

Anatomical and Statistical Analysis of Six Parasitic Loranthaceae Species

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Abstract

Anatomical leaf and stem characteristics of six Loranthaceae species growing naturally in Saudi Arabia, *Plicosepalus acacia*, *Plicosepalus curviflorus*, *Phragmanthera austroarabica*, *Oncocalyx chimperi*, *Oncocalyx glabratus*, and *Tapinanthus globiferus*, were investigated and the relationships between them determined. The objective of the present study was to review the anatomical characteristics of the six Loranthaceae species, and generate comprehensive statistical information for different scholars who wish to study those species in detail. Results showed that these species possess useful biosystematics characteristics as major features that can be used to establish relationships between themselves. Interesting aspects of this study include the type of mesophyll leaves (isolateral and isobilateral), the number of main vascular bundles, the shape of supporting collenchyma tissue in the leaf, and the type of axial parenchyma in the stem. Other characteristics observed are the presence or absence of crystals in the leaves and ray widths in the stems. According to these characteristics, an artificial key explains the differences between the six investigated species. Two histograms and a graphic tree represent statistical analysis by one-way ANOVA of leaf and stem anatomical characters.

Key words: Loranthaceae, Isolateral and isobilateral Leaf, artificial key, one-way ANOVA, statistical analysis, Coefficient of variation.

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1-Introduction

The Loranthaceae family plays an important and complex role in the biological system in which these species live by interacting with insects, birds, and mammals (Watson, 2001). This family comprises four genera: *Phragmanthera*, *Oncocalyx*, *Tapinanthus*, and *Plicosepalus*, which grow naturally in Saudi Arabia. These genera include six species that spread north, west, and south of the kingdom: *Plicosepalus acacia*, *Plicosepalus curviflorus*, *Phragmanthera austroarabica*, *Oncocalyx schimperi*, *Oncocalyx glabratus*, and *Tapinanthus globiferus*. This research is based on the hypothesis that the variation in leaves and stems anatomy is significant and revealed that

they possess many attributes of potential taxonomic importance that is diagnostic at the genus and species levels (Mbagwu and Edeoga, 2006; Nwachukwu and Mbagwu, 2007). Although the usefulness of use vegetative and anatomical features in the biosystematics consideration of various taxa have been reported by Edeoga and Eboka (2000) and Edeoga and Ikem (2001), there is no specific investigation conducted on the anatomical features of leaves and stems of family Loranthaceae hence this study reports the anatomical characters of the six species of loranthaceae growing naturally in Saudi Arabia. It assesses the relevance of and discusses the extent to which the anatomical features might be used in biosystematics consideration of these loranthaceae species. Based on these anatomical features an artificial key use major and minor characters for the diagnosis species was developed.

2- Materials and Methods

2-1. Plant material

Fresh materials of 6 Loranthaceae species were collected from south western, west and north of Saudi Arabia between 2010 and 2011 (Table 1). Attention was paid in studying specimens that were collected from different localities representing the geographical range of each species. The collected materials were identified according to Migahid (1987), Collonette (1998) and Chaudhary (2001). Samples of the identified materials were kept at the Botany Department, Faculty of Science King Abdul-Aziz University (Girls section).

2-2. Sample preparations:

For anatomical investigation specimens were fixed in formalin-glacial acetic acid – ethyl alcohol. Leaves blade and stems were sectioned using microtome serially at 10-15 μ after being embedded in paraffin wax. Sections were stained in saffranin and light green dehydrated in alcohol-xytol series, cleared in clove oil and mounted in Canada balsam, photographed by Nikon Microscope at different power.

Table 1: Location and date of collection of the six investigated species

Species	Genus	Locality	Date of collection
<i>Plicosepalus curviflorus</i>	<i>Plicosepalus</i>	Abha -Al Faria	March and November 2010, March 2011
<i>Plicosepalus acacia</i>	<i>Plicosepalus</i>	Alola - Geda	December 2010, January 2011
<i>Phragmanthera austro arabica</i>	<i>Phragmanthera</i>	Abha – Khamis mushat	March and November 2010, March 2011
<i>Oncocalyx schimperi</i>	<i>Oncocalyx</i>	Abha -Asser	June and November 2010
<i>Oncocalyx glabratus</i>	<i>Oncocalyx</i>	Al-Taef, Gabel Ibrahim	January 2010, May 2011

Table 2: Quantitative Leaf anatomical characteristics of the six species of family Loranthaceae growing in Saudi Arabia

Characters Species	Mesophyll Length	Main Vascular bundle			Lateral vascular bundle		
	In μm	Number	Length μm	Width	Length	Width μm	Number
<i>Plicosepalus acacia</i>	6.12-6,5- 5,9-5,7- 6,12-6,11- 6,5-6,12- 6,11-6,12	1-1-1-1- 1-1-1-1- 1-1	4.05-4- 4,1-4- 3,9-3,8- 4-3,7- 3,9-3,7	4.26- 4- 4,5-4,3- 4,5-4,2- 4-4,2- 4,5-4,4	2.46 -2- 2,4-2,5- 2,6-2,4- 2,4-2,5- 2-2,7	2.16-2- 2,2-2,3- 2,2-2,1- 2-2,1- 2,7- 2,16	30-30- 30-29- 30-29- 30-30- 30-30
<i>Plicosepalus curviflorus</i>	6.9-6,9-6,7- 6,8-6,6-7,1- 6,8-6,8-6,5- 6,6	1-1-1-1- 1-1-1-1- 1-1	2.45- 2,5-2,3- 2,4-2,1- 2-2,4- 2,2-2- 2,2	2.79- 2,8-3- 3,2-3,3- 3-2,9- 2,8-2,7- 2,8	1.38- 1,4-1,5- 1,2-1,6- 1,3-1,4- 1,7-1,5- 1	1.41- 1,5-1,3- 1,4-1,3- 1-1,5- 1,6-1- 1,4	10-9-10- 10-8-10- 10-10- 10-9
<i>Phragmanthera austroarabica</i>	6.21-6,22- 6,24-6,21- 6,20-6,19- 6,21-6,21- 6,23-6,22	1-1-1-1- 1-1-1-1- 1-1	2. 6 - 2,5-2,4- 2,7-2,9- 2,5-2,6- 2,7-2,8- 2,6	2.7 - 2,8-2,7- 2,5-2,6- 2,5-2,9- 2,8-2,5- 2,9	1.56- 1,7-1,6- 1,8-1,9- 1,9-1,8- 1,5-1,6- 1,6	1.79- 1,8-1,9- 2-1,7- 1,8-1,7- 2-1,9- 1,8	16-15- 16-16- 17-15- 16-16- 16-15
<i>Oncocalyx schimperi</i>	3.21-3,22- 3,3-3-3,4- 3,5-3-3,2- 3,2-3,2	7-6-7-6- 7-6-7-7- 7-7	2.31- 2,2-2- 2,1-2-2- 2,3-2,5- 2,3-2,3	1.56- 1,4-1,5- 1,6-1,7- 1,5-1,6- 1,7-1,5- 1,6	0.54- 0,4-0,3- 0,2-0,5- 0,7-0,9- 0,5-0,5- 0,3	0.86- 0,9-0,7- 0,9-0,8- 0,8-0,9- 0,7-0,8- 0,9	40-39- 38-40- 39-40- 40-40- 39-40
<i>Oncocalyx glabratus</i>	3.93 -4 -3,5 -3,8-3,7-3,9- 4-3,9-3,9- 3,7	3-3-4-3- 3-4-3- 3-4-3	1.74- 1,5-1,6- 1,4-1,2- 1,8-1,9- 1,8-2- 1,7	2.19-2- 2,2-2,3- 2,5-2- 2,1-2,3- 2,4-2,1	0.74- 0,8-0,9- 1-0,7- 0,5-0,4- 0,7-0,8- 0,9	0.88- 0,7-0,6- 0,9-0,8- 0,5-0,6- 0,9-0,8- 0,7	25-25- 24-23- 24-25- 25-25- 24-25
<i>Tapinanthus globiferus</i>	3.40-3,3- 3,5-3 -3,2- 3,9-3,4-3,4- 3,3-3,3	1-1-1-1- 1-1-1-1- 1-1	2.35- 2,2-2,5- 2,4-2- 2,6-2,9- 2,3-2,3- 2,5	2.89-3- 2,9-2,8- 2,9-3,2- 2,7-3,3- 2,8-2,9	0.54- 0,6-0,4- 0,5-0,5- 0,7-0,9- 0,8-0,5- 0,3	0.65- 0,6-0,5- 0,4-0,6- 0,7-0,7- 0,5-0,6- 0,7	27-26- 25-27- 27-27- 27-25- 27-27

Table 3: Quantitative stem anatomical characters of the six species of family Loranthaceae growing in Saudi Arabia

Characters Species	Vessels		Rays		Fiber wall thickness in μ
	Number of vessels/ 10 μm^2	Diameter in μ	Height in μ	Width in μ	
<i>Plicosepalus acacia</i>	30-29-34-30-32-31-29-36-35-35	0.16-0,19-0,20-0,16-0,17-0,18-0,29-0,29-0,21-0,25	5.82-6,5-7,7-9,43-12,65-15,33-19,40-20,44-25,66-28.32	0.59-0,60-0,65-0,70-0,78-0,63 -0.79 -0,66-0,63-0,59	0.10-0,11-0,14-0,17-0,15-0,17-0,13-0,10-0,12-0,16
<i>Plicosepalus curviflorus</i>	13-14-15-16-17-17-13-15-16-13	0.35-0.55-0,36-0,38-0,40-0,45-0,50-0,55-0,35-0,55	3.68-4,34-5-5,35-6-3,20-5,50-6-6.02-6,70	0.22-0,30-0,50-0,90-1-1,1-1.05-0,60-0,70-0,90	0.06-0,05- -0,04-0,06-0,04-0,06-0,05-0,05-0,05-0,04
<i>Phragmanthera austroarabica</i>	14-16-17-18-15-17-19-19-15-14	0.16-0,17-0,30-0,33-0,40-0,45-0,50-0,55-0,16-0,52	7.70- 8,40-9,50-16,50-17,9-19,44-15,35-20.03-12,30-14,66	1.21-1.25-1.30-1.33-1.40-1,35-1,40-1,45-1,50-1.52	-0,03-0,03-0,05-0,06-0,05-0,06-0,05-0,04-0,05-0,06
<i>Oncocalyx chimperi</i>	22-23-24-25-26-27-27-25-23-27	0.15-0,17-0,19-0,20-0,30-0,33-0,35-0,35-0,18-0,31	3.34-3,5-4,30-4,50-4,70-5-5,5-5,9-5.98-4,40	0.46-0,50-0,55-0,60-0,65-0,70-0,73-0,58-0,73-0,46	0.07-0,06-0,05-0,06-0,07-0,08-0,05-0,08-0,07-0,05
<i>Oncocalyx glabratus</i>	14-15-17-16-19-18-17-20-20-15	0.13-0,15-0,16-0,19-0,30-0,34-0,35-0,15-0,25-0,35	6.71-7,55-9,67-10,44-13,49-15,36-14,60-16.38-11,15-10,45	0.52-0,50-0,60-0,77-0,70-0,80-0,87-0,87-0,65-0,75	0.03-0,04-0,03-0,04-0,05-0,03-0,04-0,05-0,03-0,05
<i>Tapinanthus globiferus</i>	16-17-18-19-19-16-17-18-19-16	0.27-0,29-0,30-0,33-0,39-0,40-0,43-0,44-0,38-0,40	8.77-10,70-11,34-13,37-15,20-17-18,50-20,50--23,40-27.81	1.62-1,70-1,75-1,80-1,85-1,9-2-2,2-2.19-1,6	0.9 -0,9- 0.10 -0, 11-0,12-0,10-0,12--0.120, 11-0,10

Table 4: The significant difference between the seven groups for Leaf anatomical characters

Mesophyll Length	Between groups	132.464	5	26.4928	534.44	0.000
	Inside groups	2.6769	54	0.0496		
	Total	135.1408	59			
Main Vascular bundle number	Between groups	271.1333	5	54.2267	697.20	0.000
	Inside groups	4.2000	54	0.0778		
	Total	275.333	59			
Main Vascular bundle Length	Between groups	28.7566	5	5.7513	154.78	0.000
	Inside groups	2.0065	54	0.0372		
	Total	30.763	59			
Main Vascular bundle width	Between groups	41.2283	5	8.2457	289.78	0.000
	Inside groups	1.5366	54	0.0285		
	Total	42.7649	59			
Lateral vascular bundle Length	Between groups	28.2669	5	5.6354	152.54	0.000
	Inside groups	2.0014	54	0.0371		
	Total	30.2683	59			
Lateral vascular bundle width	Between groups	21.1335	5	4.2267	196.8	0.000
	Inside groups	1.1598	54	0.0215		
	Total	22.2933	59			
Lateral vascular bundle number	Between groups	5545.083	5	1109.017	2385.93	0.000
	Inside groups	25.100	54	0.456		
	Total	5570.183	59			

Table 5: The significant difference between the five groups for stem anatomical characters

Stem Anatomical characters	Variation	Sum of squares	Degree of freedom	Average squares	F Value	Significance level (P)
Number of vessels/ 10 μm^2	Between groups	2226.48	5	445.3	116.45	0.000
	Inside groups	206.5	54	3.82		
	Total	2432.98	59			
Diameter in μ	Between groups	0.41279	5	0.08256	9.5	0.000
	Inside groups	0.46931	54	0.00869		
	Total	0.8821	59			
Rays Height in μ	Between groups	1325.6	5	256.1	11.81	0.000
	Inside groups	1212.1	54	22.4		
	Total	2537.7	59			
Rays Width in μ	Between groups	13.1809	5	2.6362	83.8	0.000
	Inside groups	1.6988	54	0.0371		
	Total	14.8797	59			
Fiber Wall thickness in μ	Between groups	0.3967	5	0.0793	4.24	0.003
	Inside Group	10095	54	0.0187		

Table 6: Qualitative Leaf anatomical characteristics of the six species of family Loranthaceae growing in Saudi Arabia

Characters Species	Mesophyll Type	Sclerenchyma cell	Crystals	Supporting tissue Collenchyma cell
<i>Plicosepalus acacia</i>	Isolateral	present	clusters	Complete sheath surround v.b.
<i>Plicosepalus curviflorus</i>	Isolateral	absent	absent	Complete sheath surround v.b.
<i>Phragmanthera austroarabica</i>	Isobilateral	absent	solitary	Below vascular bundle
<i>Oncocalyx chimperi</i>	Isolateral	present	& solitary clusters	Below vascular bundle
<i>Oncocalyx glabratus</i>	Isolateral	present	& solitary clusters	Above and below the vascular bundle
<i>Tapinanthus globiferus</i>	Isobilateral	absent	solitary	Above and below the vascular bundle

Table 7: Qualitative Stem anatomical characters of the six species of family Loranthaceae growing in Saudi Arabia

Characters Species	Vessels Arrangement	Axial Parenchyma	Type of ray
<i>Plicosepalus acacia</i>	Single &radial ,cluster	Paratrachel &apotrachel in group	one type of ray
<i>Plicosepalus curviflorus</i>	radial &Cluster multilpe	Paratrachel	one type of ray
<i>Phragmanthera austroarabica</i>	radial &Cluster multilpe	Paratrachel & Diffuse apotrachel	one type of ray
<i>Oncocalyx schimperi</i>	,Single &radial cluster	Paratrachel& Diffuse apotrachel	Two types of rays
<i>Oncocalyx glabratus</i>	Single &radial , cluster	Paratrachel& Diffuse apotrachel	types of rays Two
<i>Tapinanthus globiferus</i>	radial &Cluster multilpe	Paratrachel	one type of ray

3- Results

3- 1. Anatomical Results:

Tables 2-6 summarize the quantitative and qualitative characteristics of leaf and stem anatomy. Certain taxonomic concepts were applied to revise the Saudi Arabian species of Loranthaceae. The most important characteristic in leaves were mesophyll type either isolateral or isobilateral, number of main and lateral vascular bundles and shape of collenchyma as supporting tissue surrounding the vascular bundles. In the stems, type of axial parenchyma and rays considered as high important characteristics, while crystals and fiber thickness were of minor importance.

3-2- Statistical analysis results

3-2. 1. One Way ANOVA of leaf quantitative anatomical characteristics:

The mesophyll length and the number, length, width of the main and lateral vascular bundles were represented the quantitative anatomical characteristic of the leaf (Table 4). About 10 samples were recognized for each species. Testing was conducted the quantitative anatomical characteristics of the combined leaf to see whether there are significant differences in there, among the six species belonging to family Loranthaceae growing in Saudi Arabia.

3-2.2. One Way ANOVA of stem quantitative anatomical characteristics:

The number and diameter of vessels and the high and width of rays in addition to the thickness of fiber wall were represented the quantitative anatomical characteristics of the stem (Table 5). Testing was conducted to examine the quantitative anatomical characteristics of the combined stem to see whether there are significant differences in there among the six species belong to family Loranthaceae growing in Saudi Arabia.

4-Discussion

Our study was concentrated on the anatomical description of stems and leaves of the six species of family Loranthaceae under investigation. About 17 vegetative characteristics have been recorded, viz.; Xylem vessels characteristics (number, diameter, arrangement), the rays length, fiber width and type of axial parenchyma. Also, we concentrated on type and length of mesophyll in leaf, number and size of main and lateral vascular bundles and shape of collenchyma as supporting tissues. All these characteristics help to differentiate between the six species of family Loranthaceae and build up our own artificial key.

Isobilateral mesophyll characteristics were recognized in only two species *Phragmanthera austroarabica* (Fig.4 C) and *Tapinanthus globiferus* (Fig.4 F). The other four species *Plicosepalus acacia*, *P. curviflorus*, *Oncocalyx chimperi* and *O. glabratus* were described as isolateral mesophyll type and this result agree with Metcalf & Chalk (1950). Type of collenchyma as supporting tissue around main vascular bundle in leaf and axial parenchyma in stem were considered the most diagnostic anatomical characteristics. In *Phragmanthera austroarabica* leaf, collenchyma as supporting tissue was below vascular bundle (Fig.4 C) and stem axial parenchyma was of two types: paratrachel & diffused apotrachel (Fig.5 C1&C2) The

Tapinanthus globiferus leaf was specified by collenchyma supporting tissue above and below the vascular bundle, (Fig.4 F) and paratrachel stem axial parenchyma (Fig.6 F1&F2).

The isolateral mesophyll species viz. Plicosepalus acacia, Plicosepalus curviflorus, Oncocalyx schimperi and O. glabratus, (Fig.4 A, B, D & E), where the number of main vascular bundles in their leaves was different. Both Plicosepalus species were characterized by one main vascular bundle in midrib. Compared between the two Plicosepalus species we recognized two different features: the axial parenchyma in the stem and the presence or absence of crystals in the leaf. In Plicosepalus acacia the axial parenchyma was of two types paratrachel & apotrachel in groups (Fig. 5 A1&A2), and crystals were present in the mesophyll in groups. In Plicosepalus curviflorus the axial parenchyma was paratrachel (Fig.4 F) and crystals were absent in the mesophyll. Both Oncocalyx species were characterized by more than one main vascular bundle in midrib (3-7). Compared between the two Oncocalyx species we distinguished two characteristics: type of collenchyma as supporting tissue around the main vascular bundle in the leaf and fiber wall thickness in stem. In Oncocalyx schimperi collenchyma as supporting tissue was below vascular bundle (Fig.4 D) and fiber wall thickness in woody stem was between 0.07-0.08 μ , while in O. glabratus collenchyma as supporting tissue was above and below vascular bundle (Fig.4 E), and fiber wall thickness in woody stem was between 0.03 - 0.05 μ .

Statistical analysis of the leaf quantitative anatomical characteristics (Table 4) show the sum of squares and the average squares between and within groups. Also, the differences between the averages of the groups, which calculated F values and the significance level either at five degrees of freedom (between groups), and at 54 degrees of freedom (within groups). Since all the significance level (P) is less than 0.05 ($P < 0.05$). So we can reject the null hypothesis and accept the alternative hypothesis.

Statistical analysis of the stem quantitative anatomical characteristics (Table 5) show the sum of squares and the average squares between and within groups. Also, the differences between the averages of the groups, which calculated F values and the significance level either at five degrees of freedom (between groups), and 54 degrees of freedom (within groups). Since all the significance level (P) is less than 0.05 ($P < 0.05$). So we can reject the null hypothesis and accept the alternative hypothesis.

Table (6 & 7) show number of qualitative anatomical characteristics in stem and leaf. About four characters in leaves and three characters in stem.

To compare the characters, we can use the Coefficient of variation (CV) Figure (1 & 2) which was a measure of dispersion calculated by dividing the standard deviation of a distribution by its mean. The standard error of an estimate, expressed as a ratio or percentage of the estimate. In figure (1) the number of the main vascular bundle in leaf was major character (92.53), while the length of main vascular bundle was the minor character (28.75). In figure (2) Fiber wall thickness in stem was the highest characters (153.36) while number of vessels/ 10 μm^2 (31.35) was the lowest characters

The results of numerical analysis using UPGMA (Figure 3) was further divided the six Loranthaceae species into two groups and three subgroups. The first subgroup includes Phragmanthera austroarabica and Tapinanthus globiferus, the second subgroup includes the two Plicosepalus species, while the third subgroup includes the two Oncocalyx species and these results were matched the anatomical results described in the artificial key.

4-1. Anatomical keys

I- leaf Isobilateral and stem rays width between 1.2-2.1 μ

A) Collenchyma supporting tissue below main leaf vascular bundle

----- Phragmanthera austroarabica

B) Collenchyma supporting tissue above and below main leaf vascular bundle –

-----Tapinanthus globiferus

II-leaf isolateral and stem rays width between 0.2-1 μ

A) Presence of one main vascular bundle in leaf

a. Axial parenchyma of two types paratrachel & apotrachel in groups

----- Plicosepalus acacia

b. Axial parenchyma paratrachel ----- Plicosepaluscurviflorus

B) Presence of more than one main vascular bundle in leaf

a. Collenchyma supporting tissue below main leaf vascular bundle

----- Oncocalyx schimperi

b. Collenchyma supporting tissue above and below main leaf vascular bundle

-----Oncocalyx glabratus

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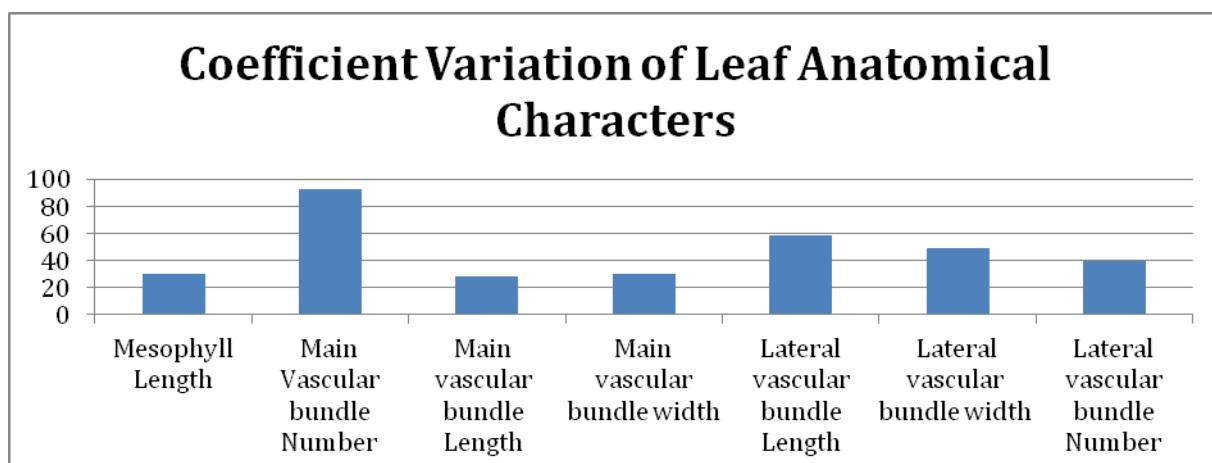
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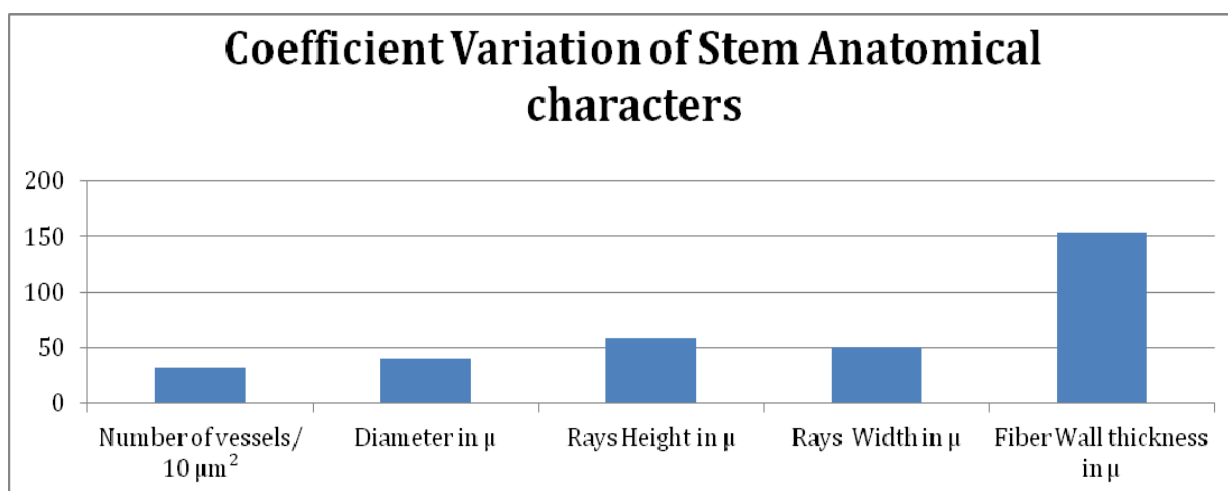
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Figure 1 : Coefficient of variation for Leaf anatomical characters



Mesophyll Length	Main Vascular bundle Number	Main vascular bundle Length	Main vascular bundle width	Lateral vascular bundle Length	Lateral vascular bundle width	Lateral vascular bundle Number
30.81	92.58	28.75	30.74	58.93	48.97	40.01

Figure 2: Coefficient of variation for stem anatomical characters



Number of vessels/10 μm ²	Diameter in μ	Rays Height in μ	Rays width in μ	Fiber Wall thickness in μ
31.35	39.42	58.35	50.90	153.36

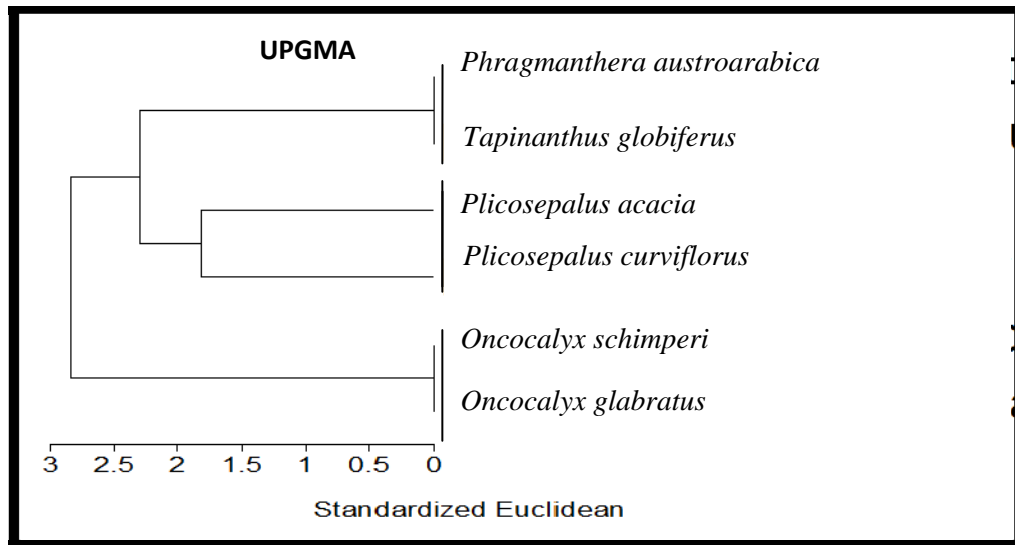


Figure (3) Graphic tree by UPGMA processor shows the relationship between the six Loranthaceae species under investigation based on anatomical characteristics.

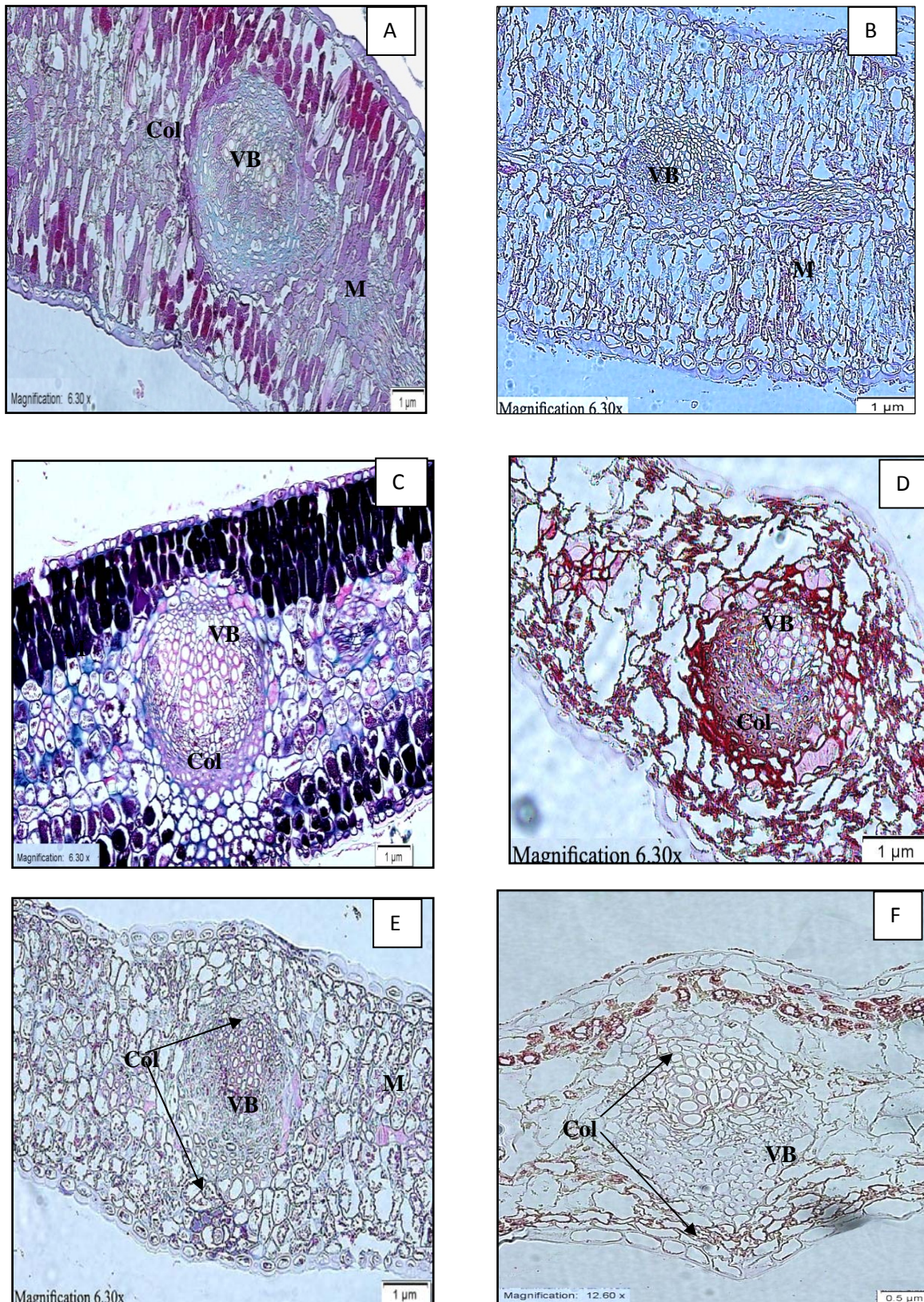


Figure (4): T.S.in Leaf of six species of family Loranthaceae : (A) *Plicosepalus acacia* (B) *Plicosepalus curviflorus* (C) *Phragmanthera austroarabica*, (D) *Oncocalyx schimperi* (E) *Oncocalyx glabratus*, (F) *Tapinanthus globiferus* . VB= Vascular bundle, M = Mesophyll, Col. =Collenchyma

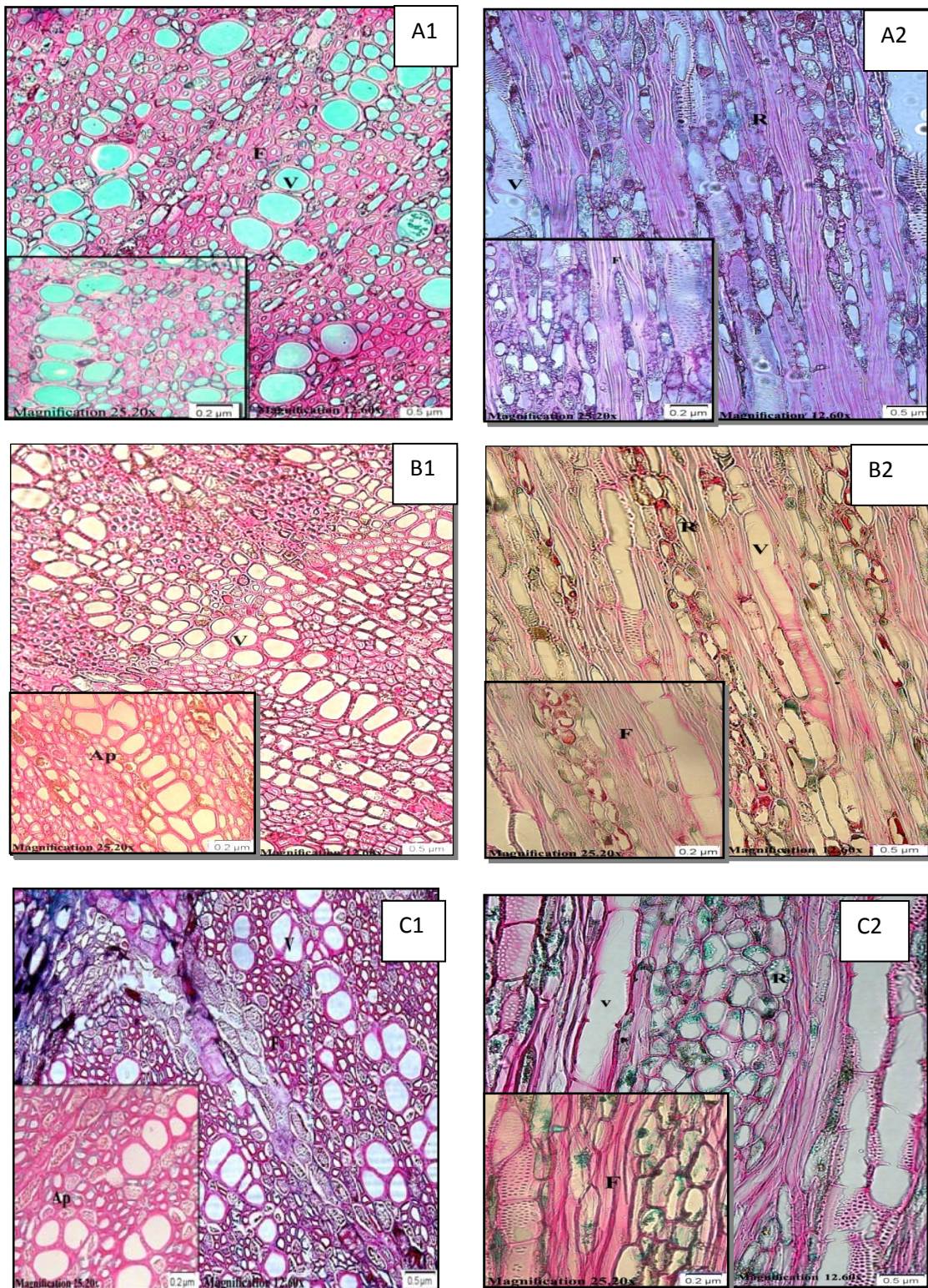


Figure (5) T.S. & L.S. in stems A1&A2 *Plicosepalus acacia*, B1&B2 *Plicosepalus curviflorus*, C1&C2 *Phragmanthera austroarabica* V=Vessels, R= Rays, F= Fiber, Ax=Axial parenchyma

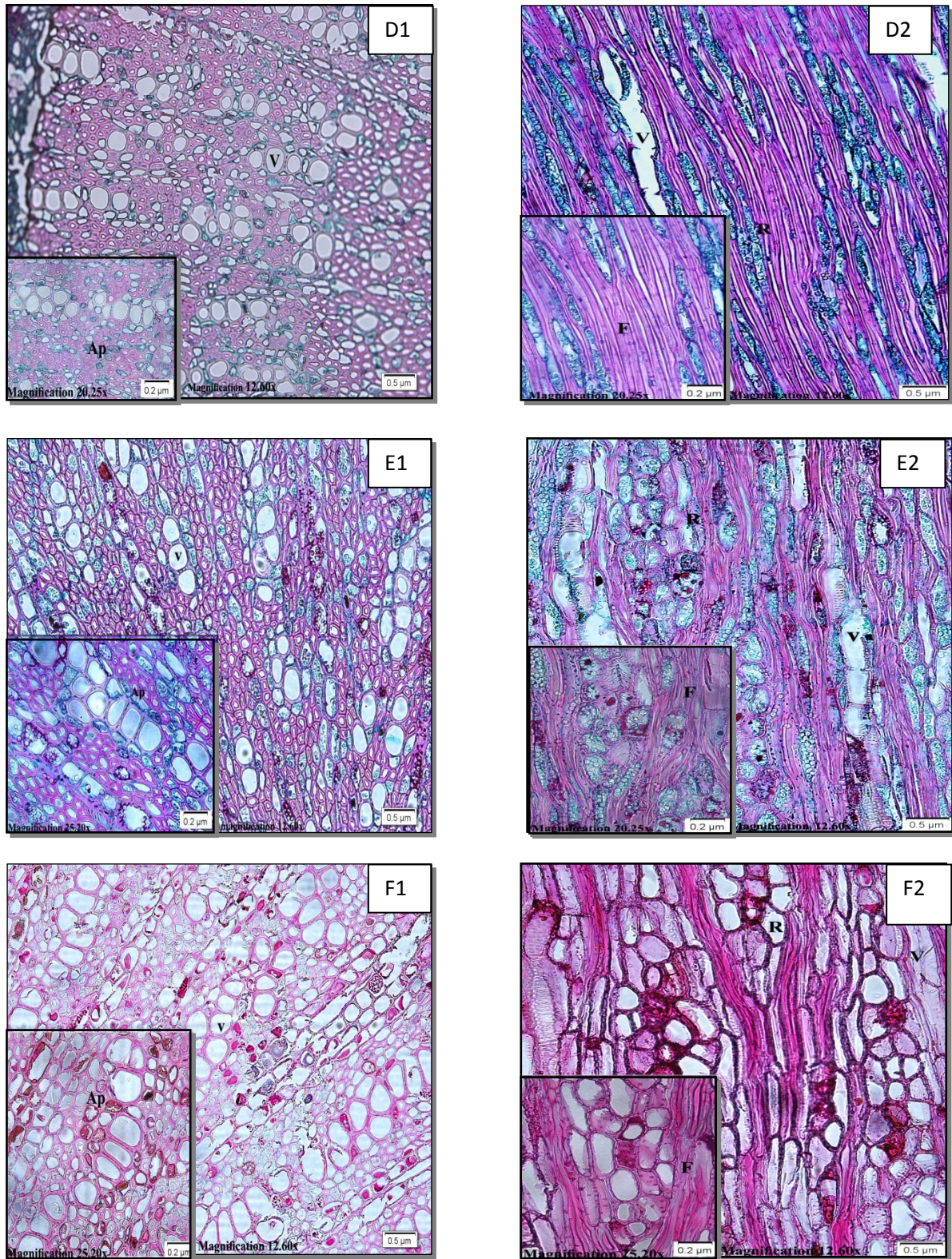


Figure (6) T.S. & L.S.in stems D1&D2 *Oncocalyx schimperi* , E1&E2*Oncocalyx glabratus*, F1&F2 *Tapinanthus globiferus*, V=Vessels, R= Rays, F= Fiber, Ax=Axial parenchym