

DISCUSSION

5.1. Significant findings of the study

A total of 10 taxa i.e. 6 species and 4 varieties of the genus *Glochidion* have been collected in Assam during the present study.

5.1.1. Critical notes on the genus *Glochidion* in Assam

The following findings have been made after a thorough literature review and physical and online consultation of herbarium specimens. Kanjilal et al. (1940) mentioned that the genus *Glochidion* had 16 species from the erstwhile Assam. After a thorough analysis, it was found that some of the species from the literature provided have no reports on their distribution inside the current political boundary of Assam and some of the species are now treated as synonyms.

G. acuminatum Müll.Arg.: The distribution of *G. acuminatum* reported by Kanjilal et al. (1940) was found to be in the North Cachar Hills i.e. present-day Dima Hasao district from Assam and Khasi and Jaintia Hills i.e. from Meghalaya. There were only herbarium specimens collected by Kanjilal (1915) from Khasi and the Jaintia Hills, Meghalaya state, rather than any specimens from Assam that had been gathered by Kanjilal et al. (1940). Chakrabarty & Balakrishnan (2018) also stated the distribution of *G. acuminatum* in Assam but they mentioned that without the exact locality collected by G. Watt. Moreover, no records of the species were found throughout the present study.

G. coccineum (Banks) Müll.Arg.: Kanjilal et al. (1940) reported the distribution of *G. coccineum* from Sibsagar district of Assam. However, no herbarium specimens collected by Kanjilal have been observed at ASSAM and CAL herbaria. Deori & Talukdar (2013) mentioned the distribution of *G. coccineum* in Laokhowa Wildlife Sanctuary, Assam (specimen deposited at ASSAM). They deposited two specimens at ASSAM herbaria from different locations but their collected specimens resemble *G. multiloculare* and *G. ellipticum* respectively. But throughout the current investigation, no record of the species was found.

G. daltonii (Müll.Arg.) Kurz and ***G. gamblei*** Hook.f.: Kanjilal et al. (1940) described both the species i.e., *G. daltonii* and *G. gamblei* separately in 'Flora of

Assam' but now *G. gamblei* is treated as a synonym of *G. daltonii*. The distribution of *G. gamblei* reported by Kanjilal et al. (1940) was found in Garo Hills, Meghalaya. Chakrabarty & Balakrishnan (2018) mentioned the distribution of *G. daltonii* in the Kamrup district, Assam collected by Prain. No herbarium deposition has been observed at ASSAM and CAL herbaria. However, no records of the species were found during the present investigation.

G. khasicum (Müll.Arg.) Hook.f.: Kanjilal et al. (1940) revealed the distribution of *G. khasicum* in the Khasi and Jaintia Hills of Meghalaya. All the herbarium specimen deposited in ASSAM was collected by Kanjilal (1914), Dey & D.C.F. (1940), Balakrishnan (1965), and Rao (1974) which were from Khasi and Jaintia Hills, Meghalaya. Sastry (1964) collected from Subansiri F.D. (NEFA), Arunachal Pradesh. Dina Nath (1936) collected from Goalpara District, Assam. After a critical review and study of the herbarium specimen, it has been found that the specimen deposited by Dina Nath (1936) are more closely allied to *G. ellipticum* (specimen deposited at ASSAM). Bora & Bhattacharya (2014) collected from Durbintila, Silchar, Assam. But their collected specimens are more resemble to *G. sphaerogynum* (specimen deposited at ASSAM). Thus, after their collection, no further specimen deposition from Assam has been recorded till now. From the above study, it can be concluded that the specimen has not been found inside the present political boundary of Assam. During the present research work, the species was not found.

G. oblatum Hook.f.: Kanjilal et al. (1940) mentioned the distribution of *G. oblatum* in North Cachar hills, Cachar, Assam. Chakrabarty & Balakrishnan (2018) also mentioned the distribution of *G. oblatum* in the Golaghat district, Assam collected by King's collectors. No herbarium specimen deposition collected by them has been observed at ASSAM and CAL herbaria. Deka (1937) misidentified *G. multiloculare* as *G. oblatum* (specimen deposited at ASSAM). Species were not found during the present research work.

G. thomsonii (Müll.Arg.) Hook.f.: Kanjilal et al. (1940) reported the distribution of *G. thomsonii* in Cachar, Assam and Garo Hills, Khasi and Jaintia Hills, Meghalaya. Although Chakrabarty & Balakrishnan (2018) revealed the occurrence of the species in Assam. But till now no collection from Assam has been reported and no herbarium specimen collected from Assam has been observed at ASSAM and CAL herbaria. The collection made by Kanjilal (1914) from Cherrapunji, Khasi and Jaintia

hills, Sharma (1938) from Mowomai, Bowmick (1975) from Khasi and Jaintia hills, Aabid Hussain Mir (2015) from Khrang all were from Meghalaya state (specimen deposited at ASSAM). Thus, the species has no distributional record from the present geographical distribution of Assam. However, no species has been encountered during the present work.

G. velutinum Wight: Kanjilal et al. (1940) described both *G. velutinum* and *G. heyneanum* separately in the ‘*Flora of Assam*’. However, *G. velutinum* is now considered a synonym of *G. heyneanum*.

5.1.2. New distribution record

One of the varieties of *Glochidion zeylanicum* (Gaertn.) A.Juss. i.e. *Glochidion zeylanicum* var. *paucicarpum* Chakrab. & N.P. Balakr. is recorded for the first time from Assam during the present research work. This variety was first reported by Chakrabarty & Balakrishnan (2018) from Andaman and Nicobar Island, India as an endemic variety of *G. zeylanicum*. The variety is also not recorded in the ‘*Flora of Assam*’ (Brahma & Baruah, 2023). The present research reports that this variety from the Kokrajhar district is a new distributional record from Assam, India.

5.1.3. Morphology of the genus

Morphological characteristics i.e., both vegetative and reproductive characteristics of the genus *Glochidion* play a significant role in segregating and delimiting the taxa.

Vegetative characters

Habit and habitat: The present recorded species of the genus show mainly shrub or small tree to tall tree habit characters. Almost all the studied taxa grow in small to large trees except some taxa viz., *G. multiloculare* var. *multiloculare* and *G. multiloculare* var. *pubescens* are medium bushy shrubs habit. *G. multiloculare* var. *multiloculare* is the most commonly found and widespread species in grassland and swampy areas. *G. multiloculare* var. *pubescens* is endemic to Assam and Sikkim (Chakrabarty & Balakrishnan, 2018) and it is also commonly found in grassland, swampy or stream areas, mixed forests, and *sal* forests areas. Species of *Glochidion* are mainly found in moist deciduous forests, evergreen forests, swampy areas, stream areas, mixed and *sal* forests, and often secondary forests and roadside areas (**Plate 54**).

Stem and branches, bark: All the members have eminent branched stems with drooping or bushy branches. Branchlets are angled, terete, and straight. The outer appearance of barks is usually green at young and dark brown at mature. In some members viz., all the varieties of the species *G. zeylanicum* and the species *G. lanceolarium* bark show red inside. Other members viz., *G. ellipticum*, *G. heyneanum*, *G. sphaerogynum*, *G. multiloculare* var. *multiloculare* and *G. multiloculare* var. *pubescens*, bark show whitish creamy to greenish white inside. During the study, it has been observed that if the specimen's fruit colour is white creamy to greenish then the colour inside the bark will also be the same as the fruit colour.

Petiole: Petiole length and colour also differs in species of *Glochidion*. Red colour has been observed in the members of *Glochidion zeylanicum* var. *arborescens*, *G. zeylanicum* var. *paucicarpum*, *G. zeylanicum* var. *tomentosum*, *G. zeylanicum* var. *zeylanicum*. Other members i.e., *G. ellipticum*, *G. heyneanum*, *G. lanceolarium*, *G. sphaerogynum*, *G. multiloculare* var. *multiloculare*, and *G. multiloculare* var. *pubescens* have the green colour of the petiole.

Leaves: Leaves are usually simple, alternate, and petiolate with prominent veins. There is significant dissimilarity in the shape and size of the leaves. Some species have cordate, ovate-elliptic, round at base and long petioles and others have elliptic-lanceolate, asymmetric or oblique base and short petioles. The margin of the leaves is normally entire, curl at the margin when dry (*G. multiloculare* var. *multiloculare*, *G. multiloculare* var. *pubescens*) and the tip is acute or short and long acuminate. Leaf surface may be coriaceous or membranous and the colour of leaves may be green, dark green or pale green, reddish, and yellowish green. Hairs present on the both lower and upper surfaces of some species (*G. heyneanum*, *G. multiloculare* var. *pubescens*, *G. zeylanicum* var. *arborescens*, *G. zeylanicum* var. *paucicarpum*, *G. zeylanicum* var. *tomentosum*).

Reproductive characters

Inflorescence: *G. zeylanicum* var. *zeylanicum*, *G. zeylanicum* var. *paucicarpum*, *G. zeylanicum* var. *tomentosum*, and *G. zeylanicum* var. *arborescens* show supra-axillary or pedunculate and rarely axillary inflorescence while rest of the taxa exhibit axillary inflorescence. When dried, most of the leaves of the members curl at the margin or the edge of the leaf.

Peduncles and pedicels: In all the taxa, the peduncles and pedicels of all the female flowers are shorter compared to male flowers.

Flowers: Both male and female flowers have been observed in the same axils as well as in different axils in the members of *Glochidion*.

Male flower: Male flowers of the taxa reveal remarkably similar traits, but the number of anthers separates them.

Female flower: Female flowers represent different characteristics from male flowers.

Ovary: The number of locules in the ovary varies by taxon, and style characters also play a key role. Styles are persistent.

Capsule: Characteristics of capsules or fruits are one of the taxonomic relevance in the members of *Glochidion*. Taxa can be distinguished based on the shape, size, colour, locules, and hairy habit of the capsule. Certain taxa like *G. zeylanicum* var. *zeylanicum* and their varieties can be easily identified through their completely unlobed and ambiguously lobed capsule. *G. multiloculare* and *G. sphaerogynum* exhibit deeply or conspicuously lobed capsules while *G. ellipticum* possesses a superficially lobed capsule. The fruit without the stalk or the non-pedicellate i.e. sessile capsule can be seen in *G. lanceolarium* which we can differentiate this species from other taxa.

5.1.4. Taxonomic significance of anatomical characters

5.1.4.1. Foliar epidermal study

One of the challenges in taxonomy is distinguishing between species that exhibit high morphological similarity. With foliar leaf epidermal characters at both qualitative and quantitative levels, taxonomists can identify subtle differences that may not be apparent through conventional methods. This detailed analysis helps to overcome the problem of similarity among taxa by revealing unique morphological traits that can be used for species differentiation. Overall, the integration of qualitative and quantitative foliar leaf epidermal character studies using LM and FESEM greatly enhances the accuracy and effectiveness of taxonomic identification and classification, particularly in addressing challenges posed by the morphological resemblance of the taxa. Based on qualitative data such as stomatal features,

epidermal cell characters, anticlinal cell walls, papillae, epicuticular wax crystals, trichomes (**Table 22**) and quantitative data such as stomatal size, area, index, trichomes size (**Table 23**), using LM and FESEM the taxa can be distinguished. All the comparative graphs are represented in **Figure 24** to **Figure 28**.

In the present study, mainly two different types of stomatal positions have been observed i.e., hypostomatic leaves having stomata only on the abaxial surface of the leaf and amphistomatic leaves having stomata on both abaxial and adaxial surface of the leaf. Taxa like *G. ellipticum*, *G. heyneanum*, *G. lanceolarium*, *G. sphaerogynum*, and the varieties of *G. zeylanicum* possess hypostomatic leaf surfaces while *G. multiloculare* var. *multiloculare* and *G. multiloculare* var. *pubescens* possess amphistomatic leaf surfaces. The dominant stomata type is anomocytic type followed by anisocytic, paracytic, and hemiparacytic types. High concentrations of stomata were observed mainly on the lower or abaxial surface of the leaf. The stomatal shape varies from elliptic, and oval to elongated. Stomatal shape exhibited significant character among the studied taxa. These types of characteristics play a crucial role in the study of phylogenetic relationships as well as their physical characteristics play a vital role in understanding the origin and classification of plants at the higher taxonomic level as well (Van Cotthem, 1970; Razzaq et al., 2021). From the quantitative analysis, we found the highest stomatal length in *G. ellipticum* ($43.14 \pm 2.340 \mu\text{m}$) and the lowest in *G. zeylanicum* var. *arborescens* ($11.70 \pm 1.112 \mu\text{m}$) (**Figure 25**) and the highest stomatal width has been observed in *G. ellipticum* ($24.756 \pm 1.432 \mu\text{m}$) and lowest in *G. zeylanicum* var. *arborescens* ($6.21 \pm 0.504 \mu\text{m}$) (**Figure 26**). The maximum stomatal area was found in *G. ellipticum* and the lowest stomatal area was observed in *G. zeylanicum* var. *arborescens* (**Figure 27**). The highest and lowest percentages of the stomatal index were observed in the variety *G. zeylanicum* var. *paucicarpum* and *G. multiloculare* var. *multiloculare* respectively (**Figure 24**). The shape of epidermal cells varied from isodiametric, pentagonal, and hexagonal to polygonal and some taxa like *G. ellipticum*, *G. lanceolarium*, *G. sphaerogynum*, *G. zeylanicum* var. *zeylanicum*, *G. zeylanicum* var. *arborescens*, *G. zeylanicum* var. *tomentosum* exhibited undulate and jigsaw shape of the epidermal cell. Maximum number of taxa exhibited sinuous and sometimes smooth, rounded, and angular anticlinal cell walls. All the taxa lack papillae except *G. multiloculare*

var. *multiloculare* and *G. multiloculare* var. *pubescens* which were rounded and present on the abaxial surface.

Epicuticular wax acts as a barrier to plant cuticles and defends the plant from excessive transpiration and UV light (Adams' et al., 1990; Koch & Barthlott, 2006). *G. multiloculare* var. *multiloculare* and *G. multiloculare* var. *pubescens*. have epicuticular wax crystals on the lower surface of the leaf. They are mostly smooth, thick, and generally present around the stomata or trichomes. A small amount of epicuticular wax crystal was found in *G. sphaerogynum*. The micromorphological study of cuticular wax is useful in taxonomic delimitation at several taxonomic levels within flowering plants (Barthlott, 1998). It is nearly impossible to determine the precise location, shape, and size of papillae and epicuticular wax crystals under light microscopy, and to detect those features, scanning electron microscopy study was employed (Duarte-Silva et al., 2013).

Taxa like *G. ellipticum*, *G. lanceolarium*, *G. multiloculare* var. *multiloculare*, *G. sphaerogynum*, and *G. zeylanicum* var. *zeylanicum* lack trichomes. Different types of trichomes have been observed in the taxa such as *G. heyneanum*, *G. multiloculare* var. *pubescens*, *G. zeylanicum* var. *arborescens*, *G. zeylanicum* var. *paucicarpum* and *G. zeylanicum* var. *tomentosum*. They are densely present on the abaxial surface rather than the adaxial surface. They are uniseriate, multicellular, unbranched, and non-glandular types. In *G. heyneanum*, they are an almost hooked shape, uniseriate, multicellular, unbranched, and non-glandular. Along with uniseriate, multicellular, unbranched, and non-glandular types, peltate types are also observed in *G. zeylanicum* var. *paucicarpum*. Glands are absent in all the taxa. The absence of glandular trichomes on the leaf suggests that some other tissues are responsible for the secretion of secondary metabolites (Lawrence et al., 2015). Trichomes have been used to resolve taxonomic conflicts and help to understand the evolutionary relationship among the species (Payne, 1978; Solihani et al., 2015). Quantitative data showed that the highest length of the trichomes in *G. zeylanicum* var. *paucicarpum* ($263.833 \pm 0.059 \mu\text{m}$) and the lowest in *G. multiloculare* var. *pubescens* ($131.336 \pm 1.170 \mu\text{m}$) on the abaxial surface and in the adaxial surface highest trichome length was observed in *G. zeylanicum* var. *paucicarpum* ($244.513 \pm 1.085 \mu\text{m}$) and lowest in *G. heyneanum* ($114.033 \pm 1.881 \mu\text{m}$) (**Figure 28**).

Table 22. Qualitative foliar epidermal study of different species of genus *Glochidion*

Name of taxa	Stomatal position	Stomatal surface	Stomatal type	Stomatal shape	Epidermal cell shape	Anticlinal cell wall	Papillae	Epicuticular wax crystals	Trichome types
<i>G. ellipticum</i>	Hypo	Ab	Ano, Ani, Para	Ellip, Ovl	Jgw, Rctg	Sin	-	-	-
		Ad	-	-	Jgw, Rctg	Sin	-	Drs, Prs	-
<i>G. heyneanum</i>	Hypo	Ab	Ano, Ani	Elg	Isd, Pntg, Hxg to Plg	Sin, Rnd, Smt, Ang	-	-	Unc, Hkd, Mlcr, Unbr, Ng
		Ad	-	-	Isd, Pntg, Hxg to Plg	Sin, Rnd, Smt, Ang	-	-	Unc, Hkd, Mlcr, Unbr, Ng
<i>G. lanceolarium</i>	Hypo	Ab	Ano, Ani, Hemi	Elg	Unl, Jgw	Sin	-	-	-
		Ad	-	-	Unl, Jgw	Sin	-	-	-
<i>G. multiloculare</i> var. <i>multiloculare</i>	Amphi	Ab	Ano, Ani, Hemi	Elg, Ellip	Isd, Pntg, Hxg to Plg	Smt, Ang, Rnd, Irrg thick	Rnd	Thck wx ppl, Smt, Upr Stm	-
		Ad	Ano, Ani	Elg, Ellip	Isd, Pntg, Hxg to Plg	Smt, Ang, Rnd, Irrg thick	-	-	-
<i>G. multiloculare</i> var. <i>pubescens</i>	Amphi	Ab	Ano, Ani, Para	Ellip	Isd, Pntg, Hxg to Plg	Rnd, Smt, Ang	Rnd	Thck wx ppl, Trch, Smt, Upr Stm	Uns, Mlcr, Unbr, Ng
		Ad	Ano, Ani	Ellip	Isd, Pntg, Hxg to Plg	Rnd, Smt, Ang	-	-	Uns, Mlcr, Unbr, Ng
<i>G. sphaerogynum</i>	Hypo	Ab	Ano, Para	Ellip	Unl, Jgw	Sin	Rnd	-	-
		Ad	-	-	Unl, Jgw	Sin	-	-	-
<i>G. zeylanicum</i> var. <i>arborescens</i>	Hypo	Ab	Ano, Para	Ellip	Jgw, Pntg, Hxg to Plg, Unl	Sin	-	-	Uns, Mlcr, Unbr, Ng
		Ad	-	-	Jgw, Pntg, Hxg to Plg, Unl	Sin	-	-	Uns, Mlcr, Unbr, Ng
<i>G. zeylanicum</i> var. <i>paucicarpum</i>	Hypo	Ab	Para, Hemi	Ellip	Isd, Pntg, Hxg to Plg	Rnd, Smt, Irrg, Slgt sin	-	-	Uns, Mlcr, Unbr, Ng, Plt
		Ad	-	-	Isd, Pntg, Hxg to Plg	Rnd, Smt, Irrg, Slgt sin	-	-	Uns, Mlcr, Unbr, Ng, Plt
<i>G. zeylanicum</i> var. <i>tomentosum</i>	Hypo	Ab	Ano, Ani	Ellip	Isd, Pntg, Hxg to Plg, Jgw, Unl	Rnd, Smt, Irrg, Sin	-	-	Uns, Mlcr, Unbr, Ng
		Ad	-	-	Isd, Pntg, Hxg to Plg, Jgw, Unl	Rnd, Smt, Irrg, Sin	-	-	Uns, Mlcr, Unbr, Ng
<i>G. zeylanicum</i> var. <i>zeylanicum</i>	Hypo	Ab	Ano, Ani, Hemi, Para	Ellip, Ovl	Jgw, Pntg, Hxg to Plg, Rctg, Unl	Sin, Bts	-	-	-
		Ad	-	-	Jgw, Pntg, Hxg to Plg, Rctg, Unl	Sin, Bts	-	-	-

Abbreviation and symbol used: Hypo = Hypostomatic, Amphi = Amphistomatic, **Ab** = Abaxial, **Ad** = Adaxial, - = Absent, Ano = Anomocytic, Ani = Anisocytic, Para = Paracytic, Hemi = Hemiparacytic, Ellip = Elliptic, Ovl = Oval, Elg = Elongated, Jgw = Jigsaw, Rctg = Rectangular, Isd = Isodiametric, Pntg = Pentagonal, Hxg = Hexagonal, Plg = Polygonal, Unl

= Undulate, Sin = Sinuous, Rnd = Rounded, Smt = Smooth, Ang = Angular, Irrg thck = Irregularly thickened, Slgt sin = Slightly sinuous, Irrg = Irregular, Bts = Buttressed, Drs = Druse, Prs = Prismatic, Thck wx ppl = Thick waxes at the papillae, Upr Stm = Upright around Stomata, Trch = Trichomes, Unc = Uncinate, Hkd = Hooked, Mlcr = Multicellular, Unbr = Unbranched, Ng = Non-glandular, Uns = Uniseriate, Plt = Peltate

Table 23. Quantitative data of foliar epidermal study of different species of the genus *Glochidion*

Name of taxa	Leaf surface	No. of stomata per area	Stomatal Density (SD)	Epidermal Cell Density (ECD)	Stomatal Index (%) (SI)	Stomatal Length (µm) (SL)	Stomatal Width (µm) (SW)	Stomatal Area (µm ²) (SA)	Trichome Length (µm) (TL)
<i>G. ellipticum</i>	Ab	67–79	72.667±1.027	229.33±1.527	24.06	43.14±2.340	24.756±1.432	838.156	-
	Ad	-	-	-	-	-	-	-	-
<i>G. heyneanum</i>	Ab	110–156	133.333±2.007	718±2.502	15.66	24.336±0.293	12.99±1.535	248.096	144.473±1.618
	Ad	-	-	-	-	-	-	-	114.033±1.881
<i>G. lanceolarium</i>	Ab	62–90	77±1.106	278.333±2.052	21.66	25.843±2.437	18.01±2.78	365.322	-
	Ad	-	-	-	-	-	-	-	-
<i>G. multiloculare</i> var. <i>multiloculare</i>	Ab	13–16	14.333±1.527	109.333±1.503	11.56	35.81±2.506	21.016±1.651	590.608	-
	Ad	7–11	9.333±0.081	84.333±1.041	10.02	33.46±2.180	20.406±1.304	535.828	-
<i>G. multiloculare</i> var. <i>pubescens</i>	Ab	14–18	16.333±2.081	111.667±1.743	12.75	34.15±1.04	20.603±1.499	552.239	131.336±1.170
	Ad	11–16	13.333±2.214	107±1.248	11.07	37.31±2.192	20.867±1.979	611.247	127.823±1.480
<i>G. sphaerogynum</i>	Ab	95–108	101.333±1.506	438.667±1.509	18.77	24.06±2.442	9.696±2.850	183.015	-
	Ad	-	-	-	-	-	-	-	-
<i>G. zeylanicum</i> var. <i>arborescens</i>	Ab	111–140	124±1.730	270±2.221	31.47	11.70±1.112	6.21±0.504	57.035	195.033±1.374
	Ad	-	-	-	-	-	-	-	151.023±1.450
<i>G. zeylanicum</i> var. <i>paucicarpum</i>	Ab	104–128	117±1.124	238±1.911	32.95	20.026±1.706	13.60±2.038	213.733	263.833±0.059
	Ad	-	-	-	-	-	-	-	244.513±1.085
<i>G. zeylanicum</i> var. <i>tomentosum</i>	Ab	80–92	86.333±1.027	212.333±2.326	28.89	14.367±0.529	7.713±1.015	86.972	176.486±2.977
	Ad	-	-	-	-	-	-	-	115.196±1.205
<i>G. zeylanicum</i> var. <i>zeylanicum</i>	Ab	80–100	91.333±0.263	246±1.442	27.08	17.507±1.847	9.34±0.598	128.308	-
	Ad	-	-	-	-	-	-	-	-

Abbreviation and symbol used: **Ab** = Abaxial, **Ad** = Adaxial, - = Absent, Data are represented as Mean±SD

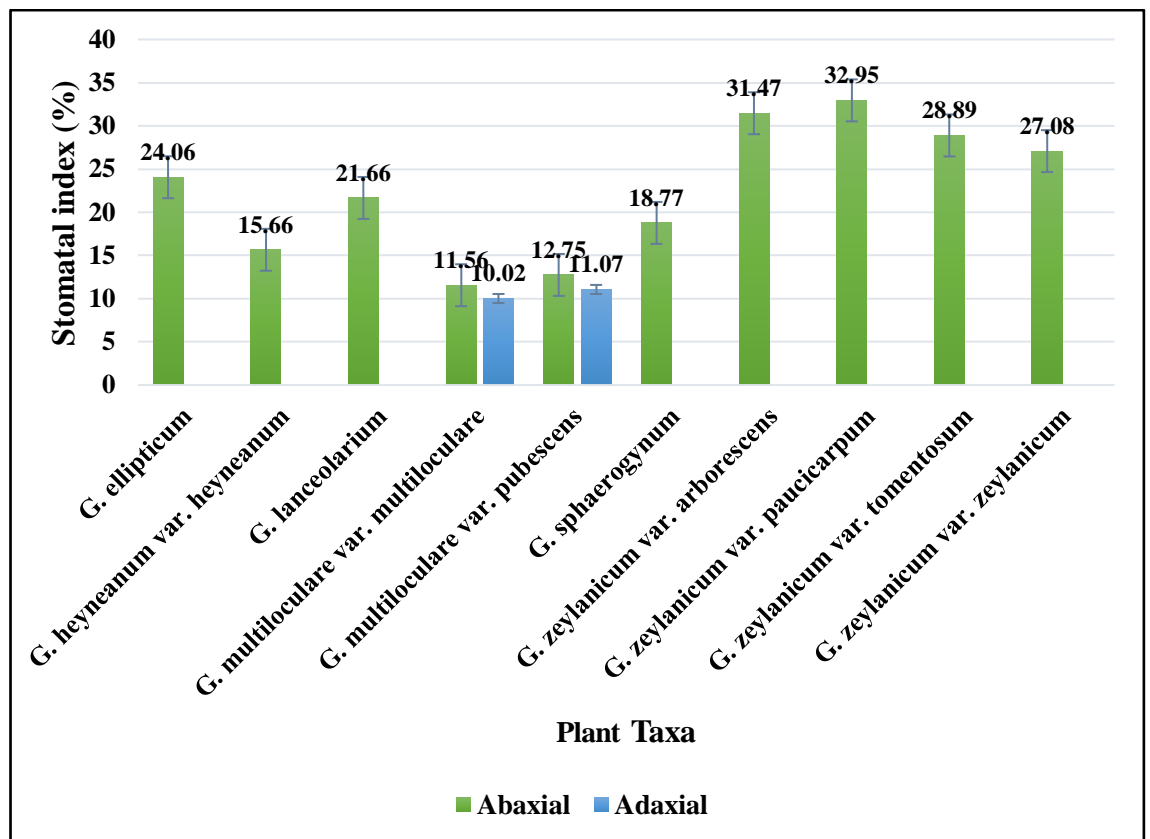


Figure 24. Comparative stomatal index of the abaxial and adaxial surface of leaf epidermal cell of different members of *Glochidion*

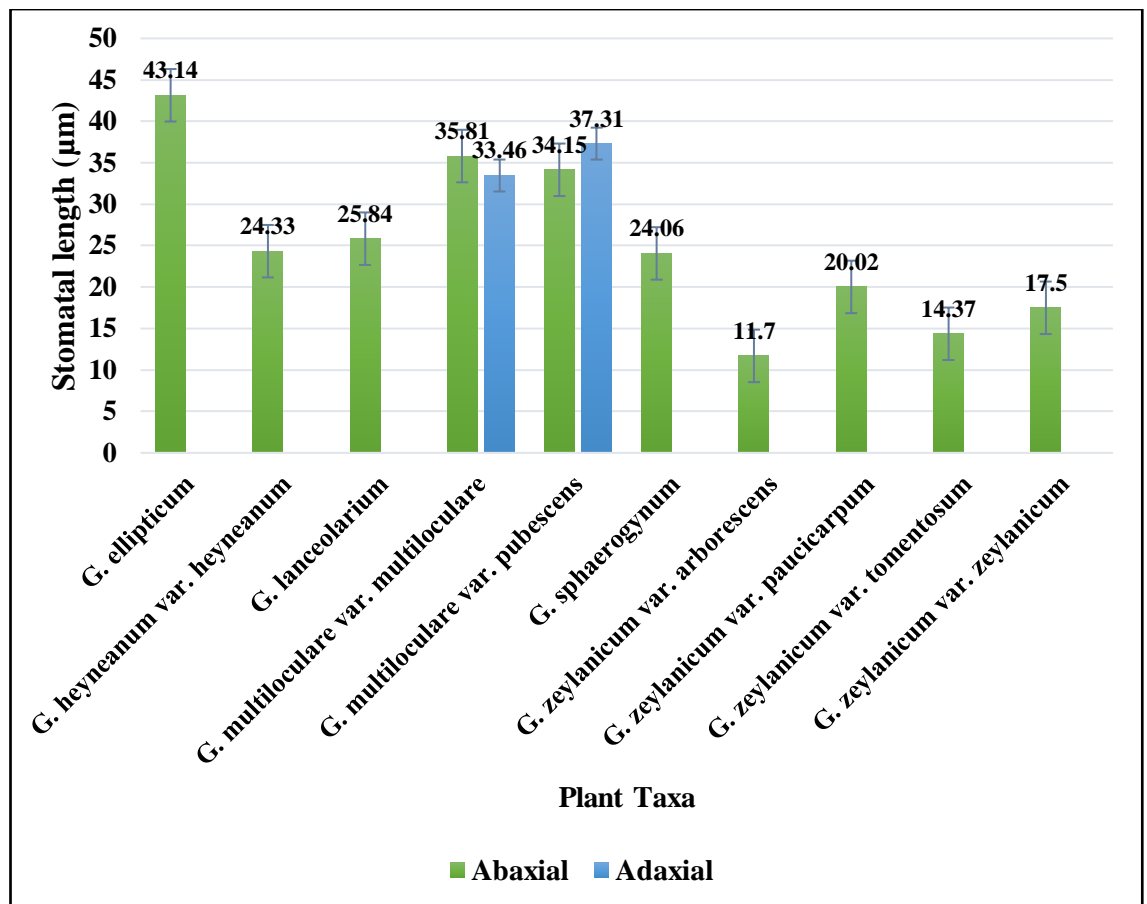


Figure 25. Comparison of quantitative data of the stomatal length of the abaxial and adaxial surface of leaf epidermal cell of different members of *Glochidion*

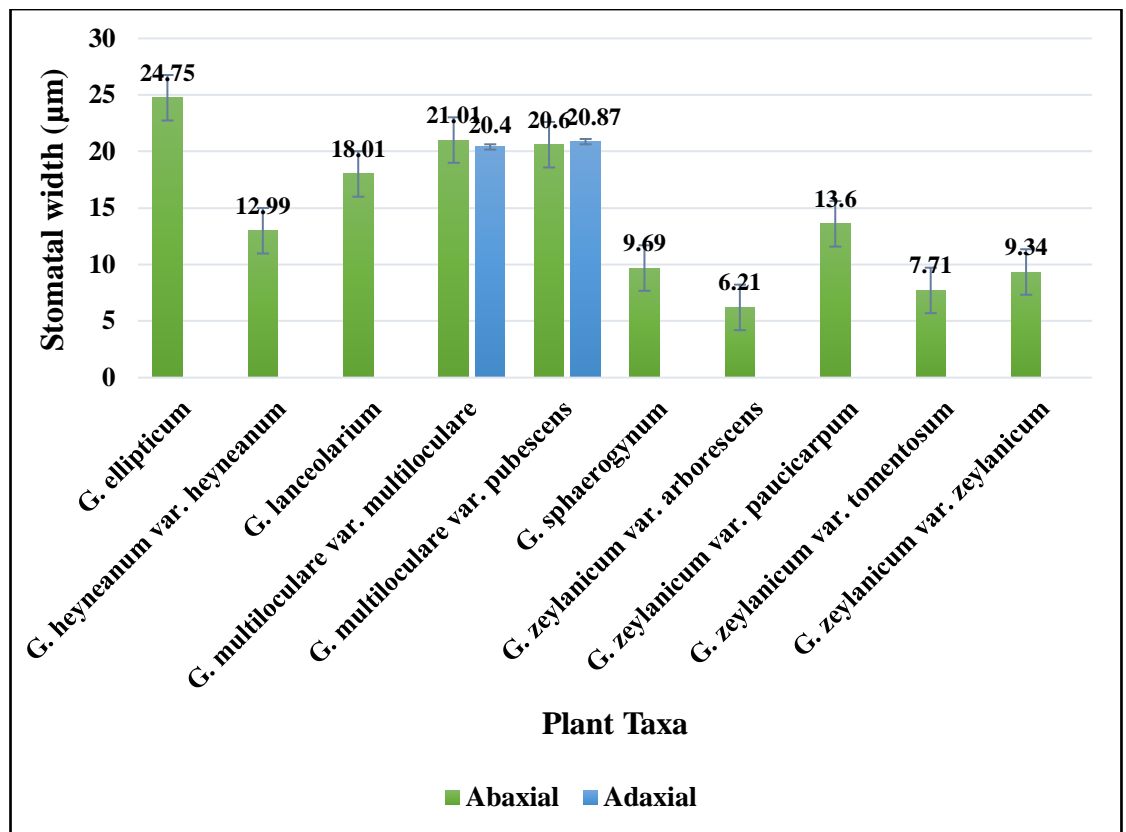


Figure 26. Comparison of quantitative data of stomatal width of the abaxial and adaxial surface of leaf epidermal cell of different members of *Glochidion*

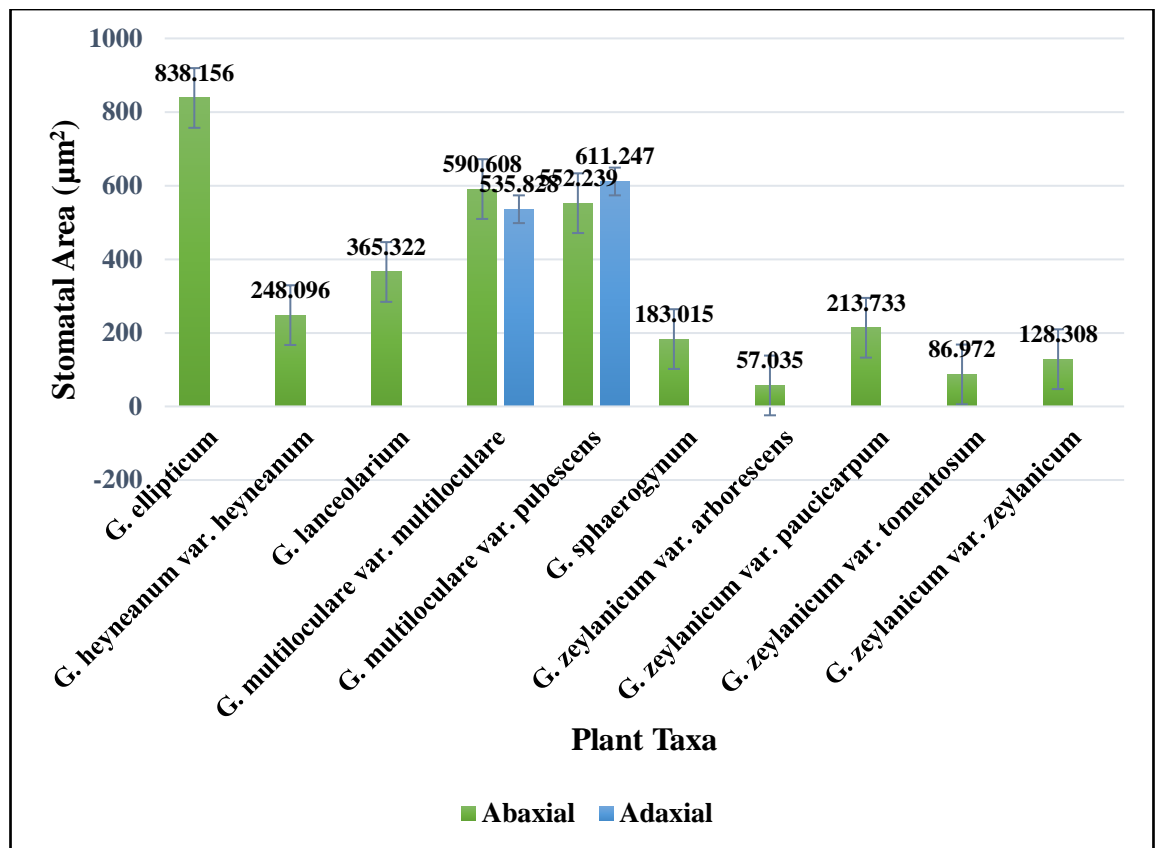


Figure 27. Comparison of quantitative data of stomatal area of the abaxial and adaxial surface of leaf epidermal cell of different members of *Glochidion*

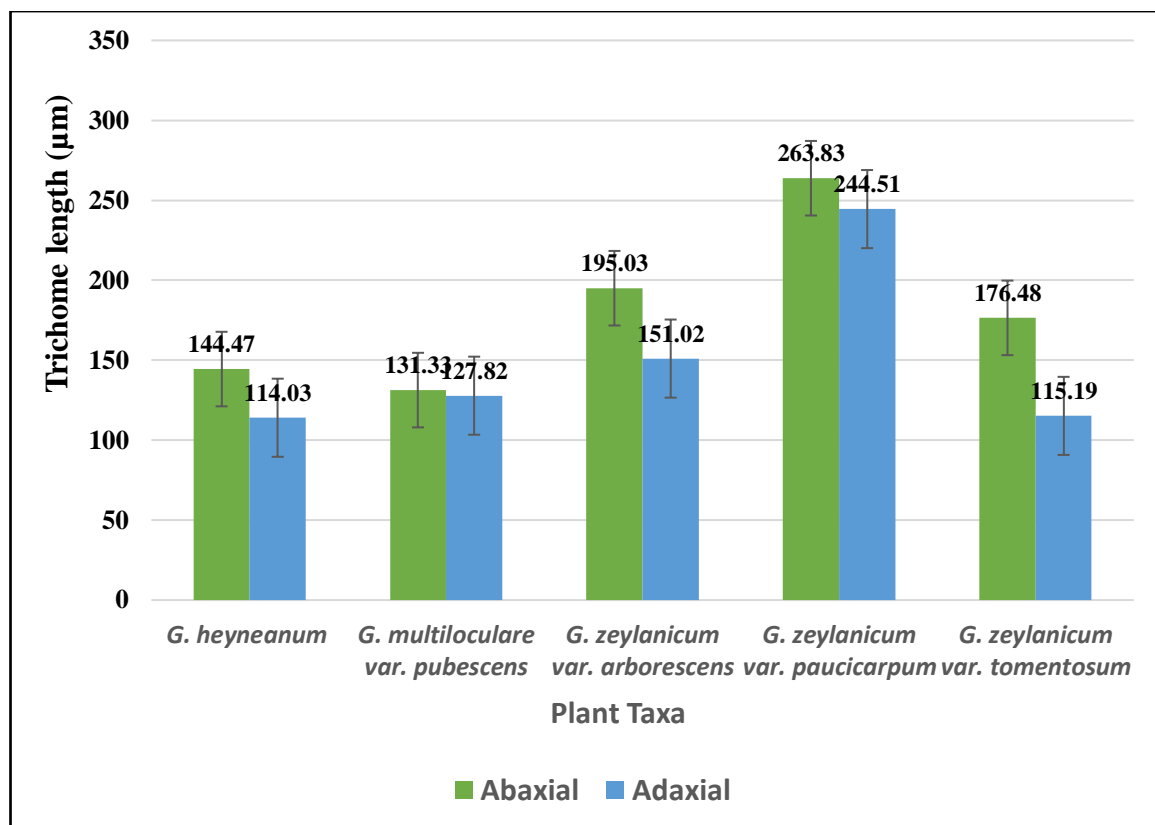


Figure 28. Comparison of quantitative data of trichomes length of the abaxial and adaxial surface of leaf epidermal cell of different members of *Glochidion*

5.1.4.2. Petiole anatomy

The transverse section of the petiole of all the studied taxa show variation ranging from circular to shield-shaped or entire in outline. The characteristics viz., number and arrangement of vascular bundles, presence or absence of glands, crystals, and epidermal hairs are particularly useful (**Table 24**). All the taxa have crystals and patches of sclerenchymatous cells present around the vascular bundles in all the studied taxa.

Table 24. Comparative anatomical features of petiole of species recorded in the present study

Name of the taxa	Outline	Hypodermis	Collenchyma	Parenchyma	Sclerenchyma	Vascular bundle	Crystals	Glands	Trichomes
<i>G. ellipticum</i>	Crc, Slgt rdg	1 lyr	2 lyr	6–7 lyr	Prst vb	Crc shp, Arc shp, 2 Dst sml vb	Drs, Prs	-	-
<i>G. heyneanum</i>	Crc, Slgt rdg	1 lyr	2–3 lyr	4–5 lyr	Prst vb	Crc shp, Arc shp, 2 Dst sml vb	Drs, Prs	Ng	Smp, Mlcr
<i>G. lanceolarium</i>	Crc	2–3 lyr	4–5 lyr	7–10 lyr	Prst vb	Arc shp, 2 Dst sml vb	Drs, Prs	-	-
<i>G. multiloculare</i> var. <i>multiloculare</i>	Crc, Shl shp	1–2 lyr	2–3 lyr	7–8 lyr	Prst vb	Arc shp, 2–4 Dst sml vb	Drs, Prs	-	-
<i>G. multiloculare</i> var. <i>pubescens</i>	Crc, Shl shp	1 lyr	2–5 lyr	2–3 lyr	Prst vb	Arc shp, 2 Dst sml vb	Drs, Prs	Ng	Smp, Mlcr
<i>G. sphaerogymum</i>	Crc, Rdg, Wvy	2–3 lyr	4–5 lyr	10–12 lyr	Prst vb	Arc shp, 3 Dst sml vb	Drs, Prs, Rpd	-	-
<i>G. zeylanicum</i> var. <i>arborescens</i>	Crc, Slgt rdg	1–2 lyr	7–8 lyr	2–3 lyr	Prst vb	Arc shp, 5 Dst sml vb	Drs, Prs	Ng	Smp, Mlcr
<i>G. zeylanicum</i> var. <i>paucicarpum</i>	Crc, Slgt rdg	1–2 lyr	6–8 lyr	3–4 lyr	Prst vb	Arc shp, Wng shp	Drs, Prs	Ng	Smp, Mlcr
<i>G. zeylanicum</i> var. <i>tomentosum</i>	Crc, Shl shp	1 lyr	6–7 lyr	2–3 lyr	Prst vb	Crc shp, Arc shp, 2 Dst sml vb	Drs, Prs	Ng	Smp, Mlcr
<i>G. zeylanicum</i> var. <i>zeylanicum</i>	Crc, Slgt rdg	1 lyr	5–6 lyr, cry	3–5 lyr, Drs cry	Prst vb	Arc shp, 3 Dst sml vb	Drs, Prs	-	-

Abbreviation and symbol used: Crc = Circular, Slgt rdg = Slightly ridged, Ridged = Rdg, Shl shp = Shield-shaped, Wvy = Wavy, Lyr = Layer, Drs cry = Druse crystal, Prst vb = Present around the vascular bundle Crc shp = Crescent-shaped, Wng = Wing, Dst sml vb = Distinct small vascular bundle, Drs = Druse, Prs = Prismatic, Rpd = Raphides, Ng = Non- glandular, Smp = Simple, Mlcr = Multicellular, - = Absent

5.1.4.3. Leaf architecture study

Leaf architecture study of the present recorded taxa show the pinnate type of venation. A comparison of various characteristic features of the leaf architecture study of the recorded taxa has been represented in **Table 25** and **Table 26** respectively. Lobation is unlobed in all the species which is a constant character of genus *Glochidion*. Bundle sheaths are well-developed in all veins and the cluster of tracheids are present at the tip of the veinlet in all the studied taxa.

Table 25. Comparative leaf characters of species recorded in the present study

Name of the taxa	Leaf arrangement	Leaf organization	Leaf length (cm)	Leaf breadth (cm)	Leaf shape	Leaf apex	Leaf base	Leaf surface	Margin	Lobation
<i>G. ellipticum</i>	Alternate	Simple	4–25	2–8	Elliptic to lanceolate, oblong to obovate	Apiculate, caudate, acuminate	Obtuse	Glabrous on both surfaces	Entire, untoothed	Unlobed
<i>G. heyneanum</i>	Alternate	Simple	3–17	2–7	Ovate to elliptic, obovate	Acute, apiculate	Obtuse or rounded	Pubescent on both surfaces and densely pubescent beneath	Entire, untoothed	Unlobed
<i>G. lanceolarium</i>	Alternate	Simple	4–27	2.5–8	Lanceolate to oblanceolate, elliptic	Apiculate, acuminate or acute	Obtuse or rounded	Glabrous on both surfaces	Entire, untoothed	Unlobed
<i>G. multiloculare</i> var. <i>multiloculare</i>	Alternate	Simple	4–16	2–6	Oblong to lanceolate, elliptic to oblanceolate	Acute, apiculate or retuse	Obtuse or rounded	Glabrous on both surfaces at mature and pubescent at young	Entire, untoothed	Unlobed
<i>G. multiloculare</i> var. <i>pubescens</i>	Alternate	Simple	4–15	2–6	Oblong to lanceolate, elliptic to oblanceolate	Acute, apiculate or retuse	Obtuse or rounded	Pubescent on both surfaces and densely pubescent beneath	Entire, untoothed	Unlobed
<i>G. sphaerogynum</i>	Alternate	Simple	3–23	1.5–5.3	Oblong to elliptic, falcate	Acuminate	Attenuate	Glabrous on both surfaces	Entire, untoothed	Unlobed
<i>G. zeylanicum</i> var. <i>arborescens</i>	Alternate	Simple	5–25	1.5–8.5	Ovate to elliptic	Acute, acuminate	Obtuse or rounded	Densely pubescent on both surfaces	Entire, untoothed	Unlobed
<i>G. zeylanicum</i> var. <i>paucicarpum</i>	Alternate	Simple	6–22	4–8	Elliptic, ovate to lanceolate	Acute	Obtuse, truncate, rarely oblique, rounded	Densely pubescent on both surfaces	Entire, untoothed	Unlobed
<i>G. zeylanicum</i> var. <i>tomentosum</i>	Alternate	Simple	5–20	3–8	Ovate to elliptic, cordate	Obcordate, acute	Obtuse, truncate, asymmetric	Densely pubescent on both surfaces	Entire, untoothed	Unlobed
<i>G. zeylanicum</i> var. <i>zeylanicum</i>	Alternate	Simple	8–20	5–8	Ovate to elliptic, cordate	Acute, apiculate	Cordate, asymmetric, truncate	Glabrous on both surfaces	Entire, untoothed	Unlobed

Table 26. Comparative leaf venation characters of species recorded in the present study

Name of the taxa	1° vein category	2° vein category	3° vein category	4° vein category	5° vein category	Areole development	Vein termination number (per mm square)	Vein islet number (per mm square)	Free ending venation	Marginal ultimate venation
<i>G. ellipticum</i>	Pinnate	Weak brochidodromous	Mixed percurrent	Irregular reticulate to mixed percurrent	Freely ramifying	Moderate to good	121–133	80–94	Dichotomously branched	Looped
<i>G. heyneanum</i>	Pinnate	Weak brochidodromous	Sinuuous to percurrent	Mixed percurrent	Irregular reticulate	Moderate to good	87–92	74–79	Dichotomously branched	Looped
<i>G. lanceolarium</i>	Pinnate	Weak brochidodromous	Mixed percurrent	Percurrent	Irregular reticulate	Moderate to good	98–105	66–72	One branched to dichotomously branched	Looped
<i>G. multiloculare</i> var. <i>multiloculare</i>	Pinnate	Weak brochidodromous to hemieucamptodromous	Mixed percurrent	Mixed percurrent	Irregular reticulate to dichotomizing	Moderate to good	127–135	97–111	One branched to dichotomously branched	Looped
<i>G. multiloculare</i> var. <i>pubescens</i>	Pinnate	Weak brochidodromous to hemieucamptodromous	Mixed percurrent	Mixed percurrent	Freely ramifying	Moderate	131–138	89–109	One branched to dichotomously branched	Looped
<i>G. sphaerogynum</i>	Pinnate	Weak brochidodromous	Mixed percurrent	Mixed percurrent	Irregular polygonal, reticulate to dichotomizing	Moderate to good	93–103	62–88	Dichotomous to dendritic branched	Looped
<i>G. zeylanicum</i> var. <i>arborescens</i>	Pinnate	Weak brochidodromous	Mixed percurrent	Irregular reticulate to freely ramifying	Freely ramifying	Good	77–95	59–68	Dichotomous to dendritic branched	Looped
<i>G. zeylanicum</i> var. <i>paucicarpum</i>	Pinnate	Weak brochidodromous	Mixed percurrent	Irregular reticulate to freely ramifying	Freely ramifying	Good	74–89	57–65	Dichotomous to dendritic branched	Looped
<i>G. zeylanicum</i> var. <i>tomentosum</i>	Pinnate	Weak brochidodromous	Decurrent	Irregular reticulate to freely ramifying	Freely ramifying	Good	80–91	65–71	Dichotomous to dendritic branched	Looped
<i>G. zeylanicum</i> var. <i>zeylanicum</i>	Pinnate	Weak brochidodromous	Decurrent	Irregular reticulate to mixed percurrent	Irregular reticulate to freely ramifying	Good	81–94	66–83	Dichotomous to dendritic branched	Looped

5.1.5. Significance in Phytochemical Study

5.1.5.1. Phytochemical screening

The preliminary phytochemical studies of different parts of the extract of *G. ellipticum*, *G. multiloculare*, and *G. sphaerogynum* show the presence of many important phytoconstituents. Across all the studied species, nearly all parts exhibit important phytoconstituents such as alkaloids, flavonoids, reducing sugar, steroids, phlobatannins, tannins, terpenoids, triterpenoids, saponin, glycosides, and phenol.

5.1.5.2. Quantitative estimation

Comparative accounts of the quantitative estimation of alkaloid, flavonoid, saponin, and terpenoid contents are represented in **Figure 29**. Comparative accounts of phenolic and tannin content are represented in **Figure 30** and **Figure 31** respectively. In *G. ellipticum*, leaves denoted the highest concentration of 27.11 ± 0.19 % yield of terpenoid contents, followed by 9.51 ± 0.38 % yield of saponin content, 8.80 ± 1.44 % yield of flavonoid content, 2.40 ± 0.40 % yield of alkaloid content (**Figure 29**) and total phenolic and tannin contents were 0.708 ± 0.003 mg GAE/g dry extract (**Figure 30**) and 3.269 ± 0.276 mg TAE/g dry extract (**Figure 31**) respectively. Bark denoted 13.30 ± 0.26 % yield of terpenoid content, followed by 4.13 ± 0.30 % of saponin content, 4.12 ± 0.61 % yield of alkaloid content, 3.87 ± 0.83 % yield of flavonoid content (**Figure 29**) and total phenolic and tannin contents were 3.180 ± 0.872 mg GAE/g dry extract (**Figure 30**) and 2.489 ± 0.148 mg TAE/g dry extract (**Figure 31**) respectively. Root denoted 17.60 ± 1.21 % yield of terpenoid contents, followed by 15.20 ± 0.35 % yield of saponin content, 1.47 ± 1.00 % yield of flavonoid content, 1.32 ± 0.61 % yield of alkaloid content (**Figure 29**) and total phenolic and tannin contents were 0.233 ± 0.002 mg GAE/g dry extract (**Figure 30**) and 5.115 ± 0.117 mg TAE/g dry extract (**Figure 31**) respectively.

In *G. multiloculare*, leaves exhibited the highest concentration of 21.16 ± 0.32 % yield of terpenoid contents, followed by 18.61 ± 0.31 % yield of saponin content, 6.92 ± 1.51 % yield of flavonoid content, 2.40 ± 0.80 % yield of alkaloid content (**Figure 29**) and total phenolic and tannin contents were 0.041 ± 0.001 mg GAE/g dry extract (**Figure 30**) and 3.212 ± 0.223 mg TAE/g dry extract (**Figure 31**) respectively. Bark exhibited 15.04 ± 0.50 % yield of terpenoid content, followed by 13.40 ± 0.42 % of saponin content, 8.12 ± 0.21 % yield of alkaloid content, 4.67 ± 0.91 % yield of

flavonoid content (**Figure 29**) and total phenolic and tannin contents were 2.168 ± 0.004 mg GAE/g dry extract (**Figure 30**) and 16.917 ± 0.757 mg TAE/g dry extract (**Figure 31**) respectively. Root exhibited 11.40 ± 0.36 % yield of terpenoid contents, followed by 9.60 ± 0.49 % yield of saponin content, 3.20 ± 0.10 % yield of alkaloid content, 1.60 ± 0.34 % yield of flavonoid content (**Figure 29**) and total phenolic and tannin contents were 1.416 ± 0.001 mg GAE/g dry extract (**Figure 30**) and 2.186 ± 0.004 mg TAE/g dry extract (**Figure 31**) respectively.

In *G. sphaerogynum*, leaves showed the highest concentration of 9.2 ± 0.60 % yield of total terpenoid content followed by 9.26 ± 1.70 % yield of total saponin content, 3.20 ± 0.40 % yield of total alkaloid content and 1.44 ± 0.61 % of total flavonoid contents (**Figure 29**). Total phenolic and tannin contents were 1.375 ± 0.001 mg GAE/g dry extract (**Figure 30**) and 5.122 ± 0.006 mg TAE/g dry extract (**Figure 31**) respectively. Bark showed a 12.93 ± 0.81 % yield of total saponin content followed by 8.66 ± 0.76 % yield of total terpenoid content, 3.73 ± 1.00 % yield of total alkaloid content, and 1.24 ± 0.80 % yield of total flavonoid content (**Figure 29**). Total phenolic and tannin contents were 0.166 ± 0.006 mg GAE/g dry extract (**Figure 30**) and 6.921 ± 0.865 mg TAE/g dry extract (**Figure 31**) respectively.

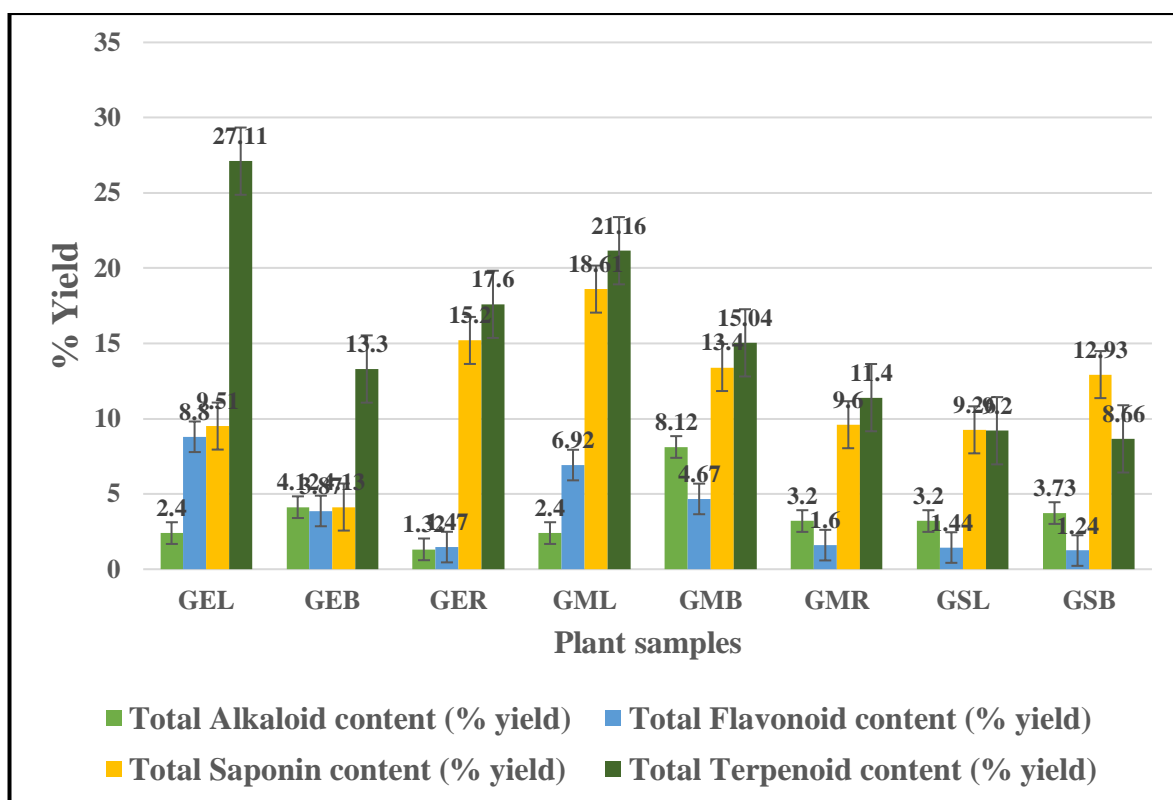


Figure 29. Comparison of % yield of total alkaloid, flavonoid, saponin and terpenoid content of the selected plant samples (GEL = *G. ellipticum* leaves, GEB = *G. ellipticum* bark, GER = *G. ellipticum* roots, GML = *G. multiloculare* leaves, GMB = *G. multiloculare* bark, GMR = *G. multiloculare* roots, GSL = *G. sphaerogynum* leaves, GSB = *G. sphaerogynum* bark)

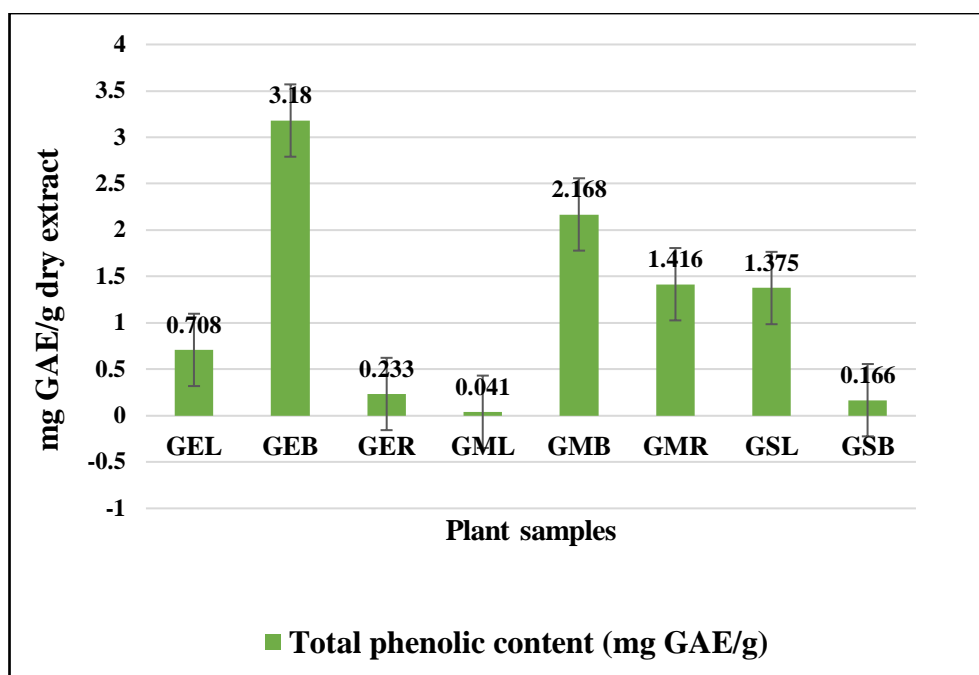


Figure 30. Comparison of concentration of total phenolic content of the selected plant samples (GEL = *G. ellipticum* leaves, GEB = *G. ellipticum* bark, GER = *G. ellipticum* roots, GML = *G. multiloculare* leaves, GMB = *G. multiloculare* bark, GMR = *G. multiloculare* roots, GSL = *G. sphaerogynum* leaves, GSB = *G. sphaerogynum* bark)

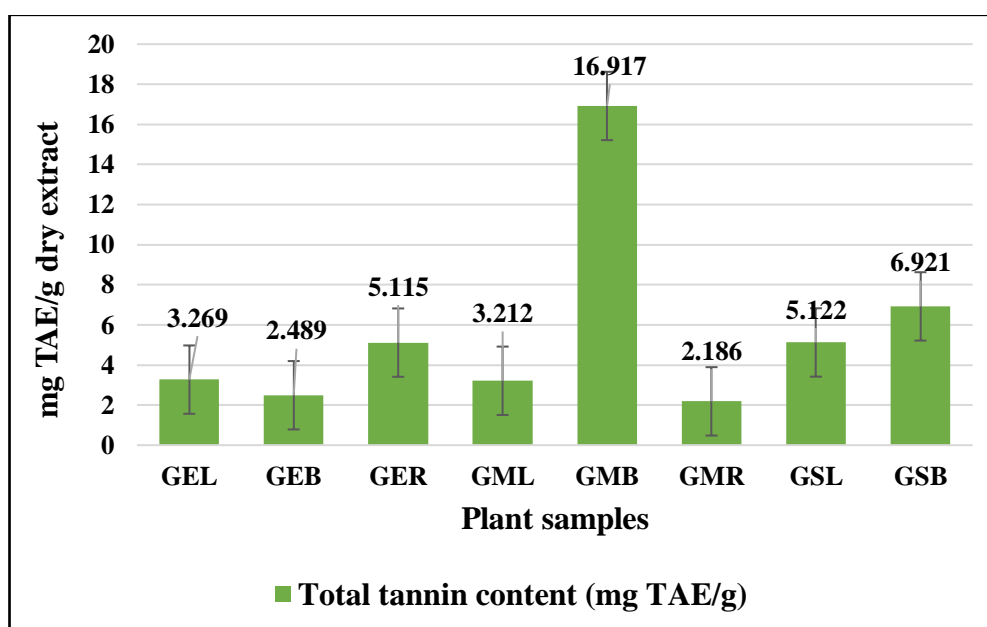


Figure 31. Comparison of concentration of total tannin content of the selected plant samples (GEL = *G. ellipticum* leaves, GEB = *G. ellipticum* bark, GER = *G. ellipticum* roots, GML = *G. multiloculare* leaves, GMB = *G. multiloculare* bark, GMR = *G. multiloculare* roots, GSL = *G. sphaerogynum* leaves, GSB = *G. sphaerogynum* bark)

5.1.6. Antioxidant activity

The DPPH free radical scavenging and the ABTS assays are used to examine antioxidant activity. Comparative accounts of the IC₅₀ value of documented species using DPPH and ABTS assay of antioxidant activity are represented in **Figure 32**.

The result showed that the concentrations of methanolic leaf extract of *G. ellipticum* had DPPH and ABTS scavenging effects. In the DPPH assay, the highest % inhibition (40.159 ± 0.594) was observed at 60 µg/mL with a 16.74 µg/mL IC₅₀ value (**Table 9**). In the study, the standard ascorbic acid exhibited $32.857 \pm 0.751\%$ inhibition at 60 µg/mL and showed the IC₅₀ value of 34.22 µg/mL (**Table 9**). In comparison with three extracts i.e., leaves, barks, and root, the highest IC₅₀ value was found in the root extract (22.50 µg/mL) followed by leaf extract (16.74 µg/mL) and bark extract (12.93 µg/mL) (**Figure 32**). But the lower the IC₅₀ value higher the antioxidant activity. Among the *G. multiloculare* leaves, bark, and root extract, the highest concentration of IC₅₀ value was observed in the leaf extract (19.17 µg/mL), followed by the bark extract (19.01 µg/mL) and root extract (10.67 µg/mL) (**Figure 32**). In *G. sphaerogynum* leaves and bark extracts, the highest concentration of IC₅₀ value was found in the leaf extract (14.45 µg/mL) followed by the bark extract (8.899 µg/mL) (**Figure 32**). The lower the IC₅₀ value the higher will be the antioxidant activity. Therefore, *G. sphaerogynum* bark extracts showed the highest antioxidant activity as compared to other extracts.

The result of the ABTS assay showed a significant amount of concentration. In the study, the standard ascorbic acid exhibited $62.703 \pm 0.181\%$ inhibition at 60 µg/mL and showed the IC₅₀ value of 47.61 µg/mL (**Table 10**). Among the leaves, bark, and root extract of *G. ellipticum*, the highest concentration of IC₅₀ value has been determined in leaf extract (45.75 µg/mL) followed by bark extract (35.60 µg/mL) and root extract (25.88 µg/mL) (**Figure 32**). Among the leaves, bark, and root extract of *G. multiloculare* the highest concentration of IC₅₀ value has been determined in the root extract (76.97 µg/mL) followed by leaf extract (75.23 µg/mL) and bark extract (31.81 µg/mL) (**Figure 32**). In *G. sphaerogynum* leaves and bark extract, the highest concentration of IC₅₀ value has been determined in the leaf extract (12.91 µg/mL) followed by the bark extract (4.059 µg/mL) (**Figure 32**). Thus, *G.*

sphaerogynum bark extract has high antioxidant activity as compared to other sample extracts.

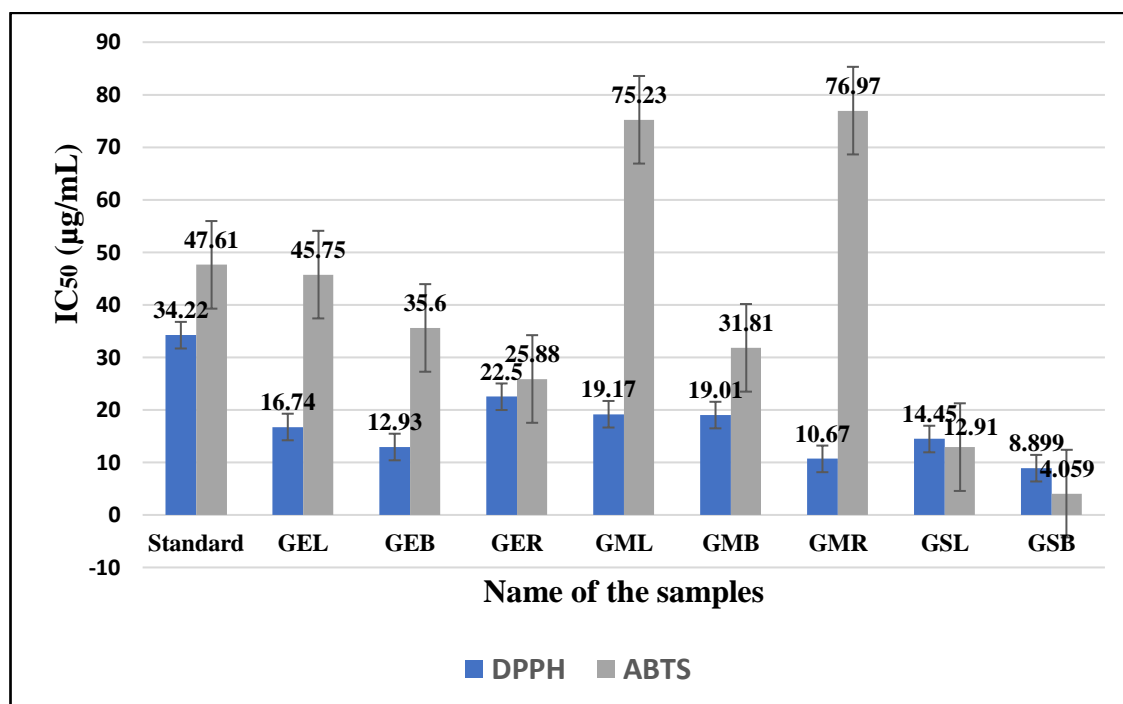


Figure 32. Comparison of IC₅₀ value of documented species using DPPH and ABTS assay of antioxidant activity (GEL = *G. ellipticum* leaves, GEB = *G. ellipticum* bark, GER = *G. ellipticum* roots, GML = *G. multiloculare* leaves, GMB = *G. multiloculare* bark, GMR = *G. multiloculare* roots, GSL = *G. sphaerogynum* leaves, GSB = *G. sphaerogynum* bark)

5.1.7. GC-MS Analysis

The result of GC-MS analysis of the documented species showed many important biologically active volatile compounds. In *G. ellipticum* leaves extract, the molecule Phytol Tetradecanoate is the major identified compound with 6.4% peak area (**Table 11**), in bark extract, 1,2-Benzenediol, 3-Methoxy- is the major compound with 16.625% (**Table 12**), in root extract, Tetradecanoic Acid, 10,13-Dimethyl-, Methyl Ester is the major compound with 4.388% peak area (**Table 13**). In *G. multiloculare* leaves extract, Lup-20(29)-En-3-One is the major compound with 16.81% peak area (**Table 14**), in bark extract, Pentadecanoic Acid, 14-Bromo is the major compound with 0.323% area (**Table 15**), in root extract, Octane, 2,2,6-Trimethyl- is the major compound with 0.936% peak area (**Table 16**). In *G. sphaerogynum* leaf extract, 1,2,3-Benzenetriol is the major compound with a 1.625%

peak area (**Table 17**). In bark extract, the molecule 1,1,6-Trimethyl-3-Methylene-2-(3,6,10,13,14-Pentamethyl-3-Ethenyl-Pentadec-4-enyl) cyclohexane with the peak area of 2.263% is the major compound (**Table 18**). The identified volatile compounds possess important biological activities (**Table 27 to Table 34**).

Table 27. Biological activities of significant compounds of *G. ellipticum* leaf extract

Name of compound	Compound Class	Biological activity
Benzene, 1-Ethyl-2-Methyl-	Toluene	Anthelmintic, insecticidal, antimicrobial (Frank et al., 2017; Morah et al., 2019), antibacterial (Safara et al., 2022)
Mesitylene	Benzene	Antifungal, antibacterial (Morah et al., 2019)
Benzofuran, 2,3-Dihydro	Coumarans	Anti-inflammatory, antimicrobial, antifungal, antihyperglycemic, analgesic, antiparasitic, antitumor, antibacterial, anti-depressant, anticonvulsant, anti-tumor, anti-HIV, anti-diabetic, anti-tuberculosis, antioxidant (Pauletti et al., 2000; Khodarahmi et al., 2015; Chand et al., 2017; Miao et al., 2019; Vandana & Deora, 2020)
Neophytadiene	Sesquiterpenoids	Anti-inflammatory, antipyretic, analgesic, antioxidant, antimicrobial, antifungal, antibacterial, anti-ulcerative, antiparasitic, anticancer, antihemolytic, antivenom, antidepressant (Adnan et al., 2019; Pratama et al., 2019; Ngoben et al., 2020; Bhardwaj et al., 2020; Jalpa & Vijaykumar, 2023)

Phytyl Tetradecanoate	Hydrocarbon	Antimicrobial (Sahu, 2019)
Z,Z-6,28-Heptatriactontadien-2-One	Aliphatic ketone	Vasodilator, antihypertensive, antioxidant (Mallikadevi et al., 2012; Deepak et al., 2017; Ralte et al., 2022)
Methyl 11-Methyl-Dodecanoate	Fatty acid methyl ester	Antibacterial (Mena et al., 2020)
Chlorpyrifos	Organophosphorus ester	Insecticidal (Supreeth et al., 2016)
Phytyl Palmitate	Fatty acid ester	Cytotoxic (Jassbi et al., 2016)

Table 28. Biological activities of significant compounds of *G. ellipticum* bark extract

Name of compound	Compound Class	Biological activity
1,2-Benzenediol, 3-Methoxy-	Methoxy phenol	Antioxidant (Nandini et al., 2021)
Hentriacontane	Alkane	Anti-inflammatory, antitumor, antimicrobial (Khajuria et al., 2017)
1,2,3-Benzenetriol	Phenol	Antimicrobial, anti-inflammatory, antioxidant, analgesic, insecticide, anticancer, cytotoxic (Beulah et al., 2018)
12-Bromododecanoic Acid	Fatty acid	Antioxidant (Khan et al., 2018)
L-(+)-Ascorbic Acid 2,6-Dihexadecanoate	Vitamin	Antidiabetic (Igwe & Okwunodulu, 2014), antioxidant, antimicrobial, antitumor, antibacterial (Karthikeyan et al., 2014; Hadi et al., 2016; Tanod et al., 2019; Kumar et al., 2021)
Piconol	Pyridine	Analgesic, antipyretic, anti-inflammatory (Mc Ateer, 2008), antitumor, cytotoxic (Bildziukevich et al. 2018)

Benzenepropanoic Acid, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy-, Methyl ester	Benzene and substituted derivatives	Antioxidant, antifungal (Akpuaka et al., 2013; Gogoi et al., 2018)
Eicosanoic acid	Fatty acid	Anticancer (Gollo et al., 2020)
N-Hexadecanoic Acid	Fatty acid	Antioxidant, hypocholesterolemic, nematocide, pesticide, anti-inflammatory, anticancer (Mazumder et al., 2020; Siswadi & Saragih, 2021)
Octadecanoic Acid	Fatty acid	Antioxidant, anti-inflammatory, antitumor, antiproliferative (Ganesh & Mohankumar, 2017; Reza et al., 2021)

Table 29. Biological activities of significant compounds of *G. ellipticum* root extract

Name of compound	Compound Class	Biological activity
Lomustine	Alkylating agent	Anticancer (Bartzatt, 2013; de Carvalho et al., 2019), antitumor (Agarwal et al., 2014)
Thymine	Pyrimidine	Antibacterial, antimicrobial, anticancer, antifungal, antimycobacterial (Klein et al., 2007; Kumar et al., 2012; Adamska et al., 2016; Fu et al., 2020; Liu et al., 2021)
Octanoic Acid, 2-Hexyl-	Fatty acid ester	Flavoring ingredient (Kushwaha et al., 2019)
3-Methyl-2-(2-Oxopropyl) Furan	Heteroaromatic	Antioxidant, antimicrobial, bacteriocide, anti-inflammatory (Ralte et al., 2022)
2-Coumaranone	Benzofuranons	Nematicidal (Sun et al., 2022)

Succinic Acid, 8-Chlorooctyl 2-Naphthyl Ester		Steroid ester	Antibacterial (Huang et al., 2022)
Z,Z-6,28-Heptatriactontadien-2-One		Aliphatic ketone	Vasodilator, antihypertensive, antioxidant (Mallikadevi et al., 2012; Deepak et al., 2017; Ralte et al., 2022)
Metoprolol, Derivative	2TMS	Tyrosols and derivatives	Act as β -1 adrenergic receptors in the heart and reduce the effects of catecholamines (such as adrenaline and noradrenaline) on cardiac function (Čižmáriková et al., 2019)
(E)-4-(3-Hydroxyprop-1-En-1-Yl)-2-Methoxyphenol		Phenol	Antioxidant (Kumar et al., 2019)
Benzoic Acid, Hydroxy-6-Methyl-, Methyl Ester	2-	Ester	Antibacterial (Lognay et al., 2000)
N-Propyl Octadecenoate	11-	Fatty acid ester	Pesticide, antimicrobial, antifungal (Alamre & Lmtair Algaraawi, 2020)
Tetradecanoic Acid, 10,13-Dimethyl-, Ester		Fatty acid methyl ester	Antioxidative, anti-inflammatory (Dulara, 2019)
N-Hexadecanoic Acid		Fatty acid	Antioxidant, antibacterial, hypocholesterolemic, nematocide, pesticide, anti-inflammatory, anticancer (Mazumder et al., 2020; Siswadi & Saragih, 2021, Ahmed et al., 2022)
Eicosanoic acid		Fatty acid	Anticancer (Gollo et al., 2020)
Cyclobarbitol		Barbiturate derivatives	Testosterone 17 beta-dehydrogenase (NADP+) inhibitor, anesthetic general, anticonvulsant, neurotransmitter antagonist, skeletal

		muscle relaxant (Brintha et al., 2017), anti-proliferative (Maitra et al., 2019; Maher et al., 2021)
L-(+)-Ascorbic Acid 2,6-Dihexadecanoate	Vitamin	Antidiabetic (Igwe & Okwunodulu, 2014), antioxidant, antimicrobial, antitumor, antibacterial (Karthikeyan et al., 2014; Hadi et al., 2016; Tanod et al., 2019; Kumar et al., 2021)
1-Nonylcycloheptane	Cycloalkanes	Antimicrobial (Ameya et al., 2022)

Table 30. Biological activities of significant compounds of *G. multiloculare* leaf extract

Name of compound	Compound Class	Biological activity
Neophytadiene	Sesquiterpenoids	Anti-inflammatory, antipyretic, analgesic, antioxidant, antimicrobial, antifungal, antibacterial, anti-ulcerative, antiparasitic, anticancer, antihemolytic, antivenom, antidepressant (Adnan et al., 2019; Pratama et al., 2019; Ngoben et al., 2020; Bhardwaj et al., 2020; Jalpa & Vijaykumar, 2023)
Phytol	Acyclic diterpene	Anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, antimicrobial, insecticidal, antidiabetic, antihyperalgesic, antitumor, antifungal, antimutagenic, anti-depression (Silva et al.,

		2014; Santos et al., 2013; Islam et al., 2018; Saha et al., 2020; Rahaman et al., 2020; Gliszczynska et al., 2021; Taj et al., 2021)
Citronellol	Monoterpene alcohol	Antimicrobial, antibacterial (Lopez-Romero, 2015; Silva et al., 2021), anti-inflammatory, analgesic (Santos et al. 2019),
Z,Z-6,28-Heptatriactontadien-2-One	Aliphatic ketone	Vasodilator, antihypertensive, antioxidant (Mallikadevi et al., 2012; Deepak et al., 2017; Ralte et al., 2022)
1-Methylene-2B-Hydroxymethyl-3,3-Dimethyl-4B-(3-Methylbut-2-Enyl)-cyclohexane	Sesquiterpenoids/sesquiterpene alcohol	Antimicrobial, anti-inflammatory, antihyperlipidemic (Nabi et al., 2022)
Lup-20(29)-En-3-One	Pentacyclic lupane type triterpene	Antileukemia (Hata et al., 2003), anticancer, anti-inflammatory, parasitic (Nistor, 2022)
Lupeol	Pentacyclic triterpenoids	Anti-inflammatory, anti-arthritic, cytotoxic, anticarcinogenic, antileukemia, hepatoprotective, cardioprotective agent, anticancer, antioxidant, antimicrobial (Wal et al., 2011; Liu et al., 2021), anti-protozoal, antiproliferative, antidiabetic, anti-invasive, anti-angiogenic, cholesterol-lowering agent (Sharma et al., 2020)
2R-Acetoxyethyl-1,3,3-Trimethyl-4T-(3-Methyl-2-Buten-1-YL)-1T-Cyclohexanol	Sesquiterpene	Antiinflammatory, antibacterial (Saravanan & Kasisankar, 2013), anticancer (Naine et al., 2016; Nabi et al., 2022)

Table 31. Biological activities of significant compounds of *G. multiloculare* bark extract

Name of compound	Compound Class	Biological activity
3-Methylpentatriacontane	Alkane	Antibacterial, antiviral, antimicrobial (Ozdemir et al., 2006)
Docosanal	Fatty alcohol	Antiviral (Katz et al., 1991)
3-Methyl-2-(2-Oxopropyl) Furan	Heteroaromatic	Antioxidant, antimicrobial, bacteriocide, antipyretic, anti-inflammatory activity (Nithyadevi & Shivakumar, 2015; Ralte et al., 2022)
Z,Z-6,28-Heptatriactontadien-2-One	Aliphatic ketone	Vasodilator, antihypertensive, antioxidant activity (Mallikadevi et al., 2012; Ralte et al., 2022)
Heptacosanoic Acid, 25-Methyl-, Methyl Ester	Fatty acid ester	Anticancer (Kandasamy et al., 2012), larvicidal (Balasubramani et al., 2015), antimicrobial (Ralte et al., 2022)
Pentadecanoic Acid, 14-Bromo	Fatty acid	Protecting cardiometabolic, immune, and liver health (Venn-Watson & Schork, 2023)
Oleic acid	Fatty acid	Antibacterial, apoptotic activity, antimicrobial, anticancer, antioxidant, anti-

		inflammatory (Fontana et al., 2013; Alabi et al., 2018; Ozsen et al., 2019)
Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15- Hexadecamethyl-	Silicon ether	Antimicrobial, insecticidal (Abdullah et al., 2018, Khalid et al., 2022; More et al., 2022)
Tetradecanal	Fatty aldehyde	Antibacterial, antifungal, antimicrobial (Nasr et al., 2022)

Table 32. Biological activities of significant compounds of *G. multiloculare* root extract

Name of compound	Compound Class	Biological activity
Propanoic Acid, 2,2-Dimethyl-, 2-Ethylhexyl Ester	Fatty acid ester	Arachidonic acid inhibitor, increases aromatic amino acid decarboxylase activity, inhibit the production of uric acid (Mohammad et al., 2022)
Octane, 2,2,6-Trimethyl-	Alkane/ Fatty alcohol/oxygenated hydrocarbon	Antioxidant, antibacterial, antimicrobial, anticancer (Ahmad et al., 2016; Wiraswati et al., 2023)
Undecanoic Acid, 11-Bromo-, Methyl Ester	Fatty acid ester	Antimicrobial, antibiofilm (Yasa et al., 2017)
Hentriacontane	Alkane	Anti-inflammatory, antitumor, antimicrobial (Khajuria et al., 2017)
Sulfurous Acid, Butyl 2-Ethylhexyl Ester	Ester	Antioxidant, antibacterial (Arulkumar et al., 2018), antimicrobial (Wiraswati et al., 2023)
2-Methylheptanoic Acid	Fatty acid	Antituberculosis activity (Martins et al., 2014)

Tridecanoic Acid, 12-Methyl-, Methyl Ester	Fatty acid ester	Antifungal, antibacterial (Elaiyaraja & Chandramohan, 2018)
9,12-Hexadecadienoic Acid, Methyl Ester	Fatty acid ester	Cytotoxic (Mazumder et al., 2020)
Methyl 11,12-Tetradecadienoate	Fatty acid methyl ester	Antibacterial (Sharma et al., 2018; Kibungu et al., 2021)
4-Dodecanol	Fatty alcohol	Antibacterial (Togashi et al. 2007), antimicrobial (Suárez-Quiroz et al., 2013; Dahiru et al., 2022), insect attractant, larvicidal (Bae et al., 2017)
7-Octynoic Acid, Methyl Ester	Fatty acid ester	Antidiabetic, antimicrobial (Suryowati et al., 2023)

Table 33. Biological activities of significant compounds of *G. sphaerogynum* leaf extract

Name of compound	Compound Class	Biological activity
1,2,3-Benzenetriol (Pyrogallol)	Polyphenol	Antibacterial (Deryabin & Tolmacheva, 2015; Oliveira et al., 2022), antimicrobial, anti-inflammatory, antioxidant, analgesic, insecticide, anticancer, cytotoxic (Beulah et al., 2018),
5-Acetoxymethyl-2-Furaldehyde	Furan	Antimicrobial, antibacterial, anti-inflammatory, analgesic, antifungal, anticancer (Saeid et al., 2023)
3-Cyclopentyl-1-Propanol	Cyclic alcohol	Antibiotic, antitumor (Alison & James, 2010)
Fumaric acid, Heptyl 3-Methylbut-3-Enyl Ester	Ester	Antioxidant (Wrona et al., 2022)
2,2-Dimethyl-Propyl 2,2-Dimethyl-Propane-Thiosulfinate	Thiosulfinic acid ester	Antibacterial, antifungal, antimicrobial

			(Sorlozano-Puerto et al., 2021)
Trifluoromethyl Disulfide	T-Butyl	Organofluorine sulfides	Antiobesity (Gooda Sahib et al., 2012; Toor et al., 2020), antimicrobial (Abraham et al., 2016), antibacterial (Mahajan et al., 2022)
Oxalic Acid, Ethyl Neopentyl Ester		Polyphenol ester	Anti-adipogenic activity (Toor et al., 2020)
Anthracene, 9-Ethyl-9, 10-Dihydro-10-Trimethylsilyl-		Furan	Anticancer, antibacterial, anti-inflammatory (Salim, 2018)
Heptalene, 7, 7-Dihydro-6,6-Bis (Trimethylsilyl)Methyl-		Polycyclic hydrocarbon	Antifungal, antimicrobial (Al-Toubi et al., 2022)

Table 34. Biological activities of significant compounds of *G. sphaerogynum* bark extract

Name of compound	Compound Class	Biological activity
Hentriacontane	Alkane	Anti-inflammatory, antitumor, antimicrobial (Khajuria et al., 2017)
Xanthosine	Purine nucleotides	Therapeutic and pharmacological properties (Kulikowska et al., 2004)
Fluorene	Hydrocarbon	Drug design (Gillis et al., 2015)
Phenanthrene	Hydrocarbon	Analgesic, antitussive, antimalarial, cytotoxic, anti-constipation, antioxidant, anti-inflammatory (Kwofie & Gupta, 2021)
Neophytadiene	Sesquiterpenoids	Anti-inflammatory, antipyretic, analgesic, antioxidant, antimicrobial, antifungal, antibacterial, anti-ulcerative, antiparasitic, anticancer,

		antihemolytic, antivenom, antidepressant (Adnan et al., 2019; Pratama et al., 2019; Ngoben et al., 2020; Bhardwaj et al., 2020; Jalpa & Vijaykumar, 2023)
Benzenepropanoic acid, 3,5-bis(1,1-dimethyl ethyl)-4-hydroxy-, methyl ester	Benzene and substituted derivatives	Antioxidant, antifungal (Akpuaka et al., 2013; Gogoi et al., 2018)
2,6,10,14-Tetramethyl-7-(3-methylpent-4-enylidene) pentadecane	Isoprenoid	Anti-inflammatory, antioxidant (Marchioni et al., 2020)
Z,Z-6,28-Heptatriactontadien-2-one	Aliphatic ketone	Vasodilatory, antihypertensive, antioxidant activity (Mallikadevi et al., 2012; Ralte et al., 2022)
1,1,6-Trimethyl-3-methylene-2-(3,6,10,13,14-pentamethyl-3-ethenyl-pentadec-4-enyl)cyclohexane	Fatty acid	Antimicrobial, anticancer, antiarthritic, anti-inflammatory, antiviral (Painuli et al., 2016; Rawat et al., 2018)
Methyl 2-hydroxy-eicosanoate	Fatty acid methyl ester	Antioxidant, anti-inflammatory (Das & Malipeddi, 2014; Ponnudurai & Peter Paul, 2020)

5.1.8. Assessment of mineral elements

Comparative accounts of the concentration of different mineral elements in the selected species using AAS are represented in **Figure 33**.

Plants have specific minerals that are advantageous for humans besides some toxic metals that are injurious to human health (Soetan et al., 2010). Various toxic heavy metals found in plants may create severe problems in the human body. Over time these metals can accumulate in the body and lead to a variety of health complications such as cancer, neurological damage, organ damage, and developmental issues (Tchounwou et al., 2012). Thus, the human body requires

essential mineral elements to maintain the body's metabolic functions. The result of the assessment of mineral composition in *G. ellipticum* leaf extract showed the maximum concentration of calcium (Ca) element followed by sodium (Na), iron (Fe), magnesium (Mg), lead (Pb), manganese (Mn), zinc (Zn), copper (Cu), chromium (Cr) and potassium (K) whereas cadmium (Cd) showed below the detection level of concentration (**Table 19**). *G. ellipticum* bark extract showed the highest concentration of Ca element followed by Na, Mg, Fe, Pb, Zn, Cr, Cu, and K whereas Mn and Cd showed below the detection level concentration (**Table 19**). Root extract showed the highest concentration of Ca element followed by Fe, Na, Mg, Zn, Pb, Cu, Cr, and K, whereas Mn and Cd showed below the detection level of concentration (**Table 19**). *G. multiloculare* leaf extract showed the highest concentration of Ca element followed by Na, Fe, Mg, Mn, K, Zn, Cu, Cr, and Cd whereas Pb showed below the detection level of concentration (**Table 20**). Bark extract showed the highest concentration of Ca element followed by Na, Fe, Mg, K, Zn, Cr, Cu, and Pb whereas Mn and Cd showed below the detection level concentration (**Table 20**). Root extract showed the highest concentration of Ca element followed by Na, Fe, Mg, Pb, Zn, K, Cr, and Cu, whereas Mn and Cd showed below the detection level concentration (**Table 20**). *G. sphaerogynum* leaf extract showed the maximum concentration of Na followed by Ca, Mn, Mg, Fe, Zn, K, Cu and the lowest number of concentrations of Cr, Pb, and Cd (**Table 21**). In bark extract, the maximum concentration amount has been determined in Na followed by Ca, K, Fe, Mg, Mn, Zn, Cu and a lesser amount of concentration of Cr, Pb, and Cd (**Table 21**).

Cr, Pb, and Cd are considered as toxic elements in plants and humans if they exhibit very high concentrations (Behera & Bhattacharya, 2016). In the present study, we found that all the sample extracts exhibit very small concentrations of Cr, Pb, and Cd elements. Based on this data, we can say that these plant samples are devoid of hazardous contaminants. Because the plants contain vital macro and trace elements, they can also be significant mineral-consuming plants.

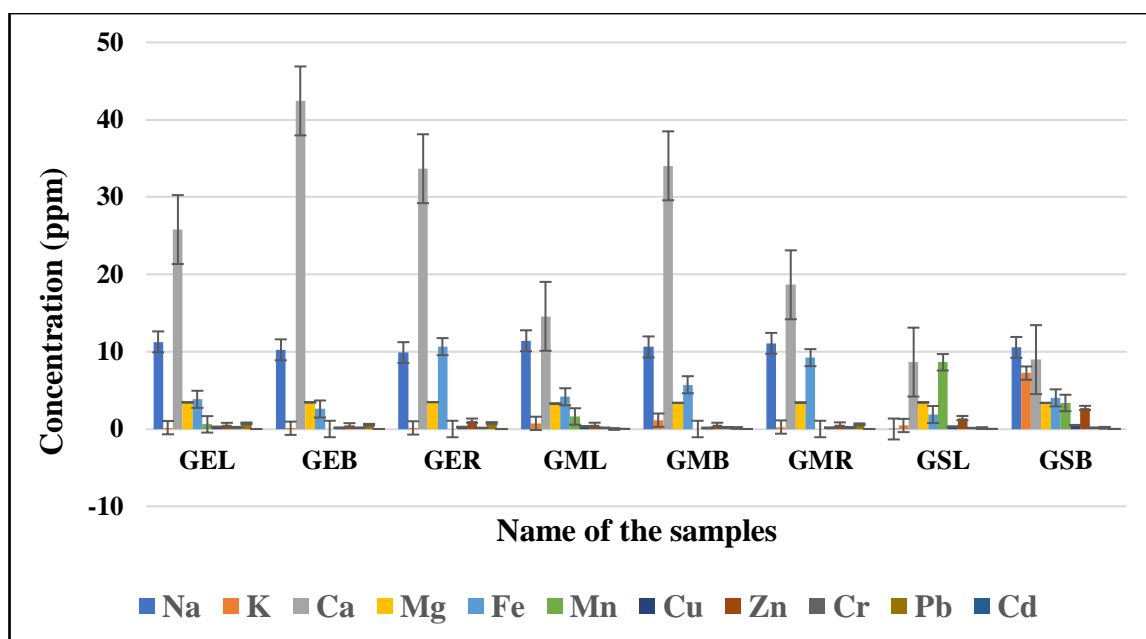


Figure 33. Comparison of concentration of different mineral elements of the selected species using AAS (GEL = *G. ellipticum* leaves, GEB = *G. ellipticum* bark, GER = *G. ellipticum* roots, GML = *G. multiloculare* leaves, GMB = *G. multiloculare* bark, GMR = *G. multiloculare* roots, GSL = *G. sphaerogynum* leaves, GSB = *G. sphaerogynum* bark)

5.2. Significant test for quantitative analysis

5.2.1. Foliar epidermal study

Table 35. One-way ANOVA test of the foliar epidermal study of the abaxial surface of leaves of the studied taxa

Parameters	DF	F	P value	Significance
Stomatal density (Abaxial)	9, 20	38.12	<.001	****
Epidermal cell density (Abaxial)	9, 20	32.75	<.001	***
Stomatal length (Abaxial)	9, 20	77.43	<.001	***
Stomatal width (Abaxial)	9, 20	57.25	<.001	***

Trichome length (Abaxial)	4, 10	6.683	0.007	**
Trichome length (Adaxial)	4, 10	2.965	0.058	*

The values are significant at $p \leq 0.05$ level

Table 36. Unpaired t-test of the foliar epidermal study of the adaxial surface of leaves of studied taxa

Parameters	df	t	P value	Significance
Stomatal density (Adaxial)	4	2.809	0.056	*
Epidermal cell density (Adaxial)	4	2.101	0.052	*
Stomatal length (Adaxial)	4	2.157	0.049	*
Stomatal width (Adaxial)	4	2.336	0.054	*

The values are significant at $p \leq 0.05$ level

5.2.2. Phytochemical study

Table 37. One-way ANOVA test of the total alkaloid, flavonoid, saponin, terpenoid, phenolic, and tannin contents of the studied sample

Parameters	DF	F	P value	Significance
Total alkaloid content	7, 16	11.95	<.001	***
Total flavonoid content	7, 16	11.77	<.001	***
Total saponin content	7, 16	18.56	<.001	***
Total terpenoid content	7, 16	8.009	0.0003	***
Total phenolic content	7, 16	11.61	<.001	***

Total tannin content	7, 16	19.77	<.001	***
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The values are significant at $p \leq 0.05$ level

5.2.3. Antioxidant activity

Table 38. One-way ANOVA test of the DPPH assay of the studied sample extracts and standard

Parameters	DF	F	P value	Significance
Standard	5, 12	800.1	<.001	***
GEL	5, 12	778.8	<.001	***
GEB	5, 12	746.7	<.001	***
GER	5, 12	44.12	<.001	***
GML	5, 12	1094	<.001	***
GMB	5, 12	919.2	<.001	***
GMR	5, 12	201.1	<.001	***
GSL	5, 12	495.0	<.001	***
GSB	5, 12	336.5	<.001	***

The values are significant at $p \leq 0.05$ level

Abbreviation used: GEL = *G. ellipticum* leaves, GEB = *G. ellipticum* bark, GER = *G. ellipticum* roots, GML = *G. multiloculare* leaves, GMB = *G. multiloculare* bark, GMR = *G. multiloculare* roots, GSL = *G. sphaerogynum* leaves, GSB = *G. sphaerogynum* bark

Table 39. One-way ANOVA test of the ABTS assay of the studied sample extracts and standard

Parameters	DF	F	P value	Significance
Standard	5, 12	314.8	<.001	***
GEL	5, 12	11.97	<.001	***
GEB	5, 12	14.38	<.001	***
GER	5, 12	284.3	<.001	***
GML	5, 12	291.8	<.001	***
GMB	5, 12	1020	<.001	***
GMR	5, 12	4405	<.001	***
GSL	5, 12	35.76	<.001	***
GSB	5, 12	54.26	<.001	***

The values are significant at $p \leq 0.05$ level

Abbreviation used: GEL = *G. ellipticum* leaves, GEB = *G. ellipticum* bark, GER = *G. ellipticum* roots, GML = *G. multiloculare* leaves, GMB = *G. multiloculare* bark, GMR = *G. multiloculare* roots, GSL = *G. sphaerogynum* leaves, GSB = *G. sphaerogynum* bark

5.2.4. Estimation of mineral elements

Table 40. One-way ANOVA test of the mineral elements of the studied sample

Parameters	DF	F	P value	Significance
GEL	9, 20	163432	<.001	***
GEB	8, 18	2411536	<.001	***
GER	8, 18	24826	<.001	***
GML	9, 20	2547	<.001	***
GMB	8, 18	425390	<.001	***
GMR	8, 18	24805	<.001	***
GSL	10, 22	27999	<.001	***
GSB	10, 22	1339	<.001	***

The values are significant at $p \leq 0.05$ level

Abbreviation used: GEL = *G. ellipticum* leaves, GEB = *G. ellipticum* bark, GER = *G. ellipticum* roots, GML = *G. multiloculare* leaves, GMB = *G. multiloculare* bark, GMR = *G. multiloculare* roots, GSL = *G. sphaerogynum* leaves, GSB = *G. sphaerogynum* bark