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IFE JOURNAL OF SCIENCE AND TECHNOLOGY

Vol 7. No. 1 (2023) 29–45

The wood of *Bougainvillea* Comm. ex Juss. and its importance in the taxonomy of the genus

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Abstract

Wood is of great importance and relevance to human existence on Earth. It has been found useful in all walks of life. This calls for documentation of the wood attributes of plants growing around us. Also, there is a dearth of information on the anatomical characteristics of the wood of *Bougainvillea* species. This study is therefore aimed at documenting the various attributes of the wood of some members of the genus *Bougainvillea* and explore wood anatomical characters of taxonomic importance among them. Five species of *Bougainvillea*, *B. glabra*, *B. alba*, *B. peruviana*, *B. glabtabilis* and *B. spectabilis* were used in the study. The transverse, tangential longitudinal and radial longitudinal sections of each of these species were cut using a microtome at a thickness of 8-15 μm and prepared on microscopic slides following standard procedure. The various wood samples were also macerated using a modified Schulz's fluid in order to study their microstructures. The unifying wood anatomical characteristics observed among the *Bougainvillea* species include the absence of growth ring, heterogeneous vascular ray type, and presence of tylose and simple pitted vessels with oblique end walls. These support the generic classification of the species. The diagnostic features observed among them which can be employed in delimiting the species include axial parenchyma type, presence of raphide bundles, vessel dimensions, fibre length and wood porosity.

***Keywords*:** Wood anatomy; *Bougainvillea*; Axial parenchyma; Wood porosity; Raphide bundles

Introduction

Wood, produced as a result of secondary growth in plants, is vital to the survival of the human race. It is of great importance in paper and pulp production, construction of shelters for humans and animals, furniture, drug production, musical instrument production, creative arts, fuel production, shipbuilding and many other life endeavours (Afiqah *et al*., 2021; Alves *et al*., 2008; Karami *et al*., 2010; Longui *et al*., 2010 and Ramage *et al*., 2017).
Genus *Bougainvillea*, a shrubby climber, is a member of the family Nyctaginaceae (Four o'clock family), and about 18 species were reported for the genus (Shilpi *et al*., 2016). The family itself is represented by about 27 genera and 350 species, which are widely distributed in the tropical and sub-tropical regions of the world with a few members in the

temperate region (Debasmita *et al*., 2015). A few examples of other genera in the family include *Pisonia* Linn., *Boerhavia* Linn. *Abronia* Juss., *Crptocarpus* Kunth, *Mirabilis* Linn., *Cuscatlania* Standl., *Commicarpus* Standl., *Neeopsis* Lundell, *Pisoniella* (Heimerl) Standl., *Allionia* Linn.

Members of the genus *Bougainvillea* have been grown all over the world especially in the tropics based on their colourful bracts, which come out fine in landscaping. They are popular ornamental plants all over the world (Salam *et al*., 2017). Some scientific studies have been carried out on the genus which includes molecular phylogenetic studies (Bautista *et al*., 2022; Chatterjee *et al*., 2007; Douglas and Manos, 2007;); in-vitro propagation of difficult-to-root *Bougainvillea* cultivars (Datta and Mandal, 2012); detailed morphological studies and cultivation requirements of the members of the genus (Kent *et al*., 2007; Shilpi *et al*., 2016; Singh, 2018); medicinal uses of members of the genus (Adebayo *et al*., 2009) and floral stage developmental studies (Iqbal *et al*., 2017 and Saifuddin *et al*., 2017). Kulshreshtha *et al*., 2009 recommended that members of *Bougainvillea* be cultivated in urbanized and industrial areas to mitigate the effect of pollutants in such areas.

Species of the genus *Bougainvillea* have been reported to undergo secondary growth which leads to wood production in plants (Carlquist, 2013; Chew, 2010). Esau and Cheadle (1969) were the first to observe and report anomalous secondary growth in the stem and root of some species of *Bougainvillea*, while studying them anatomically. Information is sparse about the attributes of the wood of *Bougainvillea* species, especially in Nigeria. Therefore, there is a need to document the characteristics of its wood. This study aims to identify and document wood anatomical characters of some *Bougainvillea* species and explore wood anatomical characters of taxonomic importance among them.

Materials and Methods

In carrying out this work, matured stems of five *Bougainvillea* species were studied. The species are: *B. glabra* Choisy, *B. alba* Comm., *B. peruviana* Humb. & Bonpl., *B. glabtabilis* Comb. Nov. and *B. spectabilis* Willd. The matured stems were collected from the species growing on different sites in Obafemi Awolowo University, Ile-Ife, Osun State (N07°31.237' E004°31.720').

Maceration

The wood of the *Bougainvillea* species was sliced into small pieces and macerated using a modified Schulz's fluid, which was obtained by mixing an equal volume of 10% Chromic acid and 5 ml Nitric acid. The macerated wood samples were washed in water and stained with Safranin O for about 3 minutes. Afterwards, the samples were mounted on a clean glass slide with a drop of 25% glycerol for microscopic examination of the microstructures of the woods.

Sectioning

Each of the *Bougainvillea* species wood was cut into cubes of about 5 cm each and boiled in water. Sodium hydroxide pellets were added to the water to aid the softening of the wood samples. Transverse Section (TS), Tangential Longitudinal Section (TLS) and Radial Longitudinal Section (RLS) of the wood of each of the species were cut at 10 microns thickness using a sledge microtome. The sections were stained with Safranin O and counter-stained with Alcian blue. They were then rinsed in water and treated in a series of ethanol (50%, 70%, 80%, and 100%) to remove water molecules and excess stain.

Microscopy

Observation of the slides was made using Olympus XSZ-107BN light microscope. The features observed were grouped into qualitative and quantitative characters. Qualitatively, the types and distribution of vessels, vessel perforation and intervascular pitting, types and distribution of axial parenchyma and their relationship with the vessels, the types of vascular ray cells and the features of the fibres were studied and recorded. The tissues and cells identification were done according to Metcalfe and Chalk (1989). Quantitatively, fibre length, ray length, vessel diameter, vessel length and pore diameter were measured. Fifty (50) measurements were carried out for each of the characters.

All microscopic measurements were taken with the aid of an ocular micrometer inserted into the eyepiece of a microscope. The ocular readings were converted to micrometer according to the magnifications. Photomicrographs of the slides were made using an AmScope camera attached to a light microscope.

Data Analysis

The mean value of each of the quantitative characters measured was calculated and subjected to Single Linkage Cluster Analysis (SLCA) and Principal Components Analysis (PCA) using the Paleontological Statistics Software Package (PAST). Percentage distribution of solitary vessels, pore clusters and radial multiple were also calculated.

Results and Discussion

Tables 1 and 2 show the qualitative and quantitative wood anatomical characters of the species, respectively. Figures 1- 4 show the photomicrographs of the transverse sections, tangential longitudinal sections, radial longitudinal sections and macerates of the *Bougainvillea* species, respectively.

Transverse section of *Bougainvillea* wood

It was observed that growth rings were absent in all the species. This is most likely because they grow in tropical forest ecological zones and the formation of continuous and uniform xylem (De Micco *et al*., 2019). According to Tarelkin *et al*. (2016), growth rings may be distinct, indistinct or even absent in plants, yet, this character is highly significant in wood identification and has been employed in many tree-ring studies. Wood porosity is a diagnostic character among the *Bougainvillea* species involved in this study. Semi-ring wood porosity was observed in *B. glabra*, while that of the other four species is diffuse. The presence of semi-ring porosity in *B. glabra* only and diffuse porosity in the other four species can be used to differentiate them. Wood porosity has been employed in the delimitation of plant species. These include the works of Michael and Peter (2011) on different wood types using mercury porosimetry and Arogundade and Onubogu (2018) on some members of the genus *Caesalpinia* Linn.

Varying vessel shapes were observed in the species, which ranges from circular or spherical to elliptical and oval. Circular to oval vessel shape was observed to be common to *B. glabtabilis* and *B. alba*, while the other three species have varying vessel shapes. The vessels of the species involved in this study expressed three types of vessel arrangements - solitary, pore clusters and radial multiple. These were observed in notable frequencies in all the species. The percentage distribution of solitary vessels observed in *B. glabra* ranges from 0% to 80%; that of pore clusters ranges from 11.1% to 77.8%, while that of radial multiple pores ranges from 0% to 71.4%. For *B. peruviana*, the percentage distribution of solitary vessels ranges from 0% to 71.43%; that of pore clusters ranges from 21.43% to 76.92%, while that of the radial multiple pores ranges from 11.11% to 57.14%. For *B. glabtabilis*, the percentage distribution of the few solitary vessels observed ranges from 0% to 41.67%; that of pore clusters ranges from 21.43% to 65%, while that of the radial multiple ranges from 15% to 55.92%. In *B. alba*, the percentage distribution of solitary vessels observed ranges from 11.11% to 66.67%; that of pore clusters from 20% to 62.50%, while that of radial multiple ranges from 11.11% to 46.15%. Lastly for *B. spectabilis*, the percentage distribution of solitary vessels observed ranges from 0% to 44.44%; that of pore clusters from 9.09% to 66.67% and that of radial multiple from 18.18% to 72.73% (Table 2).

Solitary vessels have been established to be the most primitive type of vessel arrangement (Oladipo and Oyaniran, 2013). It was observed in all the species but it has the least average percentage distribution. Pore clusters generally had the highest percentage distribution in all the species except in *B. spectabilis* where radial multiple pores had the highest percentage distribution. According to Bahadur *et al*. (2014), the presence of more radial multiple pores in a wood sample ascertains its primitiveness, it can therefore be inferred that *B. spectabilis* is more primitive than the other species. The least pore diameter or pore size of 82.80± 2.56 µm was observed in *B. spectabilis*, while the highest pore size, 111.40±3.12 µm, was observed in *B. peruviana*. According to the pore size grouping by Meier (2015), the vessels of *B. alba*, *B. glabra*, *B. glabtabilis* and *B. spectabilis* fall into the "Medium" vessel size category, while *B. peruviana* vessels are in the "Large" vessel category.

Tyloses were conspicuous in some of the cells of all the species in their transverse section. According to De Micco *et al*., 2016, tyloses are developed in plants as a result of ageing and heartwood formation and they naturally cause obstructions in vessels. Tyloses play a crucial role in limiting the spread of pathogens and wood decay organisms, as well as part of compartmentalization after wounding. The occurrence of tylose in the transverse sections of all the species studied may be due to the ageing and formation of heartwood in the woods of the species. The distribution of axial parenchyma within the woods of the species studied is also significant and can be used in their classification. The axial parenchyma is paratracheal (confluent) (Fig. 1 A – E) in all the five species which implies that the woods of the *Bougainvillea* are specialized. However, more specialized axial parenchyma type, Paratracheal Aliform-Confluent, observed in *B. peruviana*, *B. glabtabilis* and *B. spectabilis* differentiate them from *B. glabra* and *B. alba* with just Paratracheal Confluent axial parenchyma. Oladipo and Oyaniran (2013) delimited some members of the genus *Ocimum* based on their paratracheal axial parenchyma types.

Tangential longitudinal sections of *Bougainvillea* wood

A simple pitted vessel with oblique end walls, which is an advanced wood character (Lens *et al*., 2016) is found in all the species studied. Ray cells are largely biseriate and multiseriate in *B. glabra* and *B. spectabilis*; uniseriate, biseriate and multiseriate in *B. peruviana* and *B. glabtabilis*, while they are mostly multiseriate in *B. alba*. Raphide bundles are present in the tangential longitudinal sections of *B. glabra*, *B. peruviana* and *B. spectabilis*, while there are no raphide bundles in *B. glabtabilis* and *B. alba*. This delimits them from the other two species (Fig. 2 A- E; Table 1). Raphides are calcium oxalate crystals and have been discovered to function in calcium storage, defence and providing structural strength in plants (Wakata, 2003).

Radial longitudinal sections of *Bougainvillea* wood

Heterogenous or heterocellular ray cells consisting of both upright and procumbent cells were observed in the five species (Fig. 3 A – E). Van der and Baas (1974) reported that the specialization trend from heterogenous to homogenous is a reliable tool since it is unchangeable and unidirectional. Dos Santos and Miller (1997) also in their study of *Jacaranda* ascertained that species with exclusively heterogenous wood rays are primitive, while those with homogenous rays are more advanced. In this study, the wood rays of all the species are upright and procumbent (heterogenous), this suggests primitiveness in all the species studied.

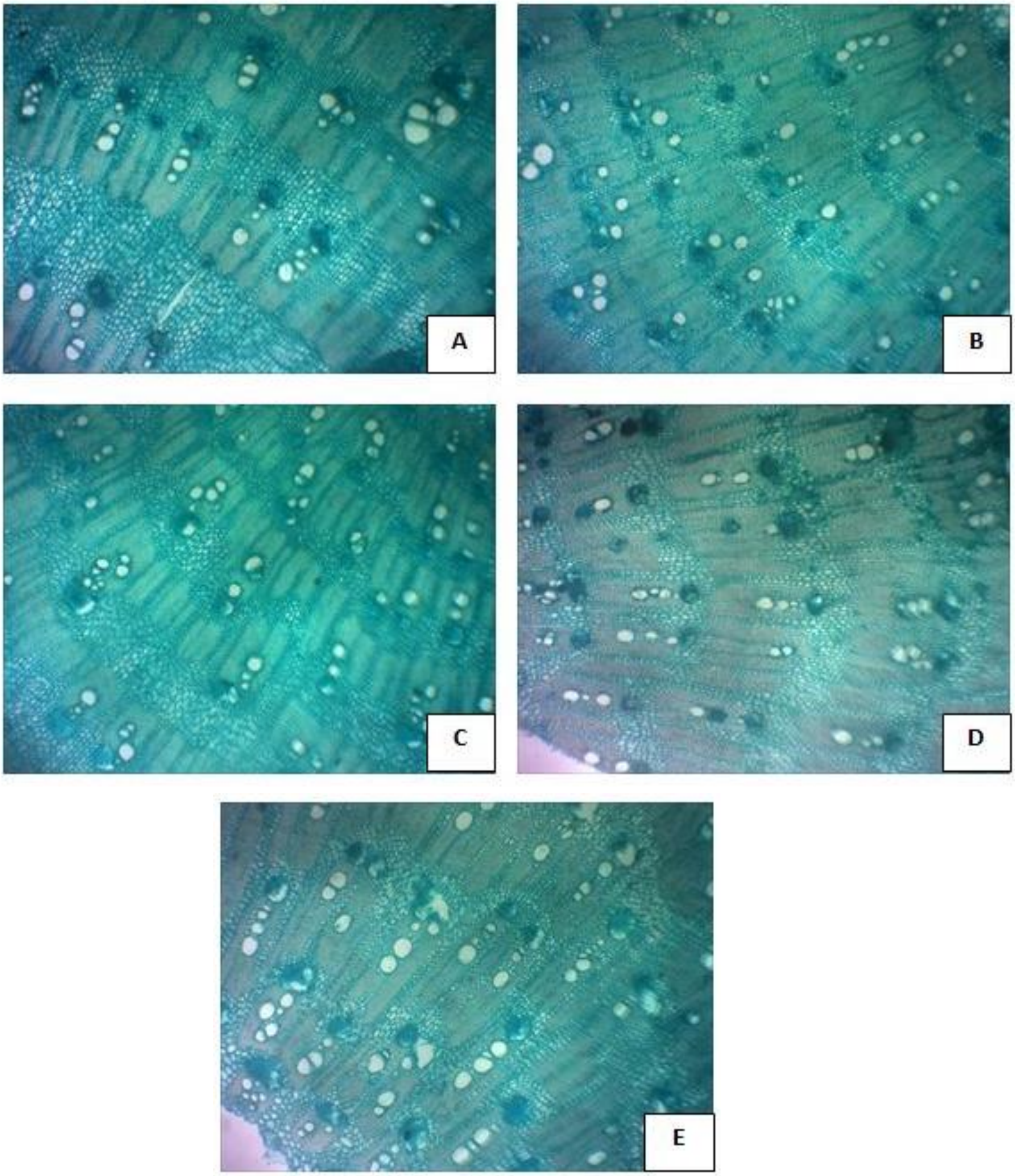
Table 1: Summary of the qualitative wood anatomical characters of the species of *Bougainvillea* studied.

Wood characters	***B. glabra***	***B. peruviana***	***B. glabtabilis***	***B. alba***	***B. spectabilis***
Axial parenchyma	Paratracheal Confluent	Paratracheal Aliform Confluent	Paratracheal Aliform Confluent	Paratracheal Confluent	Paratracheal Aliform Confluent
Growth	Absent	Absent	Absent	Absent	Absent

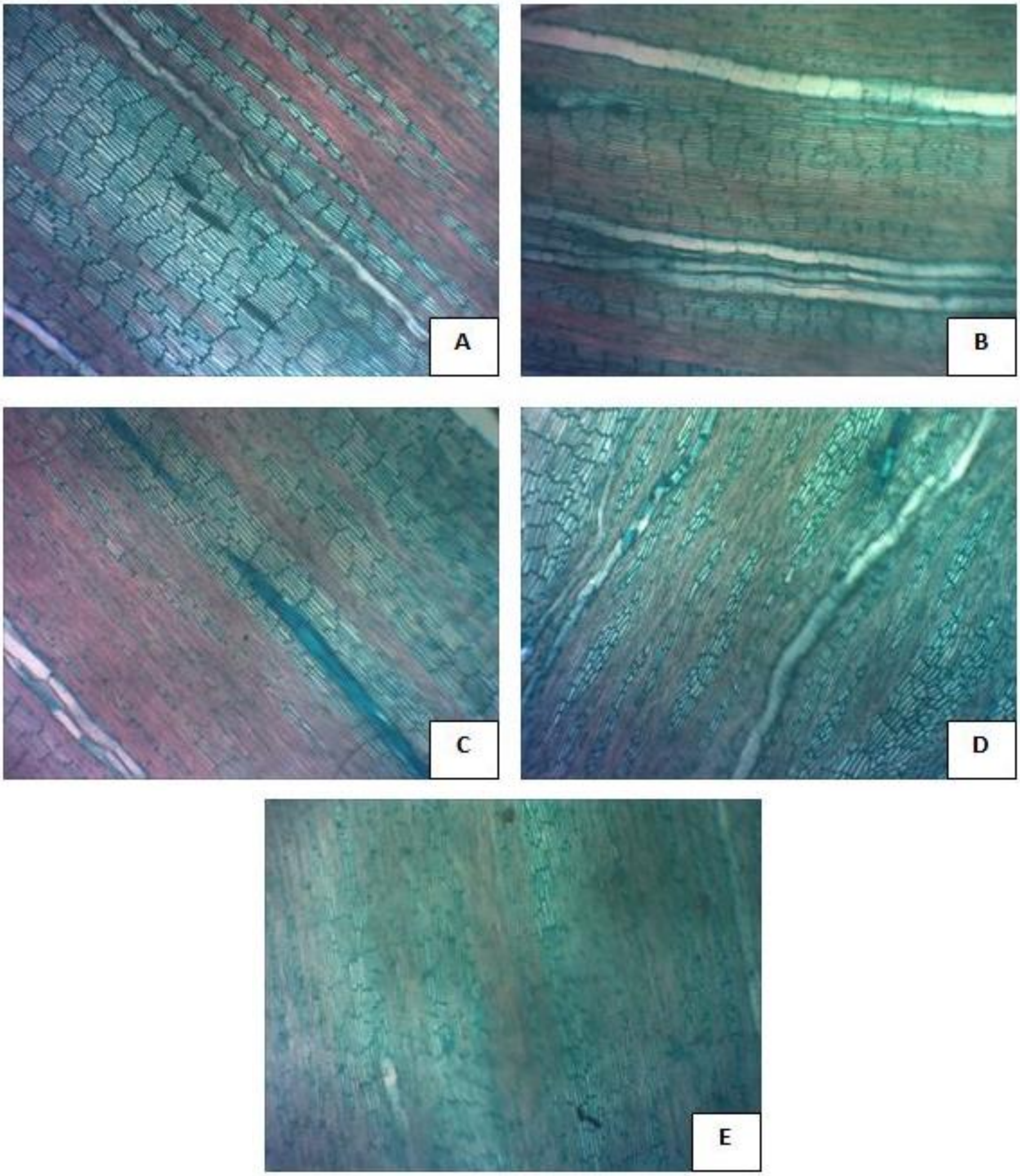
ring					
Vascular ray type	Heterogenous	Heterogenous	Heterogenous	Heterogenous	Heterogenous
Tylose	Present	Present	Present	Present	Present
Raphide bundle	Present	Present	Absent	Absent	Present
Wood Porosity	Semi-ring	Diffuse	Diffuse	Diffuse	Diffuse
Vessel Shape	Circular to Elliptical	Oval to Spherical	Circular to Oval	Circular to Oval	Elliptical to Oval
Ray Cells	Biseriate and Multiseriate	Uniseriate, Biseriate and Multiseriate	Uniseriate, Biseriate and Multiseriate	Multiseriate	Biseriate and Multiseriate

Table 2: Summary of the quantitative wood anatomical characters of the species of *Bougainvillea* studied.

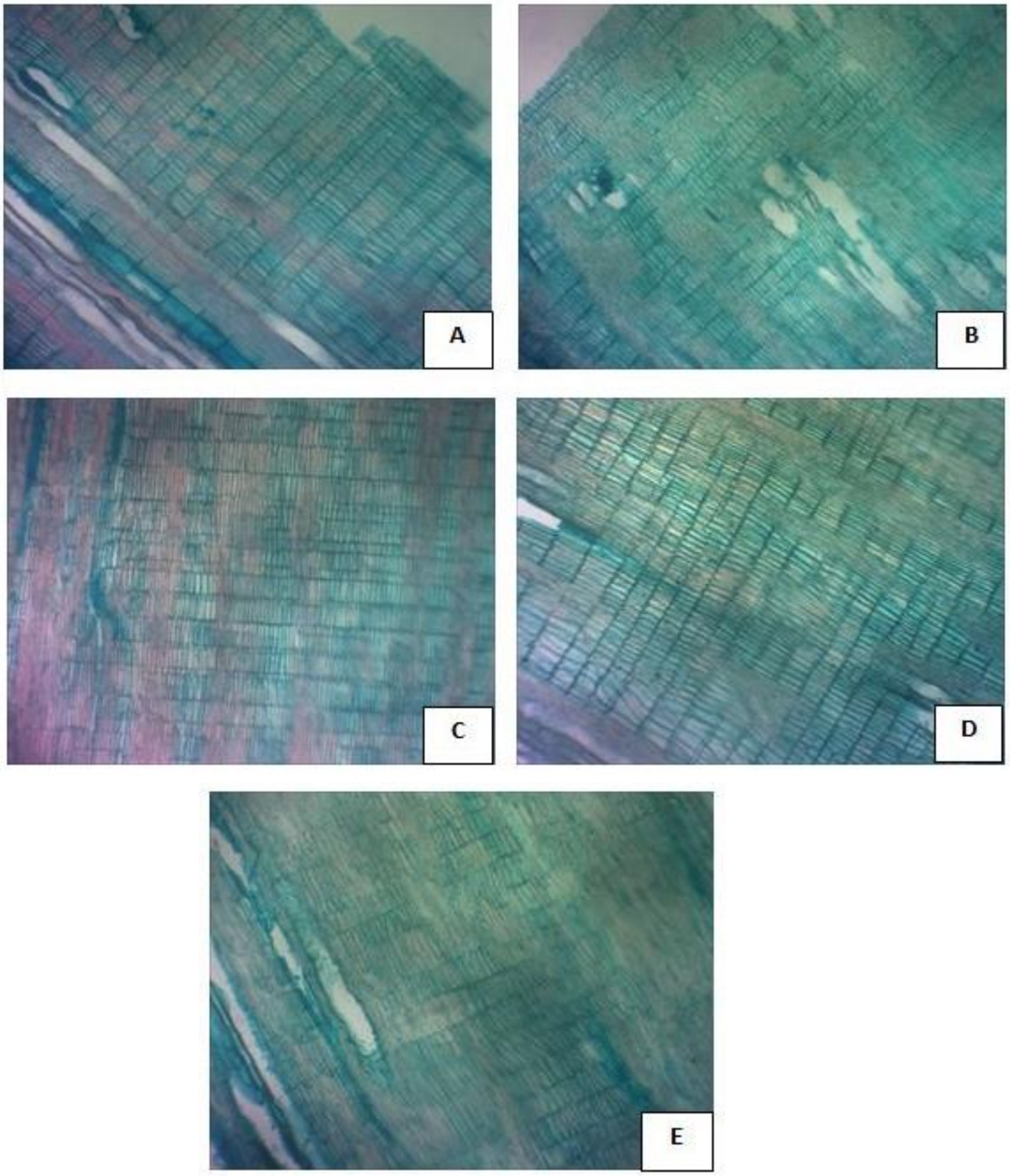
Wood characters	***B. glabra***	***B. peruviana***	***B. glabtabilis***	***B. alba***	***B. spectabilis***
Mean fibre length (µm)	608.60±17.28	531.60±16.77	232.60±7.71	526.30±12.90	576.60±15.94
Mean Vessel length (µm)	192.60±8.75	212.80±9.74	202.00±7.86	214.80±6.18	199.00±8.25
Mean Vessel diameter(µm)	83.20±3.99	85.40±4.37	67.80±4.13	75.44±4.62	82.20±3.93
Mean Ray length (µm)	155.00±4.48	151.2 ±5.31	144.00± 4.62	123.2± 4.59	139.00± 5.22
Mean Pore diameter (µm)	97.80 ±2.58	111.40±3.12	99.00± 2.45	90.60± 2.20	82.80± 2.56
Solitary vessels (%)	0-80	0-71.43	0-41.67	0-66.67	0-44.44
Pore clusters (%)	11.11-77.78	21.43-76.92	21.43-65	20-62.50	9.09-66.67
Multiple vessels (%)	0-71.43	11.11-57.14	15-76.92	11.11-66.67	18.18-72.73



Legend: A – *B. glabra*; B – *B. peruviana*; C – *B. glabtabilis*; D – *B. alba*; E – *B. spectabilis*
Figure 1 A- E: Transverse sections of the wood of the *Bougainvillea* species
Magnification: X40



Legend: A – *B. glabra*; B – *B. peruviana*; C – *B. glabtabilis*; D – *B. alba*; E – *B. spectabilis*
Figure 2 A-E: Tangential longitudinal sections of the wood of the *Bougainvillea* species
Magnification: X40

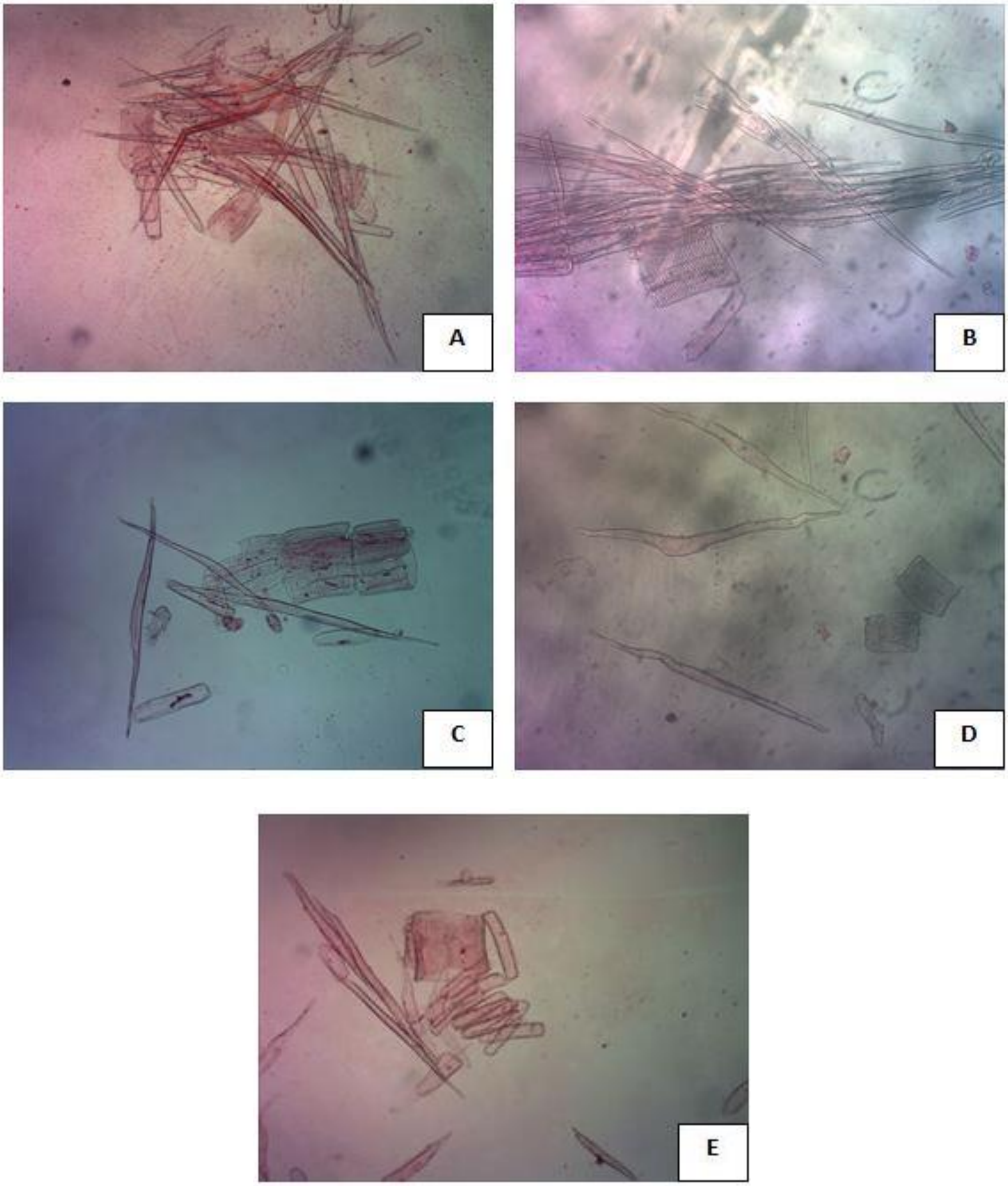


Legend: A – *B. glabra*; B – *B. peruviana*; C – *B. glabtabilis*; D – *B. alba*; E – *B. spectabilis*
Figure 3 A-E: Radial longitudinal sections of the wood of the *Bougainvillea* species
Magnification: X40

Microstructure of *Bougainvillea* wood

From the macerates which reveal the microstructure of the woods, vessels with simple perforations, simple pitting and oblique end walls were observed in all the species. The vessel length and vessel diameter of the woods are diagnostic. The highest vessel length of 214.80±6.18 µm was observed in *B. alba*, while the lowest value of 192.60±8.75 µm was recorded for *B. glabra*. For the vessel diameter, *B. peruviana* had the highest value of 85.40±4.37 µm 67.80±4.13 µm, while *B. glabtabilis* had the lowest value of 85.40±4.37 µm. Based on their measurements, the vessels of *B. alba* and *B. glabtabilis* are moderately small, while those of *B. glabra*, *B. peruviana* and *B. spectabilis* are medium-sized (Metcalfe and Chalk 1989).

The wood fibres of all the species have moderately thick walls. They are libriform, non-storied and non-septate. However, fibre length is diagnostic in the genus. The highest fibre length of 608.60±17.28 µm was recorded for *B. glabra*, while the least fibre length of 232.60±7.71 µm was recorded for *B. glabtabilis*. Generally, the woods of all the species fall into the category of short fibres as described by Metcalfe and Chalk (1989). Sadiku and Abdukareem (2019) stated the importance of short hardwood fibres in furniture making which provide good printability and stiffness to the end product. The highest ray cell length of 155.00±4.48 µm was recorded in *B. glabra*, while the lowest value of 123.2± 4.59 µm was observed in *B. alba* (Fig 4 A-E; Table 2).

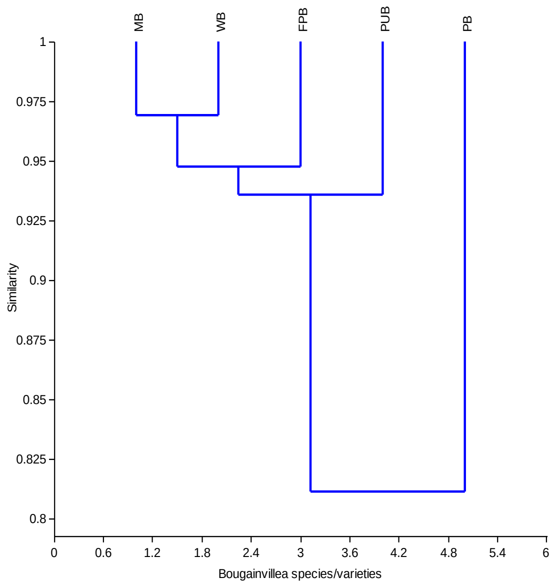


Legend: A – *B. glabra*; B - *B. peruviana*; C - *B. glabtabilis*; D - *B. alba*; E - *B. spectabilis*
Figure 4 A- E: Macerates of the wood of the *Bougainvillea* species Magnification: X100

Data Analysis

The result of the Single Linkage Cluster Analysis of the *Bougainvillea* species as shown in Figure 5, based on their similarities reveals three main clusters. *B. glabtabilis* was separated from the other taxa in the first clustering, showing a very clear distinction between *B. glabtabilis* and the other four taxa. *B. spectabilis* was separated from the other three species in the second clustering, while *B. glabra* was separated from *B. alba* and *B. peruviana* in the third clustering, leaving *B. alba* and *B. peruviana* grouped at a higher similarity level, suggesting a very close relationship between them.

The correlation among the *Bougainvillea* species was revealed from the results of the Principal Components Analysis. Figure 6 shows the graph of the Principal Components Analysis of the species based on components 1 and 2, while Figure 7 shows the graph of the Principal Components Analysis of the species based on components 1 and 3. Table 3 shows the Eigen value and the percentage of total variation accounted for by the first three components axes of ordination of the species. The first three components of the Principal Components Analysis (PCA) accounted for 99.9% of the variation among the species studied. From the PCA loadings, it was revealed that the characteristics responsible for the separations observed among the species are ray length and vessel diameter.

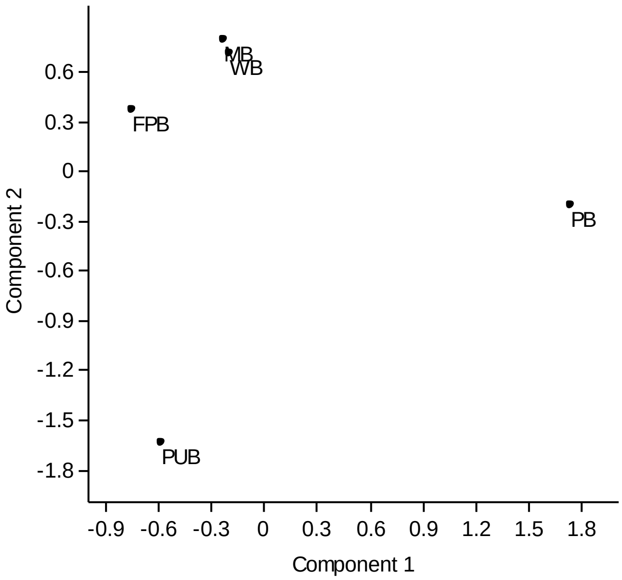


Legend:
FPB: *Bougainvillea glabra*
MB: *Bougainvillea peruviana*
PB: *Bougainvillea glabtabilis*
WB: *Bougainvillea alba*
PUB: *Bougainvillea spectabilis*

Figure 5: Dendrogram of five species of *Bougainvillea* based on quantitative wood anatomical characters.

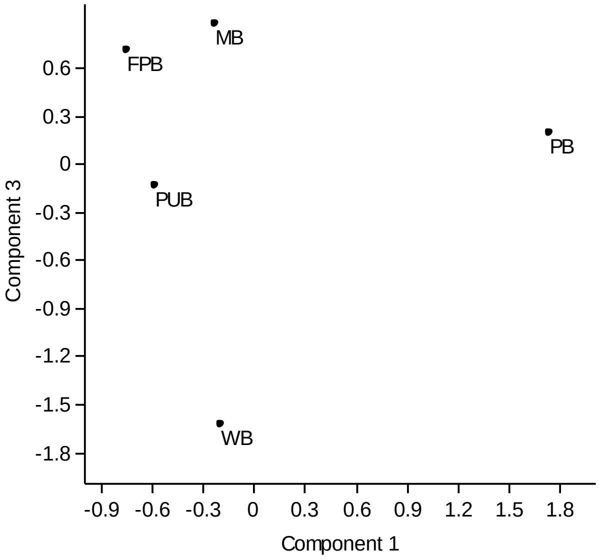
Table 3: Eigen Value and the Percentage of Total Variation Accounted for by the First Three Components Axes of Ordination of the Species.

Principal Component Axis	Eigen value	Percentage of total variation	Cummulative
1	22873.6	93.11	93.11
2	1474.39	6.00	99.11
3	202.65	0.83	99.90



Legend:
FPB: *Bougainvillea glabra*, MB: *Bougainvillea peruviana*, PB: *Bougainvillea glabtabilis* WB: *Bougainvillea alba* , PUB: *Bougainvillea spectabilis*

Figure 6: Graph of the Principal Components Analysis of the *Bougainvillea* species based on components 1 and 2.



Legend:
FPB: *Bougainvillea glabra*
MB: *Bougainvillea peruviana*
PB: *Bougainvillea glabtabilis*
WB: *Bougainvillea alba*
PUB: *Bougainvillea spectabilis*

Figure 7: Graph of the Principal components Analysis of the *Bougainvillea* species based on components 1 and 3

Conclusions

This study revealed that the wood anatomical characters of the species studied are similar although few diagnostic characters exist among them. Features like the type of axial parenchyma, raphide bundle, vessel dimensions, fibre length and porosity can be used to distinguish them. Therefore, the species of *Bougainvillea* studied can be separated and identified based on their qualitative and quantitative wood anatomical characters.

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