahexoside, tannins, proteins, carbohydrates, amino acids and saponins. In addition the pods contains 3', 5 Dihydroxy 4', 7 dimethoxy flavone, and N- Benzoyl L phenyl alaninol (Rahul *et al*., 2010; Verma *et al.*, 2013). Further, eight bioactive compounds were isolated from the 95% ethanolic extract of *A. chinensis* leaves and their structures were explained as quercetin 3'-O-beta-D-glucopyranosyl-3-O-rutinoside, kaempferol 3,7-di-O-beta-D-glucopyranoside, rutin, D-pinitol, luteolin 7-O-beta-D-glucopyranoside, (+)-lyoniresinol 3alpha-O-beta-D-glucopyranoside, (-)-lyoniresinol 3-alpha-O-beta-D-glucopyranoside, syringin (Liu *et al.*, 2009). Lupeol, epilupeol, lupenone, α-spinasterol and α-spinasterone were found to be present in *Samanea saman* (Azhar *et al*., 2009; Ragasa *et al.*, 2014).

A few main active phytoconstituents of some members of Mimosaceae are summarized in Fig. 2.1.

2.6. Molecular diversity of different Mimosoids and *Rhizobium*

2.6.1. Mimosoideae

Genetic marker stands for genetic differences between individual organisms or species that are located in close proximity to genes. All genetic markers hold specific genomic locations within chromosomes known as loci. Three types of genetic markers have been discovered so far such as, morphological markers, biochemical markers and DNA markers. The morphological markers are typically characterized by phenotypic characters including flower color, seed shape, growth habits or pigmentation (Sumarani *et al.*, 2004). Biochemical markers are the differences in enzymes that are

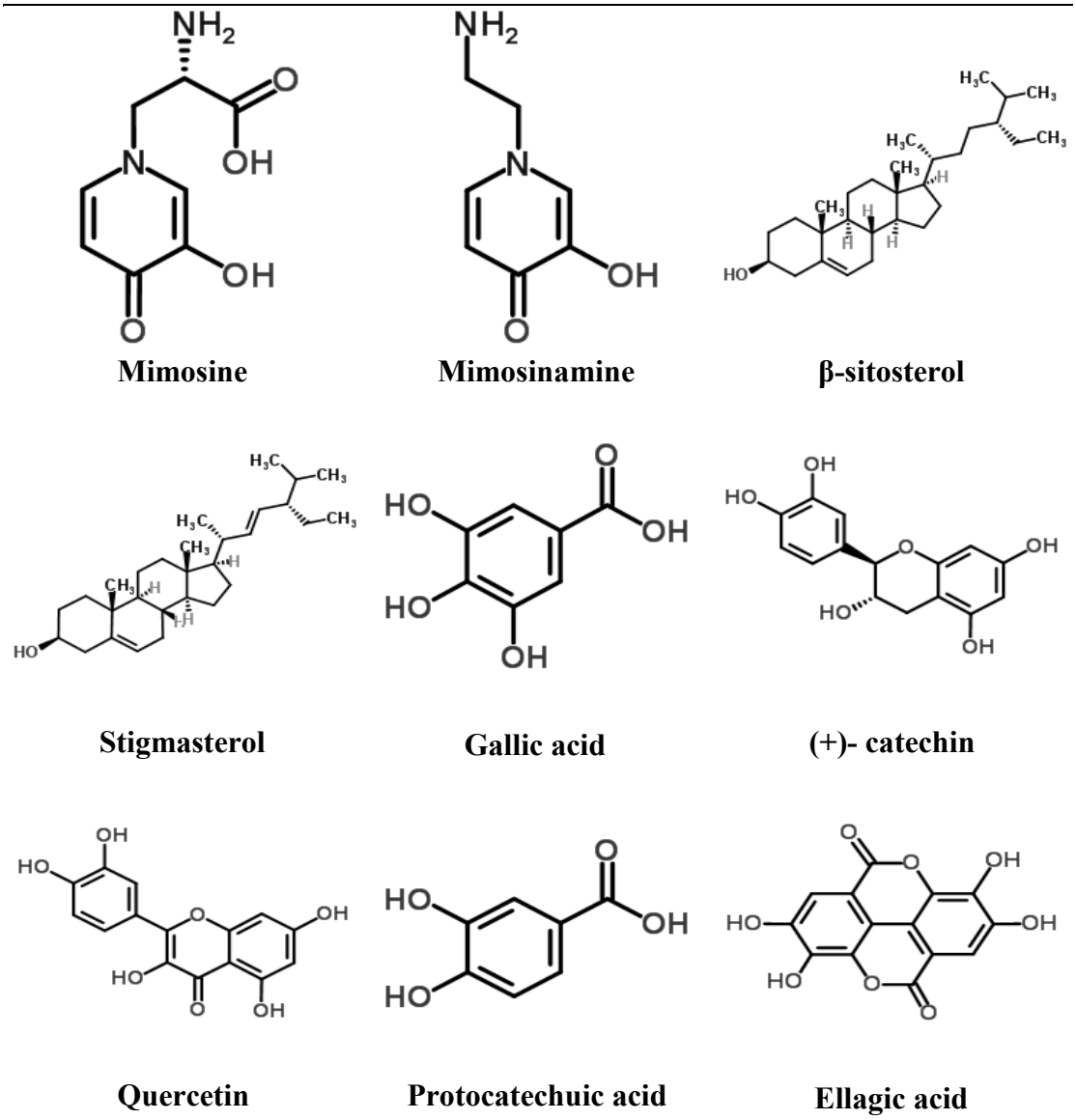

Fig. 2.1. Selected phytocompounds present in different Mimosoids.

distinguished by electrophoresis and specific staining (Pillai and Lekha, 2008). The DNA markers are the most predominant markers arise from different classes of DNA mutations such as substitutions (point mutations), rearrangements (insertions or deletions) or errors in replication of tandemly repeated DNA (Paterson, 1996). Besides, DNA markers are abundant in number and are not influenced by any environmental factors and developmental stages of the plant. Baring the use of DNA markers in the construction of linkage maps, they might be applied in plant breeding such as assessing the level of genetic diversity within germplasm and cultivar identity (Jahufer *et al.*, 2002; Winter and Kahl, 1995).

In essence, DNA markers might be broadly divided into three categories based on the method of detection: hybridization-based; PCR based and DNA sequence based (Winter and Kahl, 1995). In

addition, DNA markers may expose genetic differences between individuals of the same or different species which can be visualized by means of gel electrophoresis and staining with ethidium bromide or silver nitrate or detection with radioactive or colorimetric probes. A plethora of evidences suggested that DNA markers play a vital role in enhancing crop improvement and global food production by improving the efficiency of conventional plant breeding programs (Ortiz, 1998).

DNA markers or DNA fingerprinting techniques can be employed to resolve how closely related populations may be, as well as to classify the individuals providing better resolution of genetic relationships. Of the various DNA fingerprinting techniques developed for plant research, random amplified polymorphic DNA (RAPD), a PCR-based molecular technique, has become increasingly popular which are being used to evaluate the genetic relationship among species, cultivars and varieties (Williams *et al.*, 1990). A major advantage of RAPD markers is that it requires no prior sequence information and knowledge about any particular gene in a target taxon (Palumbi, 1996). Restriction fragment length polymorphism (RFLP) is another type of molecular technique in molecular biology to differentiate minor nucleotide sequence variations in homologous fragments of DNA. This technique relies on the specificity of restriction enzymes, which are extremely sequence-specific and cut the DNA only at their recognition sites (Heun *et al.*, 1991). In addition, RFLP is virtually used in the identification of genes for genetic disorders, genome mapping, determination of risk of disease, and paternity testing. RFLP markers are also frequently used to distinguish between two organisms or species. The DNA from different ecotypes, various geographical isolates, and different inbred lines of a species contain many RFLP.

However, these techniques are restricted only for genetic diversity, analysis of species, cultivars and varieties but not in plant identification. Recently, a new modified molecular technique i.e. DNA barcoding was developed offering a new dimension in the scientific community (Hebert and Gregory, 2005). In fact, DNA barcoding is a novel, modern and innovative technique which can be used to explore the evolution, identification and genetic relatedness of unknown plants and animal species by using a short stretch of DNA sequence. Recently, chloroplast and mitochondrial genes are being used to study the sequence variation at generic and species level. The chloroplast genes such as matK and TrnL-F have been utilized by various workers to study the plant evolutionary pattern as well as to solve the various anomalies in the taxonomic levels. The matK gene exhibits three times high rate of nucleotide substitution than that of

the large subunit of Rubisco (rbcL) and found to be six fold higher at the amino acid substitution rate (Johnson and Soltis, 1994; Olmstead and Palmer, 1994) elucidating advanced phylogenetic signal for resolving evolutionary relationships among the plant species at all taxonomic levels.

Therefore, despite of rapid development and widespread application of molecular techniques, very little is known regarding genetic variability within and among the taxa of Mimosoideae at the DNA level. Subsequently, there has always been a debate among the taxonomists regarding the taxonomic position of several populations within the order Fabales owing to similar types of morphological characters. Since, the morphological variation between species is difficult to distinguish; an appropriate knowledge of molecular documentation would help to understand the genetic relationship among the different genera in Mimosoideae.

The genetic relationships among the three species of *Mimosa* namely, *M. pudica*, *M. pigra* and *M. invisa* were analyzed using 30 RAPD primers. Out of which eleven primers revealed 83% of polymorphism in all three species with a total of 92 bands. The polymorphism percentage at interspecific level varied from 25% to 100% as well as *M. pudica* was found to be closely related to *M. pigra* and *M. invisa* (Sulain *et al.*, 2013). High degree of diversity (~70%) within the six tree species of *Acacia* were obtained by Nanda *et al.* (2004) employing 253 of distinct bands through RAPD. The result reflected that *A. farnesiana* and *A. catechu* were the closest member sharing about 30% of similarity whereas *A. auriculiformis* shares about 28% similarity with *A. farnesiana* and *A. catechu*. In addition, *A. mollissima* shares about 18% of similarity with *A. arabica*. Further, the genetic variability of nine *Acacia nilotica* subspecies of various origins was also analyzed using RAPD by Ndoye-Ndir *et al.* (2008) exhibiting large differences between subspecies but no correlation between geographic distances and genetic distances was established. Genetic differentiation among the six varieties of *A. caven* were examined by means of RAPD supporting similar kind of trend as found in taxonomic differentiation (Pometti *et al.*, 2010). Subsequently, several RAPD and ISSR markers were employed to study the genetic diversity in Kenyan populations of *Acacia senegal* (Josiah *et al.*, 2008). Besides, phylogenetic analysis chloroplast DNA of 22 species of *Acacia* was carried out using RFLP indicating that *A. nilotica* and *A. farnesiana* are sister species, while *A. nilotica* is Afro-Asiatic and *A. farnesiana* is America in origin (Bukhari *et al.*, 1999). A few RAPD and ISSR markers were further utilized to analyze 172 individuals representing eight populations of *Albizia lebbeck* in different geographical range and the genetic diversity was found to be

ranged from 1.23 to 1.38 while the total gene variability was 0.34 (Aparajita and Rout, 2009). During the DNA barcode analysis of *Acacia*, Robinson and Harris, (2000) documented that the tribe Acacieae and genus *Acacia* are not only monophyletic but also the subgenera *Acacia* and *Aculeiferum* are sister taxa and neither of them appeared closely related to subgenus Phyllodineae. Barcoding analysis of acacias from three different continents showed that all of three cpDNA regions (rbcL, matK and trnH-psbA) distinguished and supported the newly proposed genera of *Vachellia* from *Acacia* as well as discriminated sister species within either genera and differentiated biogeographical patterns among populations from India, Africa and Australia (Newmaster and Ragupathy, 2009). Nevill *et al.* (2013) demonstrated the novel use of DNA barcoding for seed identification and demonstrated the practical potential of DNA barcoding for the growing discipline of restoration ecology of *Acacia* in the Midwest of Western Australia.

2.6.2. Rhizobium and genetic diversity

Rhizobium is a type genus of the family Rhizobiaceae of the order Rhizobiales in the class alphaproteobacteria. The genera *Rhizobium* consists of about 44 recognized species including some latest novel species like *R. sphaerophysae*, *R. pusense*, *R. vallis* and *R. herbae* (Qin *et al.*, 2012). It can be characterized as rod shaped, heterogeneous group of gram negative, aerobic, heterotrophic, non-spore forming microbe (Hirsch *et al.*, 1993). It contains granules of poly-β-hydroxybutyrate which are refractile by phase contrast microscopy and produces an acidic reaction in mineral-salts medium containing mannitol or other carbohydrates. *Rhizobium* includes the largest number of species into the family. However, the original genus *Rhizobium* has undergone several subsequent changes in recent years giving rise to many other taxa.

In Bergey's Manual of Systematic Bacteriology, *Rhizobium* was included along with other three genera namely, *Bradyrhizobium*, *Agrobacterium*, and *Phyllobacterium* within the family Rhizobiaceae and the separation of these genera was predominantly based on the ability to stimulate the production of root or leaf nodules in host plant species (Jordan, 1984). The recent taxonomy of Rhizobiaceae, as well as of any other bacterial groups is mostly supported by the phylogenetic analyses based on 16SrDNA sequences. A revision and dismemberment of the genus *Rhizobium* and its relatives of the class alphaproteobacteria was led by their phylogenetic studies. Characterization of the *Rhizobium* genome at molecular level is the most discriminating method for assessing the variability among strains and isolates of the bacteria (Demezas *et al.*, 1991; Thies *et al.*, 2001). The workers used different

type of primers to obtain 'PCR-fingerprints' to characterize rhizobial isolates at the strain level. The primers, designed to study the genetic diversity, are RAPD, 16S ribosomal RNA genes, repetitive element sequences (REP, ERIC and BOX), 16S–23S rRNA intergenic spacer regions or genes for nitrogen fixation and nodulation (De Bruijn, 1992; Thies *et al.*, 2001).

Amongst handful of work done so far, Harrison *et al.* (1992) reported that the use of RAPD primers may produce varied amplification patterns from different *Rhizobium* isolates, especially directly from nodules providing this method as a potential one for examining genetic structures or strain differentiation as well as relationships in *Rhizobium* populations. Young and Cheng (1998) further suggested that RAPD technique is a potential tool for the construction of genetic maps and useful in identification of the genetics and systematics of different populations of rhizobia. Nine soil rhizobia were isolated from different field locations and subjected to RAPD analysis revealing RAPD is a very discriminative and efficient method for differentiating and studying genetic diversity of *Rhizobium* strains (Rajasundari *et al.*, 2009). RAPD profiling was further found to be an efficient discriminatory method when different root-nodule rhizobia were collected from chickpea and green pea roots for their genomic diversity (Qureshi *et al.*, 2014). The genetic diversity of indigenous soybean rhizobia, isolated from different soil types in eastern Croatia, was studied by using different PCR fingerprinting methods such as 16S rDNA, PCR-RFLP, rep-PCR and RAPD analysis. Highly specific and reproducible patterns were found that enabled accurate strain differentiation (Sikora and Redzepovic, 2003). Apart from these, different molecular techniques such as, rep-PCR, ERIC-PCR, BOX-PCR and (GTG)5-PCR were further utilized for genotyping of different bacterial strains of *Rhizobium* (Blazinkov *et al.*, 2007; Menna *et al.*, 2009; Versalovic *et al.*, 1991).