Chapter 2

REVIEW OF LITERATURE

2.1 Taxonomy and distribution:

The taxonomic history of *Brucea mollis* Wall. *ex* Kurz can be traced back to the work of Kurz (1873) where he validly published the species. *Brucea mollis* Wall. *ex* Kurz belongs to the family Simaroubaceae and the members of the family are pantropical in distribution. The family comprises of about 30 genera and 200 species (Nooteboom *et al*. 1962). According to Clayton (2008) the family comprises of 22 genera and 109 species of North American origin with probable migration *via* Beringia. In contrast to traditional views, long-distance dispersal events are considered to be common, particularly during the late Oligocene and later (Clayton, 2008). Several genera in the Simaroubaceae are either monotypic or comprised of few species with restricted geographic distribution, the majority of such genera are from Africa (Clayton, 2008). The members of this family are mainly trees or shrubs, evergreen or almost so and usually possess very bitter substances.

The family Simaroubaceae belongs to the order Sapindales and the taxonomic status of the family has long been remained uncertain and has only recently been recircumscribed in a phylogenetic framework (Fernando and Quinn, 1995). Engler (1931) classified the family Simaroubaceae s.l. in to 4 subfamilies *viz.* Surianoideae, Simaruboideae, Picramnioideae and Alvaradoideae. The genus *Brucea* was placed under the tribe Simaroubeae of the subfamily Simarouboideae (Simaroubaceae s.s.) along with the tribes Picrasmeae and Soulameae. Tribes were

delimited by the presence or absence of appendages of filaments and the degree of fusion of carpels.

Brucea J. F. Mill was named after Bruce and the type species *B. antidysenterica* was described from Africa by Bruce (Wight, 1839). The genus has only six species (Nooteboom, 1962) or eight species (Mabberly, 1997) distributed in old world tropics. But as per 'theplantlist' (www.theplantlist.org) the genus *Brucea* has 10 accepted species (accessed on 18.09.2015). In India, the genus is represented by two species *viz*., *Brucea javanica* (L.) Merr. and *B. mollis* Wall. *ex* Kurz (Gupta *et al.,* 2004; Santapau and Henery, 1973).

Brucea mollis Wall.*ex* Kurz has five accepted synonyms in 'theplantlist', *viz.B. acuminata, B. luzoniensis* S. Vidal, *B. membranacea* Merr., *B. macrobotrys* Merr., *B. stenophylla* Merr. However, it is reported that *B. luzoniensis* S. Vidal and *B. membranacea* (1906) are only synonyms (Merril, 1899). Merril considered *B. stenophylla* and *B. macrobotrys* Merr. both as different endemic species.

In India *B. mollis* is confined only to North East India except Mizoram and Tripura (Gupta *et al*., 2004). The species is listed as endangered plant in Arunachal Pradesh and Assam in the CAMP workshop held during March 2003 at Guwahati. *B. mollis* has been listed as NT (Near Threatened) species of Meghalaya by IUCN (Anonymous, 2003). The percentage of its global presence is estimated to be 5-10 (Anonymous, 2003). In Assam the plant occurs in certain localites of Karbi Anglong district only.

Kurz (1873) validated the name *Brucea mollis* provided by Wallich in 1847 based on a material collected from Sylhet (now Bangladesh). Later the species was described fragmentarily by different authors (Bennett, 1875; Kurz, 1877; Gamble, 1881; Lecomte, 1911; Brandis, 1906; Nootebom, 1962; Hua and Thomas, 2008).

Time to time *B. mollis* was given new names by same or different authors and later merged as synonyms such as *B. luzoniensis* (Vidal, 1883), *B. membranacea* (Merrill, 1906), *B. macrobotrys* (Merrill, 1915), *B. stenophylla* (Merrill, 1917) and *B. acuminate* (Li, 1943).

All the available description about the taxonomy, habit, distribution, morphology and ecology of *B. mollis* are not comprehensive. Unfortunately, descriptions of reproductive parts were based only on the male plants in most cases.

2.2 Morphology:

Comparative morphology plays a crucial role in the system of classification (Holttum, 1968; Ogura, 1964). There is limited scope of modification in the assignment of species in traditional taxonomy (Holttum, 1968).

Kurz (1873) and Bennett (1875) briefly described the leaflet, inflorescence and the fruit of the species. Morphological features of *B. mollis* have been described from time to time under different names by Merrill (1906, 1908, 1915 and 1917). However, all the species described by Merrill (1906, 1908, 1915 and 1917) were subsequently reduced to synonyms of *B. mollis*. Lecomte (1911) briefly described features of leaf, inflorescence, petals and fruit of *B. mollis* collected from Vietnam. But the description did not include any distinctive features of the reproductive parts. Li (1943) also described few morphological features such as petiolulate and inflorescence while describing the species as a new species *B. acuminata.* Nootebom (1962) described the vegetative and reproductive characters of the species only partly and in brief. However, he added a note stating the diversified morphological features exhibit by the species and remarked this phenomenon may due to the influence of different altitudes. Brandis (1906) described the leaf and fruit

of the species. Hua and Thomas (2008) briefly described the branch, leaf, leaflet, petiole, inflorescence and both male and female flowers.

2.2.1 Leaf epidermis:

Though the leaf epidermal characters are phenotypically elastic in nature, but are now considered as important attributes used extensively in plant taxonomy (Edeoga and Ikem, 2001; Mbagwu and Edeoga, 2006). Its importance has been recognized by several workers (Baranova, 1972; Metcalfe and Chalk, 1979; Baranova, 1992; Yang and Lin, 2005; Mavi *et al*., 2011; Szymura and Wolski, 2011). Edeoga (1991) and Mbagwu and Edeoga (2006) emphasized that epidermal and cuticular characters of angiosperms served as important tool in taxonomic study. Different shapes of epidermal cells, type and arrangement of stomata, size and shape of trichomes and number of vascular bundles plays important role in the study of systematics (Nwachukwu and Mbagwu, 2006).

Due to their diversity of forms and functions, stomata and trichomes represent important taxonomic characters for the recognition of certain genera, even when the plant is in the vegetative state (Paliwal, 1966; Pant, 1967; Pant and Kidwai, 1964; Shah and Kothari, 1973; Shah and Kothari, 1975; Stace, 1969a; Stace, 1969b; Lavania *et al*., 1990; Padmini and Rao, 1995; Padmini and Rao, 2001; Seibert, 1948; Fahn, 1979; Theobald *et al*., 1979). So far no study has been carried out so far on stomata and trichomes of *B. mollis*.

Since the features of stomatal apparatus are consistent these can be used for identification of species (Garg, 2010). Number, position and structure of the subsidiary cells, level of stomata in epidermal tissue, number of stomata per unit area, size, structure, spatial arrangement and mode of development of stomata are important in arriving taxonomic judgments (Solereder, 1908; Paliwal, 1966; Foster,

1949). Tribe Vicieae (Papilionaceae) is segregated into genera on the basis of stomatal character and hair morphology (Shah and Kothari, 1973). Different schemes of classification of stomata were proposed by different authors based on various features such as morphology and distribution of subsidiary cells, developmental pattern of guard cells (Rajagopal, 1973; Rajagopal and Ramayya, 1977; Rajagopal, 1979; Payne, 1979). Studies on stomatal diversities have been carried out in different taxonomic groups by different authors (Lavania, 1990; Rao *et al.*, 1985; Pravakar, 2004).

The morphology of the trichome structures can also vary greatly within a species. Trichomes are defined as unicellular or multicellular appendages, which originate from the epidermal cells and develop outwards on the surface of various plant organs (Werker, 2000). Trichomes may be non-glandular or glandular (Theobald *et al*., 1979). The non-glandular trichomes are diverse in morphology, anatomy and microstructure. Glandular trichomes or glands are epidermal appendices formed by the head of a single cell or pluricellular secretory cells and a non-glandular stalk (Fahn, 1990). Glandular trichomes vary in the chemical composition of the substances they secrete, in their structure, location and function (Werker, 2000). The structure and function of the glandular trichomes occurring in the plants are well documented in many species and they are recognized as the site of essential oil biosynthesis, secretion and accumulation (Croteau and Johnson, 1984). Trichomes may also functionally divide as nectaries, which produce nectar, and colleters, which secrete mucilage (Fahn, 1979).

Nectar-producing trichomes located on vegetative organs are extrafloral nectaries (EFNs) (Elias and Gelband, 1976). Several authors (Rivera, 2000b; Nogueira *et al*., 2013; Gama *et al*., 2013) have shown that these nectaries generally

have a large secretory head. It is reported that EFNs usually have an intense relationship with insects, particularly ants, which are attracted by the secreted energetic resource (Koptur *et al*., 1998; Heil and McKey, 2003; Oliveira and Freitas, 2004). It implies an indirect relationship between these nectaries and the protective role of the visiting ants against the herbivores (Vesprini *et al.,* 2003; Oliveira e Freitas, 2004). Studies on the occurrence, location and characterization of the trichomes can provide important insights regarding functional micro morphology and secondary metabolites producing by the plant. Furthermore, the adaptive and taxonomic value of the trichomes is of great importance in botanical studies (Abu-Asab & Cantino, 1987; Martins *et al*., 1997).

The simple unicellular non-glandular trichomes, all called prinkle hairs (Metcalfe, 1960), have smooth cell wall surfaces. These trichomes develop from single protoderm initials without any divisions (Ramayya, 1972). In contrast the wall surfaces of the simple multicellular non-glandular trichomes are covered with cuticular micro-papillae. In non-glandular trichomes, the cuticle may acquire variable thickness. The outer surface of the trichomes can be smooth or may exhibit micro-ornamentation, such as micro-papillae, warty, reticulate, seriate, etc. (Werker, 2000). According to Bathlott (1981), the cuticular micro-papillae are a continuation of the cuticular folding, present on the surface of the surrounding epidermal cells.

The glandular hairs are generally classified as capitate (clavate) or peltate (sub sessile) based on morphological characteristics (Fahn, 2000). In this study, six distinct glandular trichomes, *i.e.* short-stalked capitate, long-stalked capitate, peltate, digitiform, clavate filiform and fusiform glandular trichomes were found. According to Fahn (1988), high diversity exists in the morphology of glandular trichomes in the organ, and at the cellular and subcellular levels, which can be unicellular or

multicellular, uniseriate or multiseriate, and variously shaped (Werker, 2000). However, to date, there has been no comprehensive report on the diversity (types) of trichomes in *P. cablin*. . The glandular trichomes are known to be the primary sites of secondary metabolite biosynthesis, secretion and storage, and generally consist of either simple subcutaneous glands or of trichomes (Weiss, 1997). Henderson *et al.* (1970) also reported on the correlations between the number of external glandular trichomes and the sesquiterpenoid content of patchouli (*P. cablin*) leaf sections. The result is consistent with the findings of Werker *et al.* (1985) who noted that capitate trichomes are very variable in stalk length, glandular head shape and secretions, and can be classified into various types.

2.2.2 Leaf architecture:

Study of leaf architecture includes the study of venation pattern, marginal configuration, leaf shape, etc., allowing more rigorous comparisons among different species and regarded as taxonomically valuable (Metcalfe and Chalk, 1950; Inamdar, 1970; Dilcher, 1974; Hicky, 1973; Hicky and Wolfe, 1975; Foster, 1949; Rury and Dickison, 1977). Leaf venation pattern is one of the characters on which the primary dichotomy of angiosperms into monocots and dicots is based. Before various authors (Ettingshausen, 1861; Melville, 1976; Hicky, 1979) classified the wide variation present in leaf architecture, this important taxonomic attribute was not been considered in taxonomic and descriptive studies for long (Foster, 1961; Hicky, 1973). von Ettingshausen (1861) considered only leaf venation pattern in his classification of leaf architecture. However, Hicky (1979) considered leaf shape, structure of leaf margin, vein order etc. Hicky's (1979) classification of leaf architecture was later referred by several authors (Dilcher, 1974; Roth *et al.,* 2001). Comparative studies of mature leaf architecture in families and genera of Dicots and

Monocots have been subsequently studied by several authors (Simola, 1968; Tucker, 1964; Bharathi *et al*., 2007; Anna Mani and Prabhakar, 1991; Deokule and Kate, 2001; Inamdar *et al*., 1983).

2.3 Anatomy:

Importance of anatomical studies of plants has been discussed by several authors (Bailey, 1951; Metcalfe, 1954; Metcalfe, 1961), Dickinson, 1975; Paliwal and Anand, 1978). The elaborate work of Metcalfe and Chalk (1950) on stem anatomy of angiosperms serves as one of the important illustration used in angiosperm taxonomy. Gramineae is the most extensively investigated family where anatomical features provided very useful taxonomic features for identification and construction of keys (Davis, 1959).

No detail study on anatomy has been carried out in the members of the genus Brucea. However, presence of gum ducts, storied structure and libriform fibres in the members of the family Simaroubaceae has been described (*c.f.* Nootebom, 1962).

2.4 Floral biology:

Members of the family Simaroubaceae exhibit wide array of floral diversity. Floral morphology of the family has been briefly described by Engler (1874, 1931) and Clayton (2011). Saunders (1939), Nair and Joseph (1957), Nair and Joshi (1958) and Narayan & Sayeeduddin (1958) provided description on morphoanatomy of the flowers of members of the family Simaroubaceae. Descriptions o provided by Endress, Jenny and Fallen (1983) and Ramp (1988) consist about ontogenesis of floral parts of members of Simaroubaceae in brief. Flower of the family exhibits several evolutionary trends such as dialysepaly to gamosepaly, bisexuality to unisexuality, diplostemony to obdiplostemony or haplostemony, apocarpy to syncarpy and reduction in floral merism (Nair and Joshi, 1958). Members of Simaba

exhibits notable features such as variation in merism among flowers of the same inflorescence and sexual floral variations related to organ sterility (Alves *et al*., 2016). Endress *et al*., (1983) reported that members of Simaroubaceae exhibits apocarpy combined with postgenital fusion of carpel apices, and discussed in detail its functional implications. Ramp (1988), based on his analysis of gynoecium structure of 11 species and ten genera of the family, reported that Simaroubaceae represents an artificial group. Later, similar results were reported by Fernando and Quinn (1992) and Fernando, Gadek and Quinn (1995) based on study of fruit anatomy and in a phylogenetic analysis based on rbcL sequences. Bachelier and Endress (2008) studied the floral structure of Kirkia Oliv. (Kirkiaceae) a genus formerly placed in Simaroubaceae and now retrieved as sister to the Anacardiaceae–Burseraceae clade in molecular phylogenetic studies (Clayton *et al*., 2007). Flowers of Simaroubaceae are usually unisexual in monoecious or rarely dioecious plants (Judd *et al*., 2008). Well-developed staminodes and pistillodes are frequently observed by them. Nair and Joshi (1958) appended the list of dichlamydeous families proposed by Eames (1951) in which the ovaries remain open in various degrees with the members of Simaroubaceae. In all the members of the Simaroubaceae studied so far, the floral axis continues above the level of the carpels. This prolongation of the receptacle is very prominent in *Brucea*, where it is supplied by a well developed concentric vascular bundle that fades out near the tip. In Samadera (Nair, 1957) and Quassia (Nair, 1956), also, the receptacular vascular tissue, although not so well developed as in *Brucea,* is present above the level of the ventral carpel strands. Such findings, plus the incomplete fusion of the carpellary margins in *Alianthus* are evidence in favor of the classical interpretation of the flower.

2.5 Seed germination and viability:

There is no work has been done to study the seed germination of *B. mollis*. However some works are carried out in other members of the family Simaroubaceae. Seeds of *B. javanica* are reported to have low and slow germination (Siregar, 1999). This may be due to inadequate temperature for germination, insufficient seed moisture content, physiological age of seed may be not enough, decline of seed viability or seed dormancy (Sutarno *et al*., 2007). Effect of stratification in seed germination of *Alianthus altissima* Mill.was reported by Graves (1990) stating the method has no certain efficient result in germination. Seeds of *Simarouba amara* are not dormant and get germinate easily at high temperatures (Corbineau and Côme, 1989). Seed histology of *Eurycoma longifolia* during germination has been studied by M. Danial *et al.* in 2011. A research on the effect of seed maturity, temperature and period of storage on vigor of *Picrasma javanica* Bl. seedling germination shows that the germination time of seeds varies between 11-23 days, mature seeds had better growth than pre-mature seeds and storage in room temperature caused decrease in seed vigor (Setyowati, 2009). It is suggested to store in low temperature (5°C and 20°C) to maintain the seed viability up to 3 months. Seed germination depends on several factors such as temperature, light and substrate affect etc. Optimum level of these environmental factors can stimulate the survival and growth of the seedlings (Nogueira *et al*., 2003) and variation of them can also inhibit the germination process (Carvalho and Nakagawa, 2000). Stresses at any stage of plant growth can cause irreversible loss in yield potential (Pirasteh-Anosheh *et al*., 2011; Pirasteh-Anosheh and Hamidi, 2013). It is reported that low temperature at the time of planting usually results in poor seedling establishment because of slow germination rate and reduced growth rate after germination (Pinthus and Rosenblum,

1961; Singh, 1985). Rapid and uniform seed germination is an essential factor for quality seedling development (Parera and Cantliffe, 1994; Pirasteh-Anosheh and Hamidi, 2013). Studies revealed that a wide variability in the requirements of these parameters present for the best growth and development of seedlings which infers the necessity of such studies for lesser known species and especially the native ones (Zamith and Scarano, 2004).

2.6 Ethnobotany:

Ethnobotany can be used to know the dynamics of traditional ecological knowledge as an effort for biodiversity conservation (Pieroni *et al*., 2014). 3300 million people of under developed countries use medicinal plants as part of their traditional medicine (Dobriyal and Narayana, 1998). Several authors studied the usefulness of traditional medicines for the treatment of different ailments. Kingstone *et al*. (2009) described the use of 30 medicinal herbs for the treatment of skin diseases. Mitra and Mukherjee (2010) described 62 medicinal plants and their uses for the treatment of gastro-intestinal problems. Debnath *et al*. (2014) documented 51 herbs used for the treatment of 30 different ailments by Mog and Reang communities of Sabroom and Santirbazar Subdivision of Tripura. Singh *et al*. (2015) reported the ethno medicinal uses of 35 food plants used by the Zeliang tribe of Nagaland. Dutta and Dutta (2005) documented about 1,350 species of plants used in the preparation of traditional medicine, 665 plant species as food and 899 species for miscellaneous uses in Northeast India. Fruit or root decoction of *Brucea mollis* has long been used by the local people for the treatment of fever (Barthakur, 1976).

Assam is rich in its medicinal plant diversity. Different ethnic groups of the state have rich heritage of indigenous knowledge about medicinal plants and their use. People here still depend on medicines based on indigenous knowledge system

(Dutta and Dutta, 2005). A number of authors carried out ethobotanical studies in different parts of northeast India and documented the traditionally important plant species (Sharma and Thakur, 1999; Gogoi and Borthakur, 2001; Das and Tag, 2006; Sajem and Gosai, 2006; Buragohain and Konwar, 2007; Das *et al*., 2008; Kalita and Bora, 2008; Sikdar and Dutta, 2008; Mao *et al.*, 2009; Saikia *et al*., 2010; Sarma and Sarma, 2010; Barukial, 2011; Buragohain, 2011; Sonowal and Baruah, 2011; Abujam and Shah, 2012; Baruah *et al.*, 2012; Chakraborty *et al*., 2012; Deka *et al*., 2012; Gam and Gam, 2012; Gam, 2013; Sarma *et al*., 2013; Nath, 2014; Talukdar, 2014; Bailung and Pujari, 2016).

2.7 Phytochemicals:

Many compounds have been isolated from the members of the *Brucea viz*., quassinoids, alkaloids, triterpenoids, and flavonoids. Among these, quassinoids, a chemical class isolated only from the Simaroubaceous species, are the dominant constituents (Jian-Hua *et al*. 2009). Recently, phytochemical studies of *Brucea* has been reviewed by Liu *et al.* (2009) and recorded the various biological activities exhibiting by *Brucea mollis* such as Amebicide, Antimalarial, Antiplasmodial, Anticancer, Antileukemic, Antitumor, Antiphytoviral, Antifeedant, Antigiardial, Cytotoxic, Insecticide, Pesticide, Plasmodicide, Protisticide, Trypanocide, Pesticide, Plasmodicide, Antibacterial, CNS-Depressant, Hypotensive and cAMP inhibitor.

The antimalarial activity of the plant as been affirmed by showing biological activities of the chemical compounds like Bruceine B, Brucine D, Brusatol and Yandanziolide A, isolated from *Brucea mollis* (Bharati and Singh, 2012). These compounds also exhibited amebicidal, antiplasmodicidal, antifeedant, antigiardial, cytotoxic, insecticidal, pesticidal, antiviral and antileukemic activities (Bharati and Singh, 2012). The compounds like 11-Hydroxycanthin-6-one, Hydroxy-11-

methoxycarthin-6-one, Canthin-6-one, Yadanzi-olides T-V 10, Bruceine D and Yadanziolide B isolated from *Brucea mollis* have shown cytotoxic activities. The compound 1-Erythl-β-carboline, isolated from *B. mollis* has also been reported to be used as CNS-depressant and hypotensive, β-Carboline-1-propionic acid and Canthin-6-one have found as a cAMP inhibitor (Bharati and Singh, 2012).

Anticancer activity of *B. mollis* was also confirmed by Dhawan *et al.* (1977). Ouyang *et al*.(1994) isolated three new alkaloids from the root-wood of *Brucea mollis* var. *tonkinensis* collected in China and their structures were determined to be 11- O- beta-D– glucopyranosyl-(1-->6)-beta-D-glucopyranosylcanthin-6-one, 5-O-beta-D-glucopyranosyl-(1-6)-beta-D-glucopyranosylcanthin-6-one and 1-hydroxycanthin-6-one-N-oxide by chemical and spectral methods. In addition, two known alkaloids, canthin-6-one and canthin-6-one-N-oxide were also isolated.

Tung *et al.* (2012) collected *Brucea mollis* from the Hoa Binh province, Vietnam to see the cytotoxic effect on different cancer cell line. Ten compounds *viz*., soulameanone, isobruceine B, 9-methoxycanthin-6-one, bruceolline F, niloticine, octatriacontan-1-ol, bombiprenone, α-tocopherol, inosine and apigenin 7-O-β-D glucopyranoside were isolated from the leaves, stems, and roots of *Brucea mollis* . Their structures were determined using NMR spectroscopy and mass spectrometry. After vigorous screening and evaluation on human cell line significant cytotoxic activity of the plant has been observed. Compounds 9-methoxycanthin-6-one and niloticine have been discovered for the first time from the *Brucea* by Mai Hung Thanh Tung *et al.* (2012).

Chen *et al*. (2013) isolated ten compounds from the stems of *Brucea mollis* consisting of tirucallane triterpenoids, brumollisols A–C (1–3, resp.), together with five known analogues, (23R,24S)-23,24,25-trihydroxytirucall-7-ene-3,6-dione,

piscidinol A, 24- epipiscidinol A, 21α-methylmelianodiol and 21β-methylmelianodiol and their structures were elucidated as deacetylated isobrucein B, indaquassin X, cleomiscosin A, cleomiscosin B, (+)-lyoniresinol, (+)-epipinoresinol, (+)-pinoresinol, (+) syringaresinol, 4,5-dihydroblumenol A and adenosine on the basis of spectroscopic data analysis.