

Epidermal and Palynological Study of Some Species of Rutaceae under the Tribe Clauseneae

¹Khaleda Begum, ²Namita Nath

^{1,2} Department of Botany

Gauhati University, Guwahati, Assam, India 781014

Abstract: Epidermal structures of leaf and palynological data plays a dominant role in aggregating data to resolve taxonomic problems and helps to delimitation of taxa. The present study assesses the pollen morphology and leaf epidermal characters of five species of Clauseneae tribe of Rutaceae viz., *Murraya paniculata* (L.) Jack, *Murraya koeingii* (L) Spreng., *Micromelum minutum* J.G. Forster) Wight & Arn, *Glycosmis pentaphylla* (Retz.) Corr., *Clausena heptaphylla* Wight & Arn. Epidermal characters such as stomatal type, stomatal shape, stomatal index, stomatal frequency, trichome type etc were studied. Erdtman method (1960) of acetolysis was used to study the pollen morphology and examined under LM. In all studied genera anomocytic stomata are prominent. Pollen grains of all genera are spherical in shape except *Micromelum* in which pollen grains are elliptic in shape. Epidermal and palynological features of investigated genera strengthen their position of retaining in the same family. Both foliar morphological as well as palynological data correlate with external morphological data of all the studied taxa.

Key words: Rutaceae, Clauseneae, epidermal structure, pollen

INTRODUCTION

Engler classified the family Rutaceae into seven sub-family among them Aurantioideae is the largest group (Thorn 2000). According to Swingle and Reece classification of citrus and its allied genera, the Aurantioideae subfamily sub-divided into two tribe: Clauseneae (5 genera) and citreae (28 genera). The tribe Clauseneae comprises of five genera viz., *Micromelum*, *Glycosmis*, *Clausena*, *Murraya*, and *Merrillia*. According to Swingle and Reece (1967), genera belongs to Clauseneae tribe are more primitive in comparison to Citreae tribe of orange subfamily as all the species of the tribe does not develop spines in the axils of leaves. The odd pinnate, leaflets attached alternately to the rachis are morphological characters of the tribe Clauseneae that differ from the tribe Citreae.

The epidermal feature plays a vital role in taxonomy. These characters provide evidences to understand the interrelationships among larger groups like families and also help in establishing the affinities within the genera. The leaf epidermal characters as valuable taxonomic tool was well documented by several workers viz., Gupta (1961), Inamdar (1968), Dilcher (1974), Adedeji (2004), Patricia (2006), Paul and Devi (2013).

Different author from different part of the world worked on epidermal morphology of the Rutaceae family Mbagwu *et.al* (2007). Muntoreanu *et al* (2010) Ogundrar *et.al* (2012), Inyama *et.al.* (2015) Al-Khatib *et.al.* (2015), (Oggero *et.al* (2016). Study of leaf architecture and Epidermal structure of the Clauseneae tribe is done in this investigation to validate genus belongs to the tribe have same leaf epidermal structure as the application of epidermal characters such as shape of epidermal cells, wall pattern, type and arrangement of stomata, stomatal index, stomatal aperture, size and types of trichomes, row of cell per trichome are some of the important systematic characters used in modern biosystematic studies.

Delimitation of taxa rely on the variation of palyno-morphological characters like shape, aperture, polar unit, symmetry and sculpture of pollen grains (Huang 1972; Edeoga, 1996; Mbagwa and Edeoga, 2006). Pollen morphology of the genera of the tribe also studied to confirm or as an aid to identification or delimit the investigated genera.

MATERIALS AND METHODS

Extensive field study was carried out to collect plant specimens of tribe Clauseneae from different localities within the study area, Kamrup Metropolitan district. The collection of live specimens from their natural habitat was done at a regular interval. The specimens were collected in their flowering and fruiting stages. Voucher specimens of the collected specimens were prepared following standard herbarium techniques of Jain and Rao (1977). Voucher specimens were identified with the previously identified specimens of at GUBH (Department of Botany, Gauhati University) and also with microfilms of herbarium specimens stored online databases of various herbaria like JSTOR and KEW.

1. Epidermal structure

Foliar epidermal characters like foliar epidermal characters, stomatal type, stomatal shape, stomatal index, stomatal frequency, guard cell area, trichome type and trichome density were taken into account following methods of Boulos and Beakane (1971) with slight modification. For this mature fresh leaves were first dissolved in 10% aqueous nitric acid (HNO_3) solution for few days and then peelings from both upper surface and lower surface were made mechanically or scrapping with the help of blade. The peels of the leaves were then stained with 1% safranin and mounted in 50% glycerin and sealed with DPX.

Epidermal cells and stomata were counted at 400X magnification and microphotograph were taken with 100X and 1000X magnification under microscope.

The observation and measurement were standardized by taking the mean value of five readings taken on random basis. The classification and terminology of epidermal and stomatal morphology have been elaborated following Ramayya and Rajgopal (1980) with slight modification. In describing epidermal features, the terminology provided for epidermal cell by Stace (1965) has been followed. Absolute stomatal number was calculated by the formula given by Gupta (1961)

I. Stomatal Frequency:

The stomatal frequency was calculated by using the formula as proposed by Ghosh and Davish (1973).

$$\text{Stomatal frequency (S.F)} = \frac{S}{A}$$

Where, S= Number of stomata per field.

A= Area of the field.

II. Stomatal index:

Stomatal index was calculated by the formula of Dilcher (1974).

$$\text{Stomatal Index (S.I)} = \frac{s}{(E+S)} \times 100$$

2. Study of pollen morphology:

Pollen slides for the palynological studies were prepared by the acetolysis method as described by Erdman (1960) with slight modification of Nair (1970).

Anthers from fully bloomed flowers were collected and fixed in 70% alcohol for 24 hours as pre-treatment. Then the treated anthers were placed in a Petri dish and crushed with a glass rod. The dispersion was then sieved through a fine mesh and collected into tube. The tube was centrifuged and decanted off the alcohol, 5ml of Glacial acetic acid was added in the tube containing the pollen material. The solution was centrifuged and the supernatant was decanted off. Then 6 ml of acetolysis mixture was added to the residue. The solution was allowed to water bath at 70°C, stirred with a glass rod and again centrifuged. Thereafter the waste acetolysis mixture was decanted off. After that 10 ml glacial acetic acid was added to the tube and stir again. Centrifuged the contents and then acetic acid was removed. The pollen materials were further washed thrice consecutively, by adding 5 ml of distilled water followed by centrifuge each time. Then the supernatant was decanted off and added with 2ml of dilute glycerin. After that the solution was centrifuged and supernatant was again decanted off. The centrifuge tube was then left in inverted position over a filter paper until slide preparation and after observed under microscope.

RESULTS AND DISCUSSION

The length of epidermal cell ranges from 34.66-26.37 in abaxial surface. The epidermal cell ranges from irregular, polygonal or rectangular in shape with smooth or undulate anticlinal walls. In all taxa anticlinal walls are smooth to slightly undulate (rough) except *Murraya koenigii* in which anticlinal wall is deeply undulate on both the surface.

All studied taxa are hypostomatic. Different types of stomata are documented or observed in the studied taxa, anomocytic type of stomata is recorded as most common type of stomata, although other types of stomata such as anisocytic, animotetracyclic also observed. The gourd cells shape are generally elliptic in outline, rarely they are circular in outline. Mbagwa *et al.*, (2007) recorded anomocytic type of stomata of four species of *Citrus* of Rutaceae family. Xochicale *et al.*, (2010) and Oggero *et al.* (2016) were also observed anomocytic type of stomata for the genus *Zanthoxylum*. Stomatal frequency varies among studied taxa and ranges from 5.67-13 number of stomata per mm square. The highest stomatal index 14.44% is found on *Micromelum minutum* whereas it is the lowest 8.96% in case of *Murraya koenigii*.

Out of all the taxa studied two taxa are found to possess trichomes or epidermal hairs. These are - *Clausena heptaphylla* and *Micromelum minutum*. Among the three taxa *Clausena heptaphylla* the trichomes are nonglandular, unicellular. But in *Micromelum minutum* both unicellular and tricellular nonglandular trichomes are recorded.

Table-1: Dimensions of epidermal cells in five species of Clauseneae

Name of taxa	Surface	Epidermal cells				
		Anticlinal cell wall shape	Average Length of Epidermal cell (µm)	Average breadth of Epidermal cell (µm)	Average no of epidermal cell	L/B Ratio
<i>Clausena heptaphylla</i>	Abaxial	Undulate/Sinuous	33.04	18.60	524	1.77
	Adaxial	Straight	32.98	18.54	587	1.77
<i>Glycosmis</i>	Abaxial	Straight or Undulate	30.48	11.93	693	2.54

<i>pentaphylla</i>	Adaxial	straight	29.99	11.01	675	2.56
<i>Micromelum minutum</i>	Abaxial	Straight	26.37	14.16	501	1.86
	Adaxial	Straight	26.35	14.01	541	1.88
<i>Murraya koenigii</i>	Abaxial	Straight,slightly undulate	34.66	17.15	657	1.96
	Adaxial	Straight	34.87	17.43	641	2.00
<i>Murraya paniculata</i>	Abaxial	Deeply undulate/Sinuuous	33.32	15.49	490	2.15
	Adaxial	Undulate/Sinuuous	33.04	15.27	456	2.16



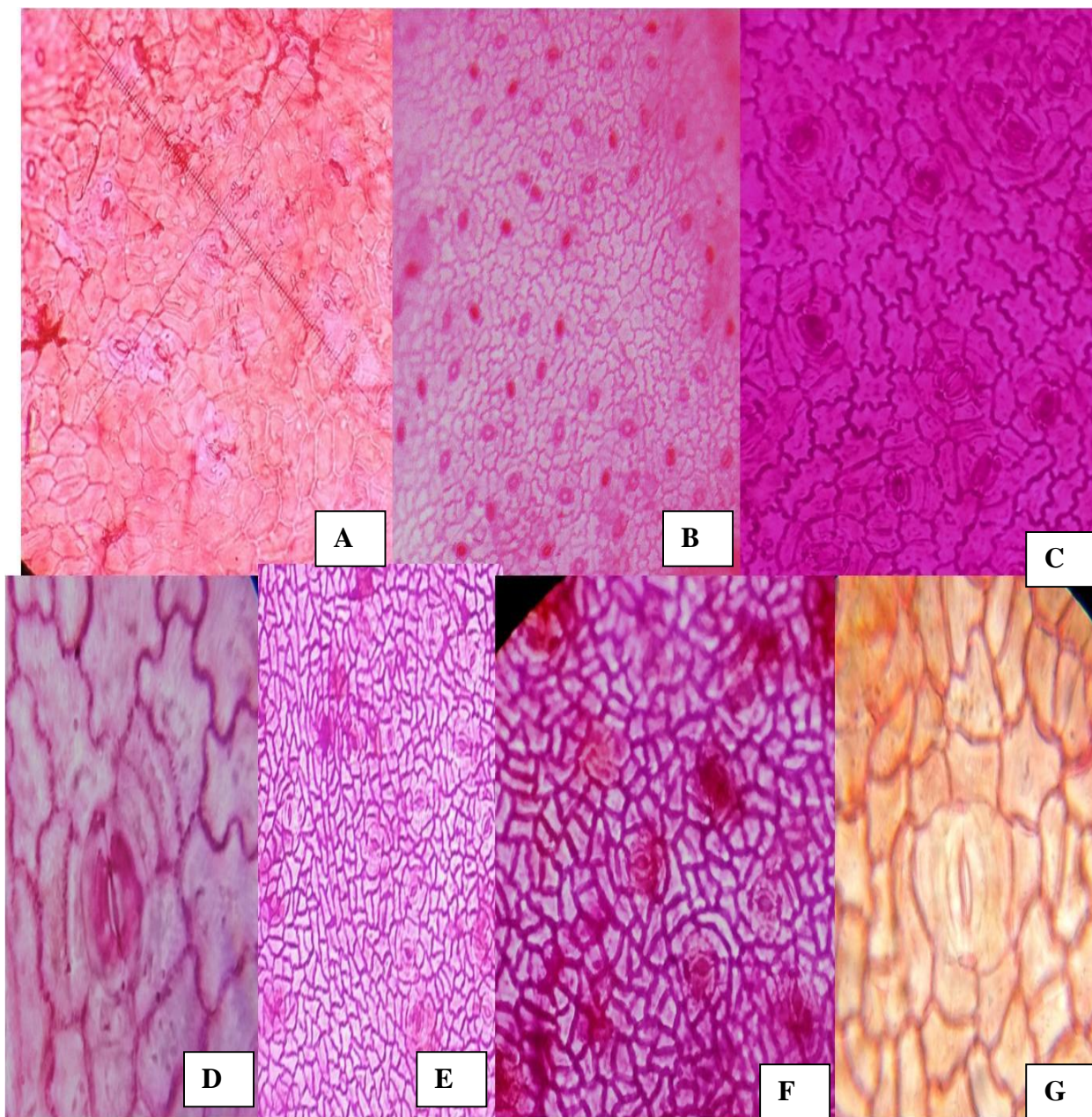


Fig 1: Foliar micro-morphology of different taxa of Rutaceae:

(a) *Murraya koenigii* -A, b) *Murraya paniculata*-B,C,D and (c) *Glycosmis pentaphylla* E,F,G

Note- B,C-10X magnification, A,C, F- 40X magnification and D,G- 1000X

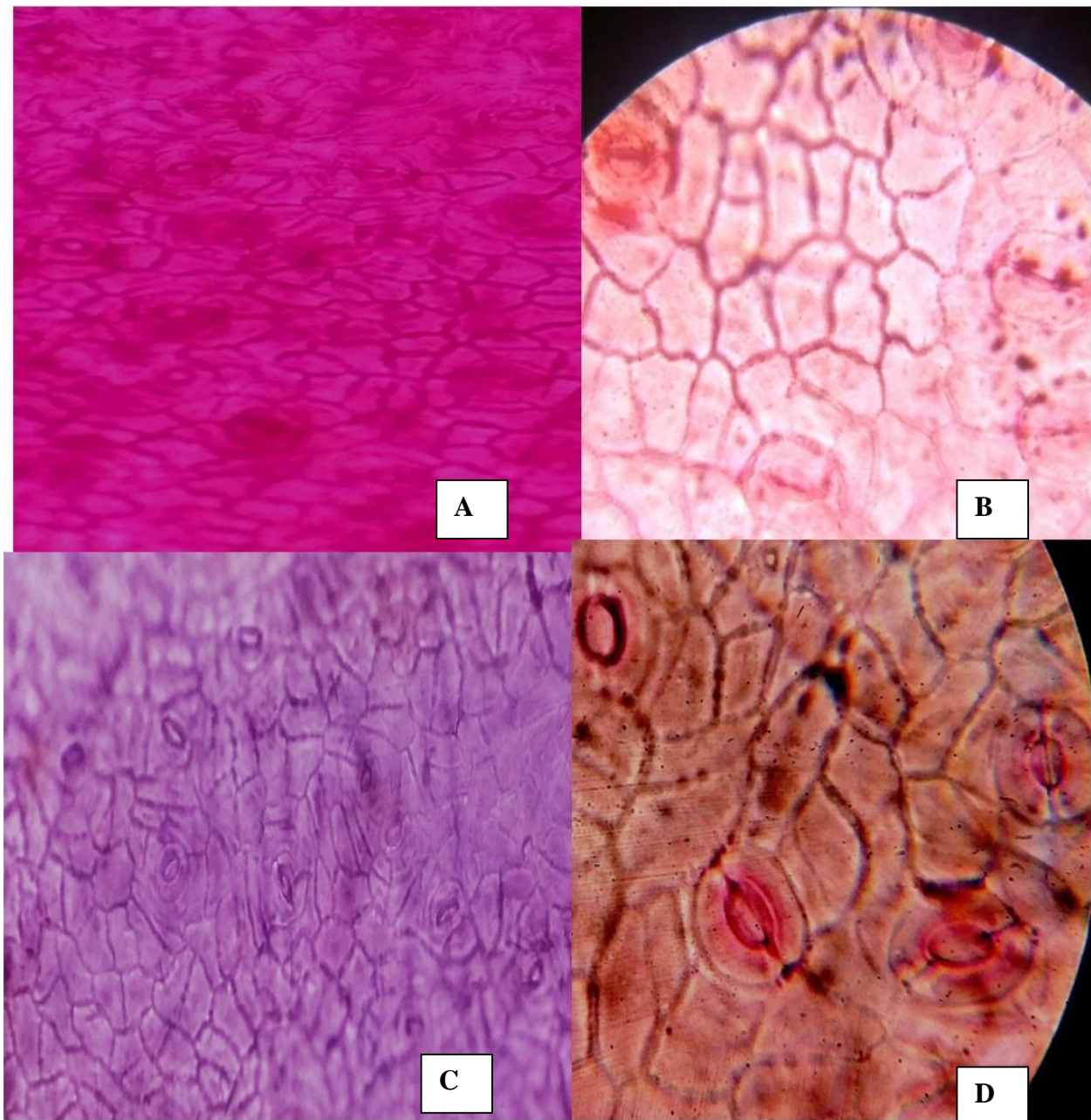


Figure 2: Foliar micro-morphology of different taxa of Rutaceae:

(a) *Clausena heptaphylla*- A,B and (b) *Micromellum minutum*-C,D

Note- A,C, - 40X magnification and B,D- 1000X magnification



Figure 3 : Trichome a) *Clausena heptaphylla*, b) *Micromelum minutum*

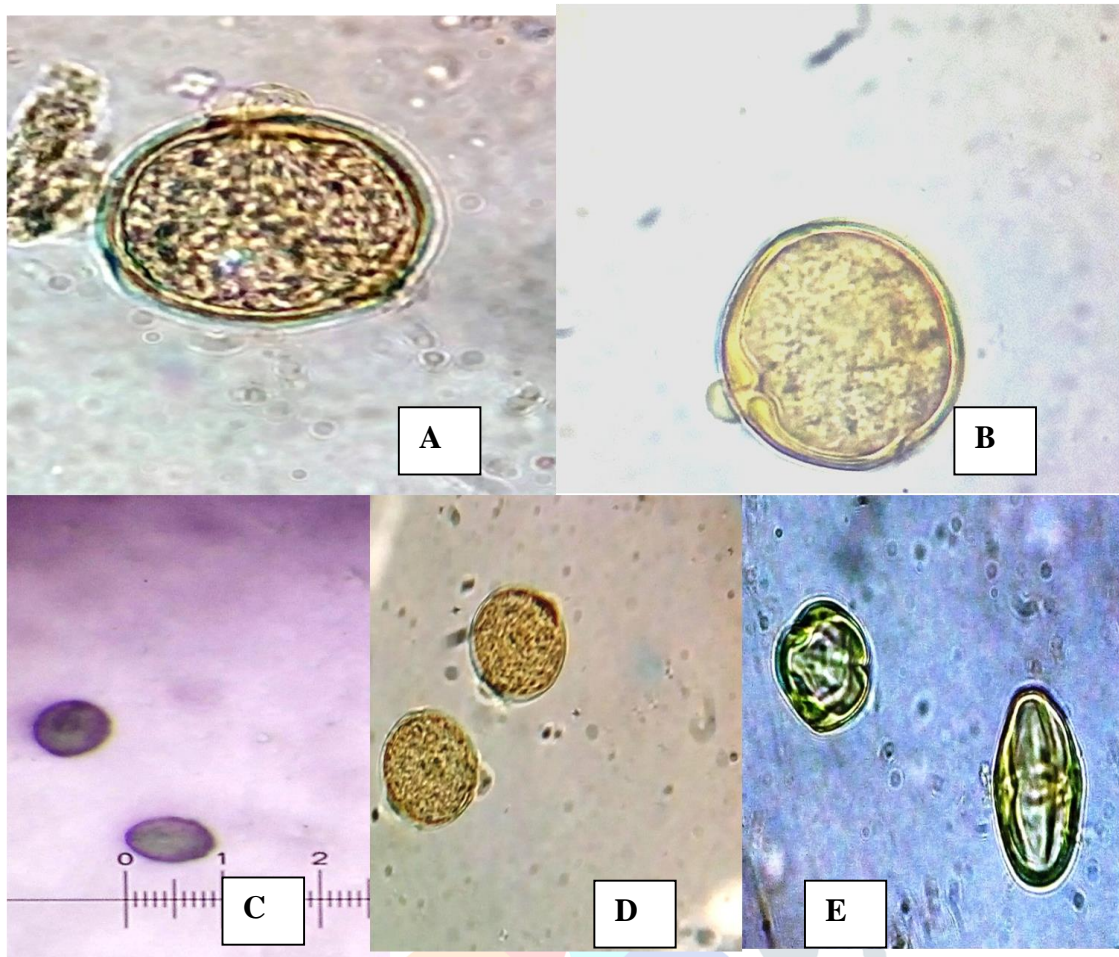


Figure 4: Pollen morphology: A) *Murraya koenigii* B) *M. paniculata* C) *Glycosmis pentaphylla* D) *Clausena heptaphylla* E) *Micromellum minutum*

Table-2: Dimensions of stomata and trichomes of five species of Clauseneae

Name of taxa	Surface	Stomata				Types of Stomata	Trichomes		
		Average no of Stomata	Stomatal frequency stomata/mm ²	Stomatal index (%)	Guard cell area(μm)		Average size of Trichome (μm)	Average no of stomata	L/B Ratio
<i>Clausena heptaphylla</i>	Abaxial	65	6.14	12.28	166.34	Anomocytic, Anisocytic,	262.39×18.32	48	14.32
	Adaxial	-	-	-	-	-			
	Adaxial	-	-	-	-	-			
<i>Glycosmis pentaphylla</i>	Abaxial	66	6.24	10	294.22	Anomocytic, Anisocytic, Amphiocytic	-	-	-
	Adaxial	-	-	-	-	-			
<i>Micromelum minutum</i>	Abaxial	76	7.18	14.54	141.73	Anomocytic, Anisocytic, Actinocytic	112.29×14.49	6	7.77
	Adaxial	-	-	-	-	-			
<i>Murraya koenigii</i>	Abaxial	60	5.67	8.96	151.63	Anomocytic	-	-	-
	Adaxial	-	-	-	-	-			
<i>Murraya paniculata</i>	Abaxial	52	4.91	9.62	183.91	Anomocytic. Anisocytic, hemiparacytic, Anomotetracyclic	-	-	-

The morphological characteristics of pollen of all the 5 studied taxa is summarized in the table-3. Pollen grains are monad in all the taxa. The shape of pollen grains varies from taxa to taxa. The pollens are Prolate –speroidal in *Murraya koenigii*, *Murraya paniculata*; Prolate in *Glycomis pentaphylla*, *Micromelum minutum*; and oblate spheroidal in *Clausena heptaphylla*. The range of polar axis varies from 21.10µm -31.08 µm, while equatorial axis ranges from 11.66 µm -29.43 µm. Three different types of apertures are found in all studied taxa. According to existing literature, mostly the family Rutaceae has 3 colporate aperture and in the present study also all the members are found to bear tricolporate pollen apertures. Exine thickness varies from 0.96 µm -1.88 µm.

Table 3- Palynological data

Name of Taxa	Equitorial Diameter (µm)	Polar Diameter (µm)	P/E	Exine thickness (µm)	Aperature type	Pollen shape classes
<i>Clausena heptaphylla</i>	17.58	18.14	0.96	0.96	4/5 colporate	Oblate-spheroidal
<i>Glycomis pentaphylla</i>	12.33	21.98	1.78	1.66	indistict	prolate
<i>Micromelum minutum</i>	11.66	21.10	1.80	1.88	3 colporate	Prolate
<i>Murraya Koenigii</i>	22.21	23.32	1.04	1.49	2/3 colporate	Prolate - Spheroidal
<i>Murraya paniculata</i>	26.65	27.21	1.02	1.72	3/5 colporate	Prolate - speroidal

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