CHAPTER 1

INTRODUCTION

1.1. General Account

The genus *Glochidion* was first established by J.R. Forster & G. Forster (1776) in 'Characteres Generum Plantarum'. The generic name '*Glochidion*' was derived from the Greek name *'Glochis'* means the expansion of anther connectives of the reproductive structure in the *Glochidion* members. The earliest record of *Glochidion* from the Indian subcontinent was that of Voigt in his 'Hortus Suburbanus Calcuttensis' in 1841. He described six *Glochidion* species, namely *Glochidion multiloculare*, *G. lanceolarium*, *G. nitidum*, *G. sinicum*, *G. hirsutum,* and *G. pinatum*. He positioned the genus in the tribe Phyllantheae Bartl. of the family Euphorbiaceae.

Glochidion J.R. Forst. & G.Forst. is one of the largest genera, mainly belonging to the family Phyllanthaceae and consisting of ca. 320 species worldwide and 22 species and 13 varieties in India (Chakrabarty & Balakrishnan, 2018). With a few species that inhabit Madagascar and tropical America, its distribution is primarily throughout the countries of tropical Asia, Northern Australia, and Polynesia (Chakrabarty & Gangopadhyay, 1995; Balakrishnan et al., 2012). The first taxonomic study on *Glochidion* with taxonomic description from India was carried out by J.D. Hooker (1890) in '*The Flora of British India*'. He described 59 species, including Assam, of which 21 species were from North East India. In '*Flora of Assam*', Kanjilal et al. (1940) described 16 species of the genus from erstwhile Assam. N.P. Balakrishnan (1971) recorded 5 species in '*Flora of Jowai*', Meghalaya. Bora & Kumar (2005) in their '*Floristic Diversity of Assam Study of Pabitara Wildlife Sanctuary*' mentioned only 2 species of the genus viz., *Glochidion multiloculare* Voight and *G. assamicum* Hook.f. In Kokrajhar district of Assam, Daimary (2011) conducted a floristic study concerning Chakrasila Wildlife Sanctuary and described 8 species of the genus. Chakrabarty & Balakrishnan (2018) described 33 species and 26 varieties of the genus in the book 'Indo-Burmese Phyllanthaceae, A Taxonomic Revision'. Borthakur et al. (2018) in their '*Flora of BTAD*' described 7 species of the genus namely *Glochidion arborescens* Bl., *G. ellipticum* Wight, *G. heyneanum* (Wight & Arn.) Wight, *G. hirsutum* (Roxb.) Voigt, *G. lanceolarium* (Roxb.) Voigt, *G. multiloculare* (Rottler ex Willd.) Voigt., *G. sphaerogynum* (Muell. Arg.) Kurz.

1.2. Taxonomy of the genus

Different workers carried out the different systematic positions of the genus. Bentham & Hooker (1883) classified the genus under the family Euphorbiaceae. Hutchinson (1973) also placed the genus under the family Euphorbiaceae. According to APG I (1998), the genus was categorized into the family Phyllanthaceae. Hoffmann et al. (2006) grouped the genus into the tribe Phyllantheae of the family Phyllanthaceae. In recent classification systems i.e., the APG IV system also kept the genus under the family Phyllanthaceae (APG, 2016). The family Phyllanthaceae is differentiated from Euphorbiaceae *sensu stricto* by the lack of latex and extrafloral nectaries on the leaves, having biovulate ovary, explosively dehiscent fruits, and ecarunculate seeds (Chakrabarty & Balakrishnan, 2018).

According to phylogenetic studies, the genus *Breynia* J.R. Forst. & G.Forst., *Flueggea* Willd., *Glochidion* J.R. Forst. & G.Forst., *Margaritaria* L.f., *Phyllanthodendron* Hemsl., *Sauropus* Blume are closely related to the type of *Phyllanthus* and placed together under the subfamily Phyllanthoideae of Phyllanthaceae family, in the Tribe Phyllantheae (Hoffmann et al., 2006; Kathriarachi et al., 2006). Later Pruesapan et al. (2012) reconstituted the genera *Breynia*, *Glochidion*, *Phyllanthodendron*, *Sauropus,* divided *Phyllanthus* into smaller genera, and accepted the generic position of *Glochidion*. Because these groups can be identified morphologically from one another. Thus, *Glochidion* was given generic status in the most current taxonomic classification (van Welzen et al., 2014; Webster, 2014; Yao et al., 2018; Xu et al., 2020).

Species of *Glochidion* J.R. Forst. & G.Forst. are mainly trees, shrubs, monoecious or rarely dioecious with glabrous or pubescent plant bodies with drooping branches. Most of these habitats are evergreen, tropical evergreen forests, moist deciduous, hilly, *sal* forests, primary forests, and some swampy regions. Leaves are mostly alternate, distichous; stipules thick and mostly persistent; petioles short; lamina simple, entire with pinnate venation. Inflorescence is axillary to supra-axillary, pedunculate, unisexual or bisexual, fasciculate, having a few to many male and female flowers. Male flowers are usually distinctly pedicellate, mostly filiform, sepals free, imbricate, petals and disc absent, stamens generally sessile with connate filaments into an oblong mass, pistillode absent. Female flowers are sessile, or shortly pedicellate, sepals usually free, imbricate, petals and disc absent, staminodes absent, ovary locular, each locule biovulate. Capsules are usually depressed or

subglobose, conspicuously lobed to superficially lobed or conspicuously unlobed (Chakrabarty & Balakrishnan, 2018). Thus, the different taxa of this genus can be identified by its unique characteristics.

1.3. Ethnobotany of the genus

Glochidion plays a substantial role in the field of traditional medicine. In China, *Glochidion coccineum* is used as a folk medicinal plant to treat influenza, dysentery, rheumatoid arthritis, dyspepsia, etc. (Xiao et al., 2008a). Various plant parts of different species of *Glochidion* have been used to cure some of the diseases in India. *G. lanceolarium* (Roxb.) Voigt bark is useful for vomiting and dyspepsia and the wood of this plant is also utilized as timber for the construction of houses (Chakrabarty & Balakrishnan, 2018). Bark and tender shoots of *G. zeylanicum* (Gaertn.) A.Juss. are mainly used in stomachic and itches (Chakrabarty & Balakrishnan, 2018). Some tribes of Khasi Hills of Meghalaya consumed fruits of *G. khasicum* (Mull.Arg.) Hook.f. (Chakrabarty & Balakrishnan, 2018).

1.4. Phytochemical Study

Alkaloids, flavonoids, steroids, tannins, terpenoids, phenol, and other chemical compounds are examples of phytochemicals, which are chemical compounds that exist in many portions of plant parts. These phytochemicals possess a variety of biochemical properties, like antioxidant, antimicrobial, antibacterial, and cytotoxic properties. These substances significantly contribute to the defense against hazardous illnesses in both plants and animals (Vinayaka et al., 2022). The genus contains different types of phytoconstituents like flavonoids, triterpenoids, saponins, glycosides, alkaloids, etc. Glucosides, lignin, and neolignan have been found in the leaf extract of *Glochidion zeylanicum* (Gaertn.) A. Juss. (Otsuka et al., 2003) . The various groups of chemical constituents identified among different plant species of the genus showed anticancer, diuretics, hypotensive, and several other biochemical properties (Sandhya et al., 2010). *G. velutinum* Wight contains carbohydrates, alkaloids, proteins, tannins, steroids, flavonoids, triterpenoids, and saponins having antidiabetic and cytotoxic activities and that is why the species is regarded as an important medicinal plant (Sandhya et al., 2010; Mallikarjuna, 2012; Hasan et al., 2016).

1.4.1. Importance of Gas Chromatography-Mass Spectrometry

The assessment of GC-MS for medicinal and herbal plants have gained a lot of popularity lately (Olivia et al., 2021). GC-MS is a scientific technique combined

with two features i.e. gas chromatography and mass spectrometry that separates the mixture from the sample and detects chemicals present inside the plant sample. The practice of GC-MS in the phytochemical analysis of medicinal plants plays a vital role in obtaining biologically active components (Hethelyi et al., 1987; Olivia et al., 2021). Many phytocomponents such as phenols, alkaloids, flavonoids, saponins, and tannins have antioxidant, anti-inflammatory, antibacterial, and antimicrobial capacities (Kabir et al., 2015; Yi et al., 2016).

1.4.2. Antioxidant activity and their significance

Antioxidants are potential reactive compounds that can control or avoid oxidative damage in the human organism (Sharma et al., 2014; Asraoui et al., 2021). Over the decades, it has been proved that oxidative stress and various human ailments can be avoided by consuming plant foods high in antioxidants like vitamins C and E or natural antioxidants such as coumarins, flavonoids, phenols, tannins, and terpenoids (Skowyra et al., 2014). By providing free radicals, antioxidants can be performed as neutralizing agents and reduce the oxidative damage that causes biological systems (Shantabi et al., 2017; Baliyan et al., 2022).

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay are the two assays that frequently use free radicals to determine antioxidant capacity in vitro. They have broadly utilized approaches in plant biochemistry that scavenge free radical activity and measure the antioxidant properties of many medicinal plants (Baliyan et al., 2022). This technique was created by Blois (1958) to evaluate the antioxidant properties similarly by employing the stable free radical DPPH. This assay depends on the estimation of the antioxidants' capacity to scavenge it (Kedare & Singh, 2011).

1.4.3. Role of phytoconstituents in antioxidant activity

Alkaloids and phenols have been confirmed to possess significant antioxidant activity in natural products (Gan et al., 2017). The major classes of phytoconstituents like terpenes, tannins, flavonoids, and saponins have been identified as aiding antioxidant activity (Cavazos et al., 2021). The largest class of secondary plant metabolites with antioxidant capabilities is phenolic compounds. Tannins are also potent anti-inflammatory agents with anti-bacterial, anti-fungal, antimicrobial, anti-diarrheal, cytotoxic, anti-proliferative, and antiparasitic effects (Huang et al., 2018). Due to their propensity to chelate metals, their capacity to delocalize unpaired electrons and produce a stable phenoxyl radical, and the occurrence of hydrogen or

electron-donating substituents that can stop free radicals from forming, flavonoids are excellent antioxidants (Musialik et al., 2009).

1.4.4. Mineral elements and their significance

Although minerals produce no energy, they play key roles in many biological functions in the human body (Soetan et al., 2010). In daily life, minerals are vital to maintaining good health. Different parts of plants contain a range of minerals required for human nutrition. The human body's physiological processes are affected by both macro and microelements. Studies of indigenous medicinal plants suggest that major and trace elements provide central roles in dealing with the treatment of an inclusive range of human ailments and if their concentration exceeds trace limitations, they may also result in severe illnesses (Subramanian et al., 2012). Na enhances nerve and muscle activity by stabilizing the osmotic pressure within the body (Paul et al., 2018). Ionic balance is preserved by K and Na, which raises blood pressure and aids in the prevention of hypertension (Radha et al., 2021). For an enzyme to operate, Mg, Cu, and Zn are essential (Serafim et al., 2012). Ca plays a key role as a component of teeth and bone formations, nerve and muscle functions in living cells, and aids in membrane and skin formation (Paul et al., 2018). Fe is associated with haemoglobin and the delivery of oxygen from the lungs to tissue cells and Mn leads to the formation of urea, aids in the development of the neurological system, and promotes appropriate bone growth (Liang et al., 2006; Saupi et al., 2009 ; Thomas & Krishnakumari, 2015). Minerals and phytochemicals present in plants are a good supply for the nutraceutical industry. Numerous studies have discovered mineral contents in different plant samples that we take as health supplements, and that macro and trace elements influence biochemical developments in the human body (Saupi et al., 2009; Radha et al., 2021). The development of plants depends on the existence of heavy metals, which also provide micronutrients. However, some heavy metals like Co, Pb, Cr, and Cd are harmful to human health (Baker, 1989; Gunavathy & Sherine, 2019). It is essential to maintain the potency, safety, and quality of plants and the products they generate to prevent serious health problems. It is well recognized that minerals may influence human health and aid in bodily biological processes and many minerals have an impact on muscular performance and metabolism. For instance, Ca, K, Na, and Fe are necessary for good muscle or physical and neurological activity, whereas Mg has a positive influence on muscle relaxation and may improve muscle performance involving protein synthesis (van Dronkelaar et al.,

2018). Ca and Zn both lead to the growth of bones and teeth and aid in enzyme stimulation (Soetan et al., 2010; Thomas & Krishnakumari, 2015). The required heavy metals, such as Cu, Mn, Mg, Fe, and Zn, have biochemical and physiological effects on both plants and animals. They participate in numerous oxidation-reduction processes and contribute significantly to many vital enzymes. But metals like Cr, Cd, and Pb are considered as non-essential metals because their biological use has not been proved through scientific research (Tchounwou et al., 2012).

1.5. Choices of the work

Glochidion J.R. Forst. & G.Forst. is generally a tough genus and its thorough taxonomic treatment is still inadequate (Yao & Zhang, 2015). From the scrutiny of the literature, it was noted that taxonomic assessment with only morphology of the genus has been done in some regional and state floras (Hooker, 1890; Kanjilal et al., 1940; Borthakur et al., 2018; Chakrabarty & Balakrishnan, 2018). No comprehensive taxonomic assessment with detailed morphology, anatomy, ethnobotany, and phytochemical study has been conducted. In a taxonomic study, different morphological and micromorphological features, including foliar epidermal study, anatomical study and leaf architecture study of the species allow us to solve some taxonomic problems. Therefore, as an aid to taxonomy, it helps in the stabilization of the taxa and the taxonomic status of the genus. Based on the literature review on the taxonomy of *Glochidion*, it confirmed that some taxa are difficult to identify because of their morphological resemblances among each other i.e., in some infraspecific taxon such as varieties level. The comparison analysis of plant structures with their morphological and anatomical characteristics have always been the backbone of plant systematics (Endress et al., 2000). Therefore, micromorphological studies like foliar epidermal studies, leaf architecture studies, and anatomical studies have been considered for the proper taxonomic assessment that would oblige in determining the taxonomic group, which is lower in the rank of species level. In addition, the study also includes the proper line drawing and photo plates of morphological characters, distribution, occurrence, and comparative studies among the different species of the genus as an aid to taxonomic treatment. Traditionally, some of the members of the genus have been used as medicine to cure various diseases. Thus, the study also brings their ethnobotanical uses consumed by the local people of the studied area, hence, no proper documentation of the genus has been done in Assam. From the

ethnobotanical study, we can gather more information about the genus and how the members of the genus have been used traditionally. Some members of the genus have been cooked as a curry and used as medicine to cure various ailments. As a result, comprehensive data for the assessment of mineral constituents is also required.

The genus contains phytochemicals like triterpenoids, saponins, glycosides, alkaloids, glucosides, and steroids (Sandhya et al., 2010). From the early studies, it has also been found that the genus has the potential to reduce cancer cells that have anticarcinogenic, antitumor, anti-inflammatory, antimutagenic, and antimicrobial activity, as well as a significant antioxidant and cytotoxic effects on prostate and breast cancer cell lines (Shah et al., 2022). Hence, the phytochemical study of plants can be considered as an important role against various diseases for human well-being. Therefore, the present research also brings a phytochemical study including antioxidant activity, GC-MS analysis, and mineral contents of the parts used of the species documented medicinally known species of the genus. Thus, the genus needs a more thorough knowledge of the evaluation of the phytochemical study and GC-MS analysis, including antioxidant activities, that will give an immense idea about the bioactive properties of the genus as well as the phytochemical constituents and phytocompounds present in the genus.

1.6. Aim and Objectives

The present study aims to prepare a taxonomic account and phytochemicals study of the medicinally known species of the genus *Glochidion* occurring in Assam.

The objectives of the study include-

- To survey and collect *Glochidion* spp. from different parts of Assam.
- To evaluate the vegetative and reproductive structures as an aid to circumscribing different taxa.
- To study the details of foliar epidermis, petiole anatomy, and leaf venation pattern for use as taxonomic criteria.
- To carry out the phytochemical analysis of documented medicinally known species of *Glochidion*.