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Comparative anatomy and trichome morphology on three medicinally important species in the family menispermaceae of southern Western Ghats

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Abstract
The Menispermaceae family of flowering plants comprises of about 71 genera and 450 species. Menispermaceae are mostly climbing plants and the majority of species can be found in countries with a tropical climate. The main feature historically used to define the family is the curved seed found in many of the genera, hence the common name moonseed family. The family contains a number of plants with scientifically recognized important pharmacological activities. The aim of this study was to determine the anatomical characteristics of stem, leaf, petiole and trichome morphology of *Anamirta cocculus*, *Cissampelos pariera* var. *hirsuta* and *Cyclea peltata.* Anatomical features are more conserve therefore useful for taxonomical studies. These characteristics proved to be useful to distinguish the species of taxonomic studies.

Keywords: morhological, anatomical, medicinal plants, *Anamirta cocculus*, *Cissampelos pariera* var. *hirsuta and Cyclea peltata*

1. Introduction
The Menispermaceae comprises of 71 genera with 450 species [1]. In Southern Western Ghats family comprises of 12 genera and 21 species [2]. Menispermaceae are mostly climbling plants and majority of species can be found in countries with tropical climate. The main feature historically used to define the family is the curved seed found in many of the genera, hence the common name moonseed family. Members of the family are mostly lianas, sometimes small trees or shrubs and occasionally perennial herbs. The leaves are alternate, petiolate, sometimes peltate and exstipulate. The lamina is simple, entire or lobed, palmately veined [3, 4]. The family contains a number of plants with scientifically recognized important pharmacological activities.
The selected species include *Anamirta cocculus* (L) Wight & Arn., *Cissampelos pariera* L. Var. *hirsuta* (Buch. -Ham. ex DC.) Forman and *Cyclea peltata* Hook. f.& Thoms. in the family Menispermaceae
Anamirta cocculus is a large, dioecious climbing plant. The stems twine into other plants for support. The leaves are large, simple, alternate, long petiolated, broadly ovate, cordate at base, having acute apex. In South-East Asia the fruit of *Anamirta cocculus* is used mainly as a fish poison and as an insecticide. *Anamirta cocculus* contain a powerful poison picrotoxin. The poisoning affects the CNS of vertebrates causes vomiting, purging, profuse sweating, dimness of vision, unconsciousness, intoxication and clonic convulsions [5].
Cissampelos pariera is sub- erect or small climbing twinner with velvety branches. Leaves are peltate, slightly broader than longer, leaf apex mucoronate, pubescent on both sides. Leaf blade is 6-12 cm long, membranous to nearly leathery, ovate to nearly round. A decoction is given in colic and dysentery, cholera and bronchitis [6]. The root acts as an antiseptic of the bladder and is used in chronic inflammation of urinary passage [7].
Traditonally, *C.pariera* is reported for its blood purifier and anti-inflammatory properties in India [8].
Cyclea peltata is a climber, Leaves are peltate, simple, alternate, hairy, ovate with apex acute. The leaves of *C. peltata* were being used as coolant, antidandruff, antipyretic and diuretic [9]. The leaves of *C. Peltata* found to contain alkaloids such as cycleanine, bebeerines, hayatinin, hayatidin and hayatin. Traditionally leaves of *C. Peltata* are used for the treatment of herpes in some part of coastal area [10].
Comparative anatomy shows evolutionary relation between organisms. The main importance of comparative anatomy is that it shows both similar and dissimilar characters of different species. It is an important tool that provides evidences for evolution. Comparative anatomical studies have archived a remarkable record. Anatomical character provides many evidences that shows inter relationships and affinities to uncertain taxonomic status. Anatomical characters are also important in Systematics and also suggested those conclusions supported by combination of characters are more reliable than those on a single character [11].

2. Materials and Methods
2.1 Present study includes the following plants
a. *Anamirta cocculus*
b. *Cissampelos pariera* Var. *hirsuta*
c. *Cyclea peltata*

Plants were observed for their morphological peculiarities at the site of occurrence. Stem, Petiole and leaves were collected and a free hand section method was carried out for anatomical and trichome studies. For this 1% safranin were used and mounted in glycerine. The slides were analysed by stereomicroscope (Leica DM 500). Photographs were taken using Leica Las EZ software.

3. Result and Discussion

The three species were collected in different part of the Southern Western Ghats. Both wild and cultivated were collected and examined for their proper identification. Collection locality was given in Table1and Figure 1(A, B and C).

Comparative anatomy of stem, petiole and leaves were performed and given in the Table 2, 3, 4 respectively. In case of stem all species have uniseriate epidermal cell with thick cuticle. In case of *Anamirta cocculus* (Fig 2A), 1-2 layers of collenchyma followed by 2-3 layers of chlorenchymatous hypodermis and it also extend and touches the sclerenchyma arches and in case of *C. Pariera* and in *C. peltata* 2-3 layers of collenchyma followed by 3-4 layers of Chlorenchymatous hypodermis (Figure 2B and 2C). The presence of two layers of hypodermis also reported by Prasanth and Sekaran [12]. Cortical region is present only in *A. Cocculus* and is seen as parenchymatous patches in between the chlorenchyma.

In case of *C. Pariera and in C. peltata* sclerenchyma fibres form arcs joined by stone cells in the vascular rays making a continuous ring. Inside the sclerenchyma, parenchyma caps are present. The presence of single layer of parenchyma layer below the sclerenchyma arcs in *C. Pariera* [13]. Open, collateral, 8-10 vascular bundle; adjacent vascular bundles are connected by parenchymatous vascular rays. Xylem contains large vessels. *Anamirta* contain 10-15 vascular bundles and parenchyma patches are not seen. Pith is large in *A. Cocculus* but in *C. Pariera* and in *C. peltata* pith is small. *C. pareira* stem showed the wavy epidermis with unicellular trichomes, lignified xylem vessels and biseriate radial medullary rays reported by Jhuma Samanta [14]. Frederic and Dario reported the general characters of the family menispermaceae as wide rays, enlarged vessel pits near the perforation plates, and pitted tyloses [15].

Detailed anatomical studies of Menispermaceae [16, 17, 4] have shown the relative uniformity, particularly with regard to the medullary rays which separate the vascular bundles and also with regard to the xylem which is characterised by large and conspicuous vessels. In young stems sclerenchyma fibres form arcs, which are nearly always joined by stone cells in the vascular rays to form a continuous ring.

Bailey explained the potentialities and limitation of wood anatomy in study of phylogeny and classification of angiosperm [18]. Kishore and Rao studied development of cambial variant and xylem structure were studied in the stem of *Cocculus hirsutus* (Menispermaceae). They also noticed the developments of several collateral vascular bundles which are joined by interfascicular cambium resulting in the formation of a complete cambial cylinder in the early stages of stem [19]. Successive cambium is the characteristics feature of stem of Menispermaceae [20] and mostly the successive cambium orginate from the internal cortical layer.

The petiole of *A. Cocculus* (Fig 3A) consist of Chlorenchymatous cortex which is scattered followed by 3-4 layers of parenchyma whereas in case of *C. Pariera* (Fig 3B) contain 3-6 layers of collenchymatous cortex where as Sudhakaran reported collenchymas cortex is followed by 2-3 layer of parenchyma and in *C. peltata* (Fig 3C) contain 3-4 layers of collenchymatous cortex [21]. In *Anamirta* Sclerenchyma form a continous ring. Concentric endarch and 10-15 vascular bundle, adjacent vascular bundles are divided by wide bands of parenchymatous vascular rays. Number of vascular bundles are more in *Anamirta* than in *C. Pariera* and in *C. peltata*

The leaves of *A cocculus* (Fig 4A) contain multiseriate epidermis where as in *C. Pariera* and in *C. peltata* contain uniseriate epidermis. In all the three case Mesophyll is differentiated into palisade and spongy cells. The palisade cells occur towards the upper epidermis in one layers. Palisade cells are usually elongated and densely packed while the spongy parenchyma cells are more or less isodiametric and loosely packed. Interconnections between the spongy parenchyma cells are present. In *A. Cocculus* few sclerenchyma cells are present at the two ends of the bundles. The bundles are collateral. 4-5 vascular bundles are seen where as in *C. Pariera* and in *C. peltata* (Fig 4B and 4C) the bundles are collateral and seen in central portion of the midrib sclerenchymatous bundle sheath was observed surrounding the vascular bundle region. Sandhya *et al*. reported the similar feature [22].

Trichomes are hair like appendages that develop from cells of the aerial epidermis and are produced by most plant species [23] generally in mesophytic community. Leaf trichomes can serve several functions including protection against damage from herbivore [24]. Metcalfe and Chalk provided useful information on structure, function, classification of trichome with their significance in comparative anatomical studies [10]. In *Anamirta cocculus* trichomes are multicellular, unbranched, thick walled with narrow lumen, tip pointed. In *C. Pariera* and in *C. peltata* trichomes are unicellular, unbranched and thick walled and is shown in the Table5 and Figure 5A, 5B and 5C. The major difference is that *C. Pariera* trichomes are with acute apex and bulbous base. *C. Pariera* trichomes are uniseriate and bicellular [21]. In *Cyclea peltata* the majority of the trichomes observed were unicellular, long and hook shaped [25].

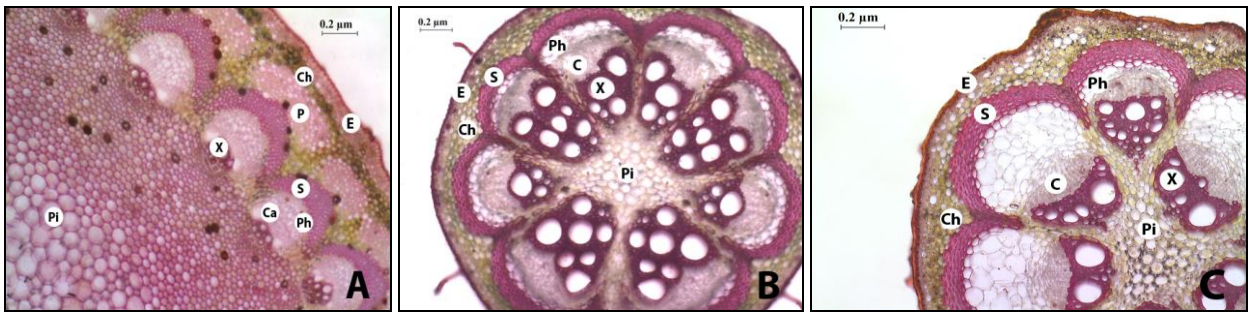
Table 1: Locality of collected species

Taxon	Habitat	Locality
Anamirta cocculus	Wild	Bonacaud (8° 40.6045' N 77° 6.0863' E)
Cissampelos pariera var.*hirsuta*	Wild	Wayanad 11° 47.2021' N 75° 59.2966 E
	Cultivated	Kesavadasapuram (8° 31.5808' N 76° 56.0434' E)
Cyclea peltata	Wild	Nedumangad (8° 37.0609'N 76° 58.5420E)
	Cultivated	Palode (8° 43.466' N 77° 1.4869' E)

Fig 1: A- *Anamirta cocculus*, B- Cissampelos pariera var.*hirsuta*, C- *Cyclea peltata*

Table 2: Comparative anatomy of stem of three different species

Taxa	Epidermis	Hypodermis	Cortex	Vascular Bundle	Pith
Anamirta cocculus	Single layered epidermis with thick cuticle	1-2 layers of collenchyma followed by 2-3 layers of Chlorenchymatous hypodermis and it also extend and touches the sclerenchyma arches	Patches of Parenchymatous cortex seen in between the Chlorenchymatous band	Sclerenchyma form arcs joined by stone cells, making a continuous ring. Open collateral 10-15 vascular bundle, adjacent vascular bundles are connected by parenchymatous vascular rays	Pith is large
Cissampelos pariera var.*hirsuta*	Epidermis single layered.outer walls of cells are cuticularised	2-3 layers of collenchyma followed by 3-4 layers of Chlorenchymatous hypodermis	Cortical region is absent	Sclerenchyma fibres form arcs joined by stone cells in the vascular rays making a continuous ring. Inside of the sclerenchyma, parenchyma caps are present. Open collateral 8-10 vascular bundle; adjacent vascular bundles are divided by parenchymatous vascular rays. Xylem contains large vessels.	Pith is small and composed of large parenchyma cells
Cyclea peltata	Single layered epidermis. Trichomes present	2-3 layer of collenchymatus and 3-4 layers of Chlorenchymatous hypodermis	Cortical region is absent	Sclerenchyma form arcs joined by stone cells in the vascular rays making a continuous ring. Inside of the sclerenchyma, single layer of parenchyma cells are present. Open collateral 6-8vascular bundle are present. Large, conspicuous vessels characterise the xylem	Pith is small

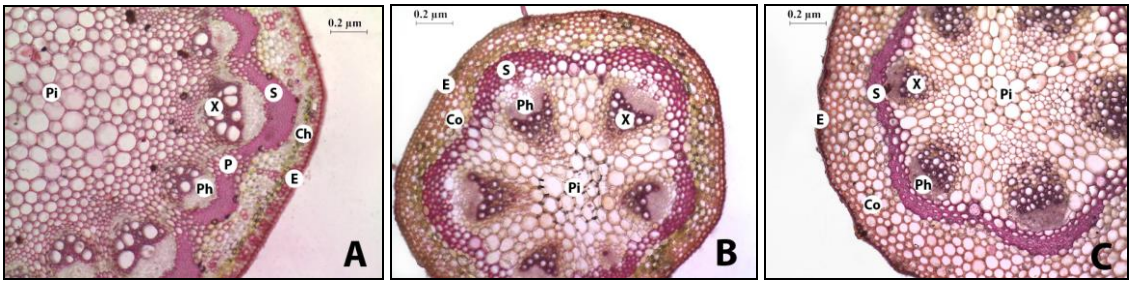


E-Epidermis, Ch-Chlorenchyma, S-Sclerenchyma, P-Parenchyma, Ph-Phloem, C-Cambium, X-Xylem, Pi-Pith

Fig 2: A-Stem anatomy of *Anamirta cocculus*, B- Stem anatomy of *Cissampelos pariera* var.*hirsuta*, C- Stem anatomy of *Cyclea peltata*

Table 3: Comparative anatomy of petiole of three different species

Taxa	Epidermis	Cortex	Vascular Bundle	Pith
Anamirta cocculus	Single layered epidermis. Hairs present.	Chlorenchymatous cortex is scattered followed by 3-4 layers of parenchyma	Sclerenchyma form a continous ring.concentric and endarch 10-15 vascular bundle, adjacent vascular bundles are divided by wide bands of parenchymatous vascular rays	Pith is large
Cissampelos pariera var.*hirsuta*	single layered Epidermis.	3-6 layers of collenchymatous cortex present	Sclerenchyma forms a continuous undulating ring. concentric and endarch 5-8 vascular bundles are present.	Pith is small
Cyclea peltata	Single layered epidermis.	3-4 layers of collenchymatous cortex present	Sclerenchyma form a continous bundle cap in the form of ring is present. concentric and endarch 6-8 vascular bundles are present	Pith is small

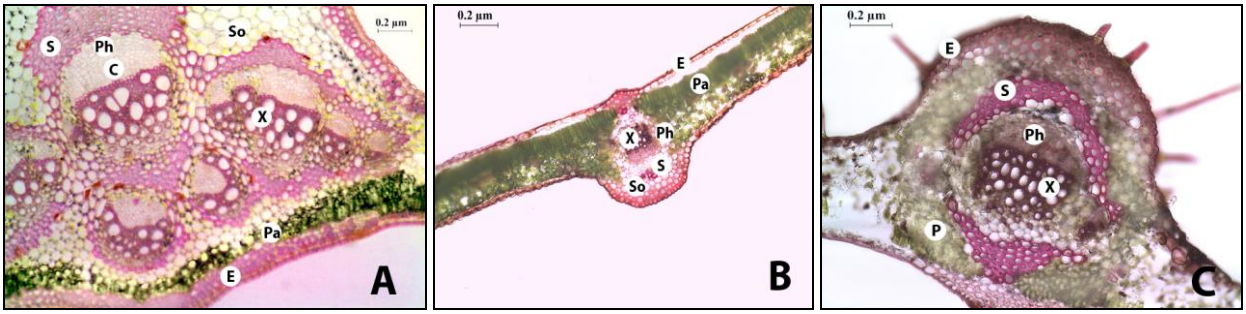


E-Epidermis, Ch-Chlorenchyma, Co-Collenchyma, P-Parenchyma, Ph-Phloem, C-Cambium, X-Xylem, Pi-Pith

Fig 3: A-Petiole anatomy of *Anamirta cocculus*, B- Petiole anatomy of *Cissampelos pariera* var. *hirsuta*, C- Petiole anatomy of *Cyclea peltata*

Table 4: Comparative anatomy of leaves of three different species

Taxa	Epidermis	Mesophyll	Vascular Bundle
Anamirta cocculus	Epidermal layers are multiseriate with thick cuticle	Mesophyll is differentiated into palisade and spongy cells. The palisade cells are comparatively much smaller than spongy	A few sclerenchyma cells are present at the two ends of the bundles. The bundles are collateral.4-5 vascular bundles are seen.
Cissampelos pariera var.*hirsuta*	Uniseriate Epidermis.	Mesophyll is differentiated into palisade and spongy cells. The palisade cells occur towards the upper epidermis in one layers. Palisade cells are usually elongated and densely packed while the spongy parenchyma cells are more or less isodiametric and loosely packed. Interconnections between the spongy parenchyma cells are present.	These are collateral. Elongated secretory cells, are generally associated with the vascular bundles Patches of sclerenchyma occur on the upper and lower sides of the bundles. Collateral closed vascular bundles are seen.
Cyclea peltata	Epidermal layers are single layered	Mesophyll is differentiated into palisade and spongy cells but the palisade cells were discontinued in the upper epidermal region	The bundles are collateral and seen in central portion of the midrib sclerenchymatus bundle sheath was observed surrounding the vascular bundle region



E-Epidermis, Pa-Palisade tissue, So-Spongy tissue, P-Parenchyma, S-Sclerenchyma Ph-Phloem, C-Cambium, X-Xylem, Pi-Pith

Fig 4: A-Leaf anatomy of *Anamirta cocculus*, B- Leaf anatomy of *Cissampelos pariera* var.*hirsuta*, C- Leaf anatomy of *Cyclea peltata*

Table 5: Trichome morphology of three different species

Micro-morphology	***Anamirta cocculus***	***Cissampelos pariera* var. *hirsuta***	***Cyclea peltata***
Trichome	Multicellular, unbranched, thick walled with narrow lumen, tip pointed.	Unicellular, unbranched, thickwalls with acute apex, bulbous base.	Unicellular, unbranched thick walled with narrow lumen

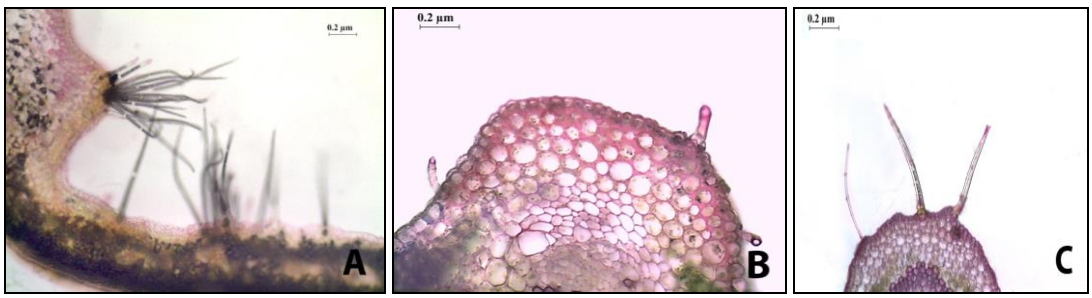


Fig 5: A-Trichome of *Anamirta cocculus*, B- Trichome of *Cissampelos pariera* var.*hirsuta*, C- Trichome of *Cyclea peltata*

5. Conclusion

Anatomical features are more conserve and therefore useful for taxonomic studies. These characteristics proved to be useful to distinguish the species for taxonomic studies.

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