A STUDY OF SKELETONISATION TECHNIQUES AND LEAF DIVERSITY AT BMC CAMPUS”

Project submitted

TO

MAHATMA GANDHI UNIVERSITY

In partial fulfillment of the requirement in degree of

BACHELOR OF SCIENCE IN BOTANY

Submitted by

HADIYA ABDUL KHADER

Register no : 210021022668

May 2024

DEPARTMENT OF BOTANY

BHARATA MATA COLLEGE

THRIKKAKARA

KOCHI-682

CERTIFICATE

This is to certify that this project work entitled "**A STUDY OF SKELETONISATION TECHNIQUES AND LEAF DIVERSITY AT BMC CAMPUS**" is a bona fide piece of project work done by **Hadiya Abdul Khader (Reg.no:210021022668)** in the Department of Botany, Bharata Mata College, Thrikkakara under my guidance and supervision for the award of Degree of Bachelor of Science in Botany during the academic year 2021-2024. This work has not previously formed the basis for the award at any other similar title of any other university or board.

Place : Thrikkakara **Dr. SHAHINA NK**
Date : Supervising teacher

HOD, Department of Botany
Bharata Mata College

DECLARATION

I hereby declare that this project entitled ""**A STUDY OF SKELETONISATION TECHNIQUES AND LEAF DIVERSITY AT BMC CAMPUS**" is the result of work carried out by me under the guidance of **Dr.Shahina NK,** Department of Botany,Bharata MataCollege, Thrikkakara. This work has not formed on the basis for the award at any other similar title of any other university of board.

HADIYA ABDUL KHADER

ACKNOWLEDGEMENT

I express my heartfelt gratitude to God for His blessings.

Special thanks to Dr. Newby Joseph, Head of the Botany Department at Bharata Mata College, for providing essential support and facilities.

I express my sincere gratitude to our project guide Dr. Shahina N K (Assistant Professor) for continuous guidance and support throughout the project.

I also extend my thanks to other faculties of our department Dr. Lins Simon, Dr. Abin Kurian, and Mr. M J Pauli for their assistance.

I acknowledge the Central iInstrumentation Facility of Bharata Mata College, Thrikkakara for Analysis which is funded by DST-FIST(SR/FIST/College-313/2016 dt 08-02-2018), KSCSTE-SARD(23/2019/KSCSTEdt04-01-2019) and DBT STAR (HRD-11011/22/2022-HRD-DBTdt 24-08-2022)

Special thanks to my parents, team members, and friends for their unwavering encouragement.

CONTENTS

CHAPTER 1

INTRODUCTION

Leaves are anatomical structures found in vascular plants, characterized by their flattened, green morphology originating from the stem. They serve as the primary sites for photosynthesis, a fundamental biochemical process wherein light energy is converted into chemical energy to synthesize organic compounds.

Leaves are integral components of the plant's stem system, interconnected through a continuous vascular network facilitating the exchange of nutrients, water, and photosynthetic by-products such as oxygen and carbohydrates. Initiation of leaves occurs at the apical bud, alongside stem tissues. Certain morphologically distinct organs, like cactus spines, pine needles, and asparagus scales, derive from the same developmental process as conventional green leaves.

The central function of leaves is to execute photosynthesis. Chlorophyll, the pigment imparting the characteristic green hue, absorbs light energy critical for this process. Internally, the leaf's structure is safeguarded by the leaf epidermis, which seamlessly extends from the stem epidermis. Within the leaf, the mesophyll, comprised of parenchyma cells, constitutes the bulk of the leaf tissue. Approximately one-fifth of the mesophyll comprises chlorophyll-containing chloroplasts, pivotal in capturing sunlight and catalyzing the decomposition of water into hydrogen and oxygen through enzymatic pathways.

The liberated oxygen from photosynthesis replenishes atmospheric oxygen consumed by both plant and animal respiration, as well as combustion processes. Simultaneously, hydrogen from water combines with carbon dioxide in the enzymatic cascade of photosynthesis to synthesize sugars, serving as essential organic substrates for cellular metabolism. Oxygen generated during photosynthesis exits the leaf through stomata pores, facilitating gaseous exchange (Bolhar and Draxier, 1993).

Leaf morphology varies widely across plant species, encompassing diverse sizes, shapes, and venation patterns. Leaves may be classified as simple, comprising a single blade attached to the stem, or compound, featuring lobes or multiple leaflets. Leaf margins exhibit distinct characteristics, ranging from smooth to serrated. Reticulate venation is common in dicotyledonous plants with a branching network of veins throughout the leaf, resembling a net-like pattern. Reticulate venation allows for efficient nutrient and water distribution, as well as structural support. Whereas parallel venation is the characteristic of monocotyledonous plants. Parallel venation is efficient for transporting nutrients and water over long distances. Examples of plants with parallel venation include grasses, lilies, and bamboo. The intricate network of veins within the leaf not only supports its structure but also facilitates the transport of water, nutrients, and photosynthetic products throughout the plant .

Leaf venation system is flexible and helps plants adapt to different environments. By studying how leaf veins have changed over time, scientists can learn a lot about how plants have evolved. This knowledge isn't just interesting; it also helps in many practical ways. For example, it can help farmers grow better crops and inspire new technologies.

Understanding leaf venation gives us insights into how plants work and how we can use this knowledge to benefit both nature and people (Sack,and Scoffoni, 2013) .

Importance of leaf skeleton

Leaf skeletonization is the process of removing the soft tissue of a leaf, leaving behind the intricate network of veins. By studying the pattern of veins in leaf skeletons, scientists can gain insights into various aspects of plant biology and ecology:

Nutrient and Water Transport: The veins in leaf skeletons serve as conduits for the transportation of water and nutrients throughout the leaf. Understanding the arrangement and connectivity of these veins can provide valuable information about how efficiently plants can uptake water and nutrients from the soil.

Photosynthesis: Leaf veins also play a crucial role in supporting photosynthesis, the process by which plants produce sugars using sunlight. The intricate network of veins ensures that water and nutrients reach the photosynthetic cells in the leaf, while sugars produced during photosynthesis are transported to other parts of the plant for storage and growth.

Plant Adaptation: Leaf venation patterns can vary among different plant species and can be influenced by environmental factors such as light intensity, temperature, and humidity. Studying leaf skeletons can provide insights into how plants have adapted to different environments over time, allowing them to thrive in diverse habitats.

Paleobotany: Leaf skeletons found in fossils provide important clues about ancient plant species and ecosystems. By examining fossilized leaf skeletons, scientists can reconstruct past environments and track changes in plant diversity and distribution over geological time scales.

Aesthetic Enhancements: Skeletonized leaves add unique beauty to floral arrangements and crafts. Various venation patterns enhance visual appeal and texture in bouquets and wreaths.

Preserved skeletonized leaves inspire creativity in botanical art and mixed media projects.They can be preserved and incorporated into various art forms, such as collage and botanical illustration.

OBJECTIVES OF THE STUDY

1. To produce leaf skeletons from Bharata Mata College Campus plant leaves using a 5% NaOH solution.
2. Compare the structural and venation patterns of the skeletonised leaves
3. Investigate the efficacy of natural and synthetic dyes in coloring the leaf skeletons.
4. Evaluate the effectiveness of glycerin as a preservation agent over time.

SIGNIFICANCE OF THE STUDY

- Preparing leaf skeletons with a 5% NaOH solution helps us understand leaf structure and chemical treatments' effects. This knowledge is crucial for botanical research and education.
- Trying out natural and synthetic dyes on leaf skeletons provides insights into art and preserving botanical specimens. This connects science with art, encouraging collaboration and creativity.
- Preserving leaf skeletons with glycerin makes them last longer, aiding long-term studies and art projects. This makes botanical collections more accessible and helps preserve biodiversity.
- Using leaf skeletons in art shows their versatility and beauty. By demonstrating different ways to use them, we inspire people to appreciate nature and care for the environment.

CHAPTER 2

REVIEW OF LITERATURE

Leaf venation patterns not only serve as structural elements for plant vascular systems but also play a crucial role in plants' adaptation to changing environments, as documented by Sack and Scoffoni (2013). Variations in these patterns among species reflect adaptive strategies to diverse habitats, highlighting the importance of understanding leaf venation in ecological contexts and paleobotanical reconstructions.

A study by Badron et al. (2014) investigated the variation of leaf venation patterns in 21 taxa of the genus Ficus in Peninsular Malaysia. They identified eight distinct leaf venation patterns based on veinlets, the ultimate marginal and areolar venation. The findings suggest that variations in leaf venation patterns can be a valuable tool for not only identifying a taxon group but also differentiating between species within the genus Ficus.

In the preparation of leaf skeletons, various methods are employed, each with its advantages and considerations. One common technique, natural decomposition, involves allowing fresh leaves to decay slowly over time, facilitating microbial breakdown of tissue while preserving the vascular structure (Hättenschwiler et al., 2005). Maceration, another method, utilizes microbial decomposition through submerging leaves in water, leading to the gradual removal of fleshy tissue while retaining vein networks (Ferreira et al., 2020). Boiling leaves in water accelerates tissue degradation but may distort delicate vein structures (Llano et al., 2003). Chemical maceration with NaOH or KOH offers precise control and speed but requires careful handling due to the hazardous nature of these chemicals.

The leaf skeleton study by Mir et al., 2015 investigated the impact of baker's yeast fermentation on leaf maturity, focusing on determining the optimal age group of leaves suitable for fermentation (Mir & Jana, year). Using Ficus religiosa L. leaves as the model substrate, the study also explored the optimal fermentation time for skeletonizing leaves of other plant species like Bauhinia purpurea, Tectona grandis, Ficus benjamina, and Hiptage bengalensis (Mir & Jana, year). Standardizing the yeast concentration with a 2.0% solution, the study found that only matured leaves aged 4-5 months were suitable for baker's yeast fermentation under the experimental conditions

Now researchers are turning to nature for new materials. Plant leaf skeletons, with their intricate patterns, are getting attention. These skeletons, abundant and eco-friendly, are mainly made of cellulose, hemicellulose, lignin, and silica. Despite being treated as waste, they have potential. They help in water and nutrient transport and provide support to leaves. Finding ways to use them efficiently could address environmental issues.

The research conducted by Periasamy et al. (2020) explores the innovative application of plant leaf skeletons as scaffolds for three-dimensional cell culture. By treating these skeletons with sodium hydroxide, a microporous surface conducive to cell growth is created. This surface, composed of hemicellulose, cellulose, and lignin, along with the intricate venation architecture, promotes nutrient absorption and cell-cell interaction. The study demonstrates the potential of leaf skeletons in various biomedical applications such as tissue engineering, wound healing, and drug screening, while also emphasizing their role in utilizing agro-wastes sustainably.

The application of leaf skeletons in fabricating bioinspired heaters offers promising prospects for various practical uses (Sharma et al., 2022). Researchers have developed highly flexible, fast-heating, and biodegradable heaters using silver nanowires and leaf skeletons from different plant species (citation). These leaf skeletons serve as transparent substrates with a high surface-area-to-volume ratio, allowing for uniform dispersion of silver nanowires and excellent transmittance properties (citation). The resulting heaters demonstrate rapid heating capabilities at low voltages, making them suitable for wearable, medical, and industrial heating applications (citation). Furthermore, the performance of these bioinspired heaters is dependent on the microscale fractal structures present in the leaf skeletons (citation).

Research by Sharma et al. (2022) explores a novel application for plant leaf skeletons in tissue engineering**.** By treating *Ficus religiosa* leaf skeletons with sodium hydroxide under pressurized conditions, researchers created microporous scaffolds primarily composed of hemicellulose, cellulose, and lignin. Human mesenchymal stem cells (hMSCs) exhibited enhanced attachment and proliferation on these scaffolds, attributed to their biocompatibility and porous nature. The venation architecture of the leaf skeletons facilitated nutrient and oxygen absorption, promoting cell-cell interaction and long-term culture. This innovative scaffold holds promise for various applications, including multi-layer cell culture, drug screening, cell therapy, wound healing, and tissue engineering, while also offering a sustainable solution by utilizing agro-wastes as precursor materials for scaffold construction.

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection of Leaves

The plant materials were selected randomly from the campus of Bharata mata College, Thrikkakara, Kerala, focusing on leaves that were showcased prominent veins. Notable details including the scientific name, common name, morphology, and texture of the leaves were carefully recorded. Initially, leaves were sourced from *Ficus religiosa* tree, with subsequent experiments expanding to include leaves from additional species. (Figure 1) After preliminary trials, we concentrated on gathering fully-grown leaves with a firm texture, smooth and glabrous, to ensure their structural integrity. Leaves devoid of insect damage or environmental wear were exclusively chosen to maintain consistency in the experimental setup. This meticulous selection process aimed to ensure uniformity and reliability in the subsequent analyses and experiments.

3.2 Leaf skeleton preparation steps

Once leaves were collected, they were rinsed with water to remove any dirt or dust particles. This ensured that the leaves were clean and ready for further experimentation or analysis. The following steps outline the process of preparing NaOH-treated leaf skeletons (Chen et al.2021).

- A 5% NaOH solution was prepared by dissolving NaOH pellets or flakes in distilled water. Proper safety precautions, such as wearing gloves and goggles, were ensured while handling NaOH due to its caustic nature.

- A pot of water was brought to a boil, and the prepared 5% NaOH solution was added. The collected leaves were submerged into the boiling solution and boiled until softening occurred. The time required for softening each leaf was noted.
- The softened leaves were removed carefully from the boiling solution using tongs. Then, the leaves were thoroughly rinsed under running distilled water to remove any residual NaOH solution or debris.
- Using a soft-bristled brush, the surface of each leaf was gently scrubbed to break through the waxy layer and remove superficial tissue. Brushing continued until the leaf skeleton was fully exposed, with care taken not to damage the delicate veins.
- The cleaned leaf skeletons were rinsed once again with distilled water to ensure complete removal of any remaining debris or chemicals.
- The cleaned leaf skeletons were placed on paper towels or a clean, dry surface to air dry completely. Direct sunlight or heat sources that may cause excessive drying or damage to the specimens were avoided

3.3 Leaf-vein architectural characteristics

Leaf laminar characters and vein architectural characters such as vein order, vein category, spacing, etc were noted (Ash and Amanda, 1999).

3.4 Coloring of Skeletonised leaves

The leaves were also subjected to colouration using natural dyes (Křížová, 2015). The skeleton of the leaves were naturally coloured using the flowers and plant that we collected. Bougainvillea flower, turmeric, Indian blackberry and also the miss of bougainvillea and turmeric. Bougainvillea extract gave us the red color for the skeleton.

Turmeric gave yellow, Indian blackberry the purple color and lactophenol cotton gave the blue color for skeletons. The mix of red and yellow by bougainvillea and turmeric helped us to get an orange color for the skeleton. After coloring they were kept in a tissue for air dry and to remove the water content in the skeleton.

3.6 Preservation of Leaf skeleton

The air dried colored leaf skeletons were preserved with the help of glycerin. The leaf skeletons were dipped in the glycerin for preservation. After that they were kept for air dry in a tissue. The dried leaf skeleton showed a glazing texture hence it was dipped in the glycerin for preservation.

CHAPTER 4

RESULT

An examination of leaf samples from the BMC campus reveals a diverse plant community across 10 families. The Moraceae family (Peepal & Banyan trees) showcases unique shapes (cordate & broadly ovate) and a smooth, glossy texture. The Fabaceae family (Rain & Golden shower trees) exhibits pinnate and paripinnately compound leaves with varying textures (dull/hairy underside to smooth). The Annonaceae family's False Ashoka has distinct oblong narrow leaves with wavy margins, maintaining a smooth, glossy texture (Table 1). Morphological variation of leaves are shown in Figure 1.

Table 1. Details of Scientific name , Family and Morphology of Collected Leaves

Sl No	Common name	Scientific name	Family	Morphology	Texture
1	Peepal tree	*Ficus religiosa*	Moraceae	Cordate shape, extended drip tip, and size 10.5–17 cm long, 7–12 cm broad	Smooth and durable
2	Cannon ball tree	*Couroupita guianensis*	Lecythidaceae	oblong or broadly lance-shaped. The leaf margins can vary, being either smooth or finely toothed.5 - 35 cm long , 4 - 20 cm broad	Smooth and soft
3	Queen's crape	*Lagerstroemia speciosa*	Lythraceae	Oval to elliptic with stout petiole, acute apex 8–14 cm 4–7 cm broad	smooth and glossy upper surface, lower surface rougher
4.	Champak	*Magnolia champaca*	Magnoliaceae.	Ovate to oblong leaves. Stout petiole ,acute apex, 8–20 cm length and 4–7 cm broad	Thin leathery, glabrous, lower surface pubescent

5.	Rain tree	*Samanea saman*	Fabaceae	Pinnate leaf, 5-16 leaflets. leaflet has diamond shaped 2.2-4 cm long and 1.2-3.2 cm broad	Dull top surface and finely hairy beneath
6.	Golden shower	*Cassia fistula*	Fabaceae	Ovate leaflets, spirally arranged, paripinnately compound, 30-40 cm long and 2-5 cm broad	Medium thin and smooth
7.	Ashoka tree	*Saraca asoka*	Fabaceae	Alternate, paripinnate, copper red when young and green when mature and 30-60 cm long	Glossy, Smooth
8.	Banyan tree	*Ficus benghalensis*	Moraceae	Broadly ovate, obtuse, base cordate; lamina 10-30 cm long and 7- 20 cm wide, 1.5-7 cm long and 5 mm wide	Leathery, Glossy, Coarse
9.	Ixora	*Ixora coccinea*	Rubiaceae	Oblong leaves, 4 in (10cm) long with entire margins and carried in whorls	Glossy and leathery
10	False Ashoka	*Polyalthia longifolia*	Annonaceae	Oblong narrow leaves 10-15 cm long and 4-7 cm breadth with wavy margins.	Smooth and glossy
11	Jamun	*Syzygium cumini*	Myrtaceae	oblong or elliptic shape with a pointed tip. 5-15 cm long and 2-4 cm breadth	Smooth, glossy, robust and durable
12	Guava	*Psidium guajava*	Myrtaceae	Elliptic to oval 5-15 cm long and 3-7 cm breadth	Smooth, leathery, robust
13	Curved Flower Chassalia	*Chassalia curviflora*	Rubiaceae	Lanceolate, 3-8 cm long and 3-4 breadth	Smooth , glossy, thin and delicate
14	Yellow Tabebuia.	*Tabebuia aurea*	Bignoniaceae	Each leaflet is lanceolate 5-10 cm long and 1-3 cm breadth smooth margins.	Smooth, glossy, hard
15	Spinous Kino Tree	*Bridelia retusa*	Phyllanthaceae	Elliptic to ovate-oblong in shape 5-10 cm cm long and 3-7 breadth	Smooth leathery and robust

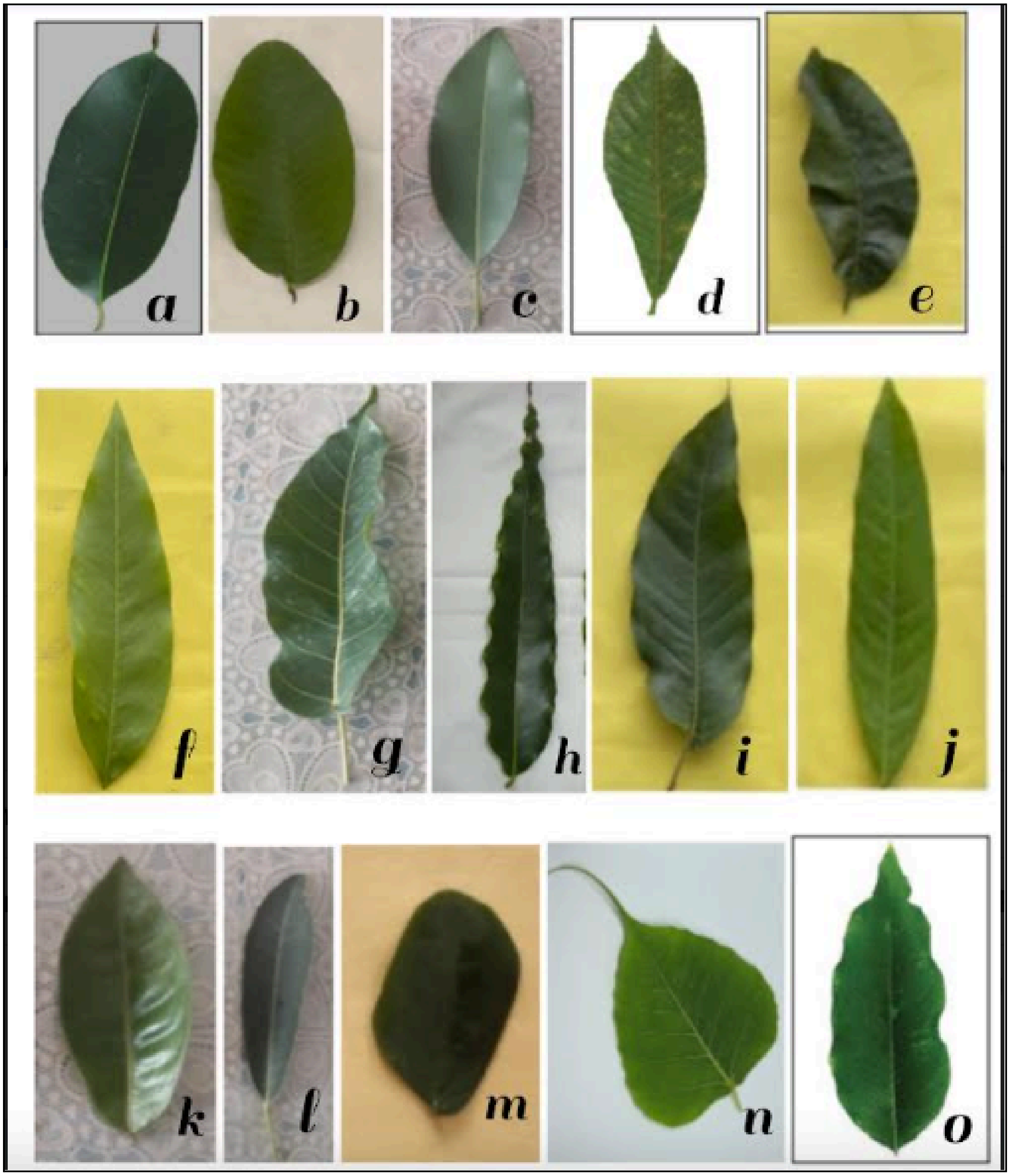

Figure 1. Morphological Variations of the Studied Leaves a. *Syzygium cumini,*b.*Psidium guajava,*c. *Bridelia retusa,*d. *Couroupita guianensis, e. Chassalia curviflora,* f. *Lagerstroemia speciosa,* g. *Ficus benghalensis,*h. *Polyalthia longifolia.*i. *Magnolia champaca,* j. *Saraca asoka, k. Ixora coccinea,* l. *Tabebuia aurea,* m. *Samania saman, n. Ficus religiosa,*o. *Cassia fistula.*

4.2 Leaf Skeletonization

Leaf skeletonization is a method used in studying plants to reveal their internal structures, particularly the veins and framework within leaves. In this study, we examine the effects of treating 15 plant species with a 5% NaOH solution to soften their tissues and produce leaf skeletons (Figure 2).Table 2 summarizes experiments where we treated 15 different plant leaves with a solution made of 5% NaOH to see how well their tissues softened and turned into skeletons. We varied the treatment times from 1 to 5 minutes. Most leaves, like the Peepal tree and Ashoka tree, softened well and became skeletons, with percentages ranging from 92% to 100%. But some, like the False Ashoka and Guava, only slightly softened, with percentages of 20% and 40% respectively. A few, like the Jamun and Guava, barely softened at all, and their tissues remained mostly unchanged. One leaf, the Curved Flower Chassalia, completely fell apart after just 1 minute. Two other leaves, Yellow Tabebuia and Spinous Kino Tree, didn't show any changes even after 5 minutes of treatment (Figure 3)

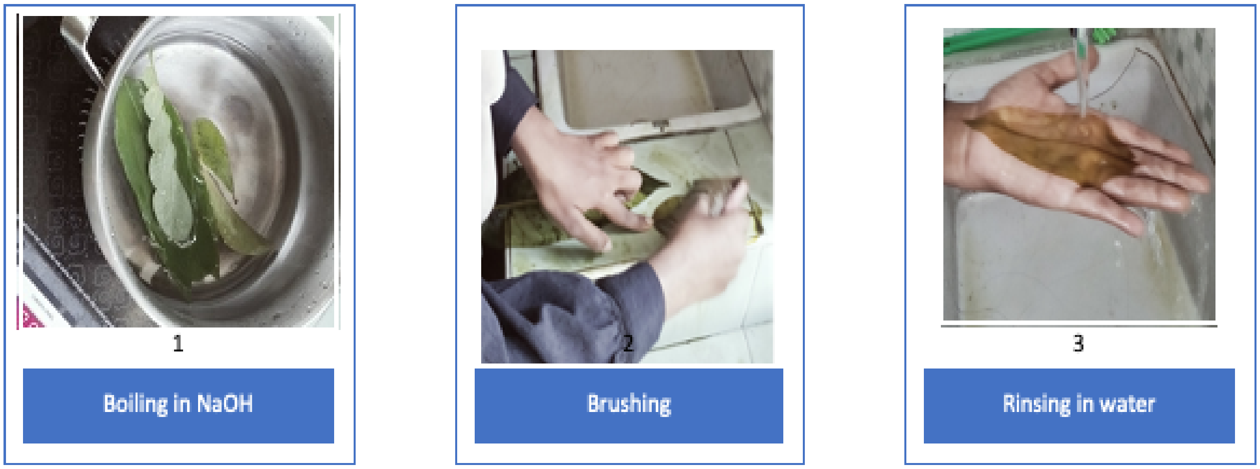

Figure 2. Steps involved in leaf skeletonisation

Table 2: Results of Leaf Skeletonization

Sl No	Plant Leaf name	Treatment time in 5% NaOH for skeletonisation	Quality of Tissue	Quality of leaf skeleton (in %)
1	*Ficus religiosa*	1 minute	Softened	98%
2	*Couroupita guianensis*	5 minute	Softened	92%
3	*Lagerstroemia speciosa*	1 minute	Softened	98%
4	*Magnolia champaca*	2 minute	Softened	98%
5	*Samania saman*	1 minute	Softened	97%
6	*Cassia fistula*	1 minute	Softened	99%
7	*Saraca asoka*	5 minute	Softened	95%
8	*Ficus benghalensis*	1 minute	Softened	100%
9	*Ixora coccinea*	1 minute	Softened	100%
10	*Polyalthia longifolia*	5 minute	Softened	20%
11	*Syzygium cumini*	5 minutes	Slightly softened	2%
12	*Psidium guajava*	5 minutes	Slightly softened	40%
13	*Chassalia curviflora*	1 minutes	Disintegrated	-
14	*Tabebuia aurea*	5 minutes	No change	-
15	*Bridelia retusa*	5 minutes	No change	-

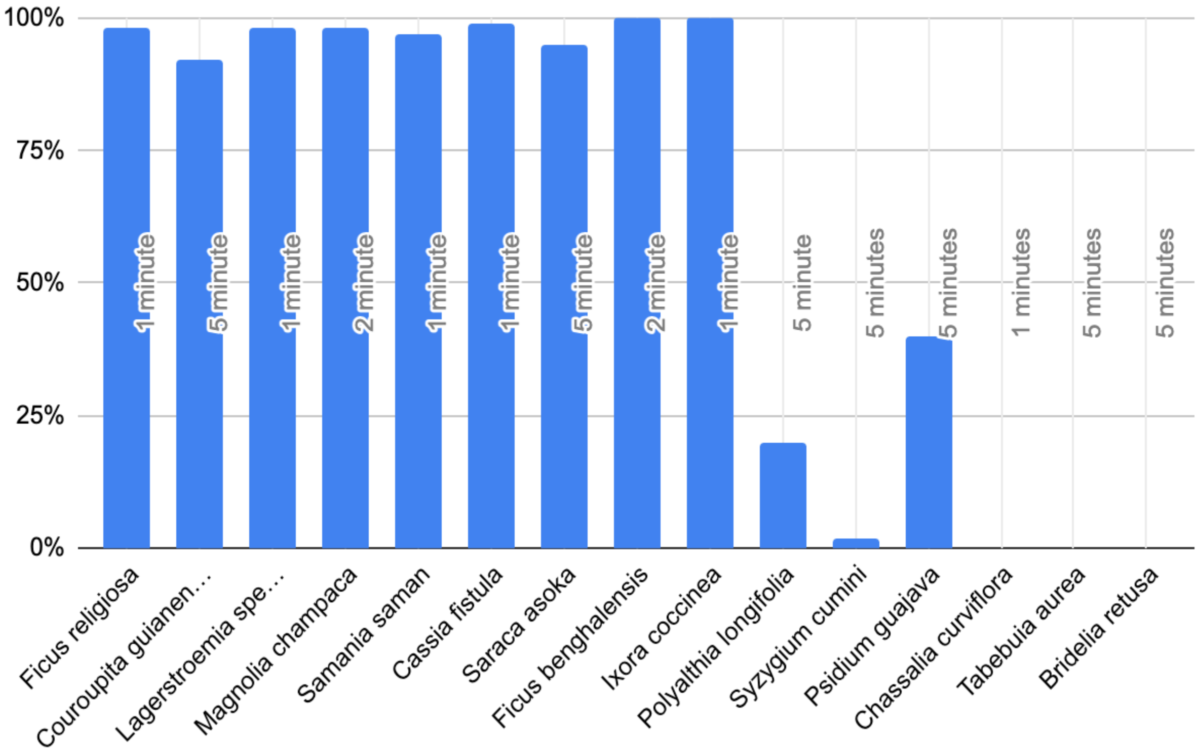

Figure 3: Comparative Analysis of Leaf Skeletonization

4.3 Laminar details of the Skeletonised leaves

The comparative analysis of the leaf characteristics of the plant vein structure, and other key attributes of skeletonised leaves were observed. The studied plant species shared similarities in leaf characteristics. For instance, *Ficus religiosa, Lagerstroemia speciosa, Cassia fistula,* and *Ficus benghalensis* all exhibited ovate leaves with symmetrical laminar symmetry. *Couroupita guianensis* and *Magnolia champaca* displayed elliptic leaves with symmetrical symmetry. Samanea saman and Ixora coccinea both showed leaves with moderately asymmetrical symmetry. *Saraca asoka* displayed oblong leaves with symmetrical symmetry. These similarities provide insights into the diverse yet interconnected nature of leaf morphology among different plant species (Table 3)

Table 3 Laminar details of the Skeletonised leaves

SL no:	Name of the plant	Laminar shape	Laminar symmetry	Base shape	Apex shape	Margin Type
1.	*Ficus religiosa*	Ovate	Symmetrical	Cordate	Acuminate	Entire
2.	*Couroupita guianensis*	Elliptic	Symmetrical	Rounded	Convex	Erose
3.	*Lagerstroemia speciosa*	Ovate	Symmetrical	Oval	Truncate	Erose
4.	*Magnolia champaca*	Elliptic	Symmetrical	Decurrent	Acuminate	Erose
5.	*Samanea saman*	Ovate	Moderately aymmetrical	Oval	Convex	Erose
6.	*Cassia fistula*	Ovate	Symmetrical	Cuneate	Convex	Erose
7.	*Saraca asoka*	Oblong	Symmetrical	Complex	Convex	Erose
8.	*Ficus benghalensis*	Ovate	Symmetrical	Truncate	Convex	Revolute
9.	*Ixora coccinea*	Elliptic	Symmetrical	Cuneate	Convex	Entire

4.4 Vein Order details

Our study reveals varied leaf vein patterns and orders, aligning with past findings showing most angiosperm leaves have four to seven vein orders (Figure 4) . Veins follow a hierarchical structure, with smaller ones branching from larger ones. As veins advance, there's a decrease in cell size and number, except for companion cells in minor veins, which enlarge to support phloem loading. This suggests an efficient distribution system within leaves . The result consistent with prior research indicating most angiosperm leaves have four to seven vein orders (Sack et al., 2012).

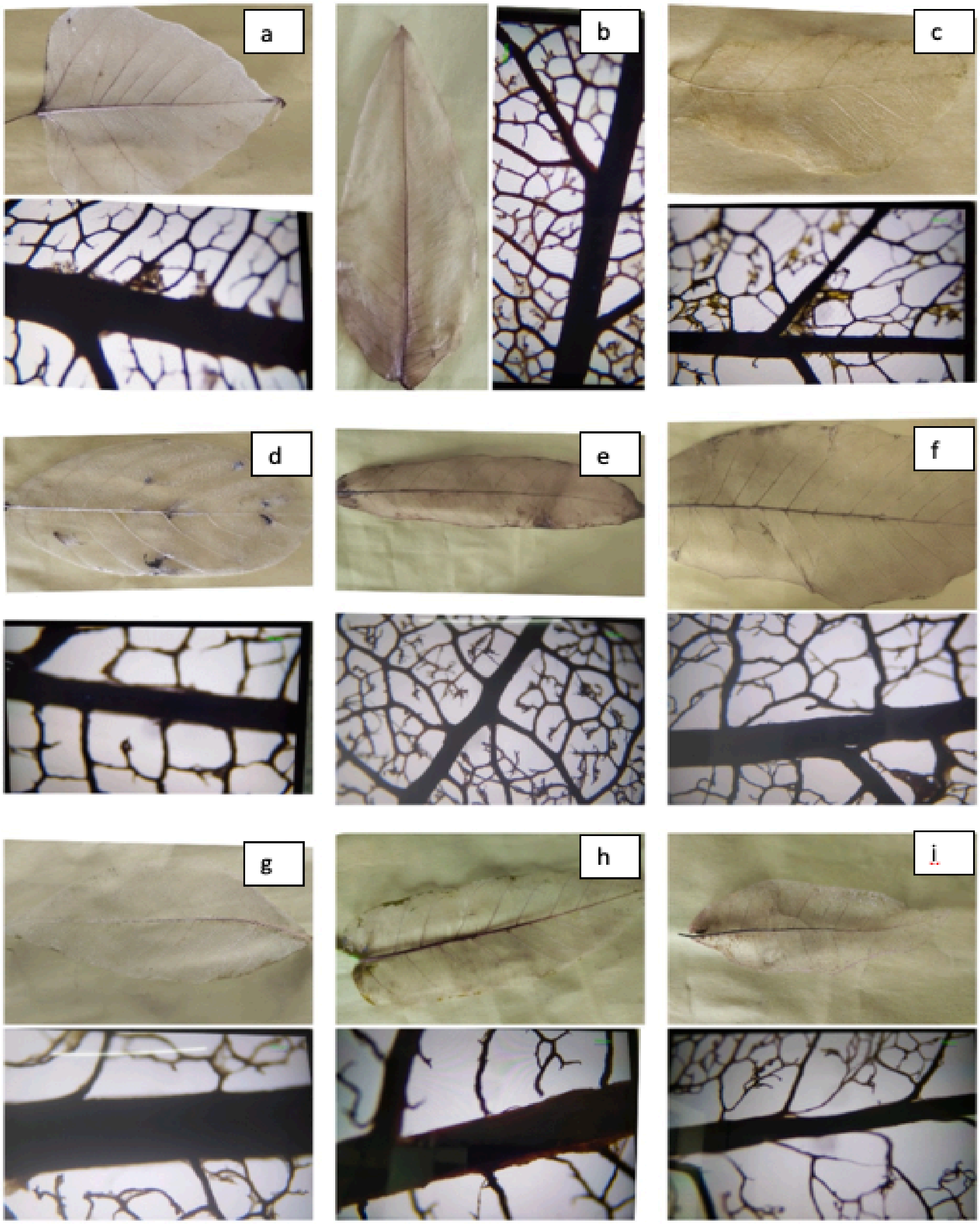

Figure 4. Leaf skeleton and vein orders

4.5 Coloring of Leaf skeleton

Leaf skeletons, with their intricate vein patterns, offer a captivating platform for artistic endeavors. In our study, we explored the use of natural and synthetic dyes to add color to these delicate structures. Table 5 outlines coloration quality, and Figure 4 compares aesthetic appeal using various dyeing agents.

Table 5 Evaluation of Dyeing Agents for Leaf Skeletons

Dying agent	Imparting colour	Color Vibrancy	Uniformity	Rinse Test	Overall Appeal (%)
Bougainvillea	Magenta	Vibrant	Even	Retained colour	98
Turmeric	Yellow	Vibrant	Even	Retained colour	100
Jamun fruit	Purple	Vibrant	Uneven	Removed slightly	78
Mixture of Bougainvillea and Turmeric	Orange	Vibrant	Even	Removed slightly	65
Safranin	Red	Vibrant	Even	Retained colour	100
Lactophenol cotton blue	Blue	Vibrant	Even	Retained colour	98
Jamun leaf	Brown	Vibrant	Even	Retained colour	100

4.6 Preservation of leaf skeletons for decoration

The preservation process for decoration involved immersing the skeletonized leaves in a glycerin-water solution, which rendered them translucent and flexible, indicating successful preservation. Subsequent drying in a hot air oven maintained the leaves' shape, translucency, and flexibility without any visible damage or discoloration, ensuring their suitability for decorative purposes (Figure 6) .

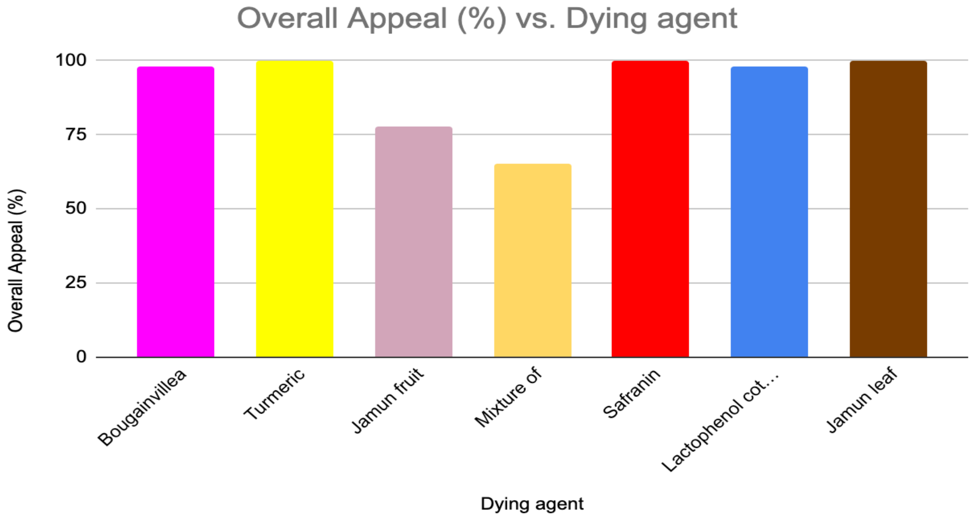

Figure 5. Effect of Dyeing Agent on Overall Appeal of Leaf Skeletons (%)

Figure 6 Coloured and preserved leaves

CHAPTER 5

DISCUSSION

The detailed study of 15 plant leaves provided valuable insights into their morphological, anatomical, and venation characteristics, aligning with the hierarchical vein system described by Sack et al. (2012). Each plant species exhibited unique features contributing to its adaptive strategies for survival. For instance, species like the Peepal tree (Ficus religiosa) and Ashoka tree (Saraca asoka) exhibited smooth and glossy leaves with symmetrical laminar symmetry, likely reflecting a well-developed hierarchical vein system facilitating efficient water and sugar transport (Sack et al., 2012). This hierarchical organization, characterized by smaller diameters but greater branching frequencies and lengths in veins of higher branching orders, was evident across the studied species. Comparative analysis of skeletonized leaves highlighted similarities in leaf characteristics among species. For instance, species like *Ficus religiosa, Lagerstroemia speciosa*, and *Cassia fistula* shared ovate leaves with symmetrical laminar symmetry, suggesting common evolutionary adaptations. This observation aligns with the general scaling across angiosperm species and correlations among vein diameters reported by Scoffoni et al. (2011) and Sack et al. (2012).

The leaf skeletonization process using 5% NaOH revealed varying degrees of effectiveness across species, which can be linked to their vein hierarchy and tissue composition. Some species achieved complete skeletonization within a short time, while others required longer treatment times or showed resistance to the NaOH treatment (Hättenschwiler et al., 2005).

CHAPTER 6

SUMMARY AND CONCLUSION

This study examined the leaves of 15 plant species belongs to 10 botanical families from the BMC campus, focusing on their morphology, anatomy, and vein structure. Leaf characteristics were documented before and after a skeletonization process using 5% sodium hydroxide (NaOH). The skeletonized leaves were then dyed and evaluated for their potential use in decorative applications.

Conclusive points

- The study revealed a diverse array of leaf shapes, textures, and vein patterns among the examined species.
- Skeletonization with 5% NaOH was successful for most species, although effectiveness varied depending on the plant's vein hierarchy and tissue composition.
- The hierarchical vein system observed aligns with previous findings on angiosperms .
- Species with similar leaf shapes (e.g., ovate with symmetrical laminae) might share evolutionary adaptations related to water and sugar transport.
- Natural and synthetic dyes can be effectively used to color leaf skeletons for artistic purposes.

- A glycerin-water solution successfully preserved the skeletonized leaves for decoration, maintaining their shape, translucency, and flexibility.

Limitations of the study are small sample size and potential method variations.

Future Scope

This study observed similarities in vein patterns among certain species. Future research could involve detailed quantitative analysis of the vein networks using image processing techniques. This could reveal more specific relationships between leaf morphology and vein structure. Understanding the relationship between vein structure and leaf morphology could have ecological applications. In future this study can pave the way for a deeper understanding of leaf diversity, vein networks, and the potential applications of leaf skeletonization techniques.

REFERENCE

1. Ash and Amanda (1999). Manual of Leaf Architecture - morphological description and categorization of dicotyledonous and net-veined monocotyledonous angiosperms by Leaf Architecture Working Group. 65p
2. Badron, U. H., Talip, N., Mohamad, A. L., Affenddi, A. E. A., & Juhari, A. A. A. (2014). Studies on leaf venation in selected taxa of the genus Ficus L.(Moraceae) in peninsular Malaysia. *Tropical life sciences research*, *25*(2), 111.
3. Bolhar-Nordenkampf, H. R., & Draxler, G. (1993). Functional leaf anatomy. In *Photosynthesis and production in a changing environment: A field and laboratory manual* (pp. 91-112). Dordrecht: Springer Netherlands.
4. Chen, H., Wu, J., Shi, J., Zhang, W., & Wang, G. (2021). Strong and highly flexible slivers prepared from natural bamboo culm using NaOH pretreatment. *Industrial Crops and Products*, *170*, 113773.
5. Ferreira, V., Elosegi, A., D. Tiegs, S., von Schiller, D., & Young, R. (2020). Organic matter decomposition and ecosystem metabolism as tools to assess the functional integrity of streams and rivers–a systematic review. *Water*, *12*(12), 3523.
6. Hättenschwiler, S., Tiunov, A. V., & Scheu, S. (2005). Biodiversity and litter decomposition in terrestrial ecosystems. *Annu. Rev. Ecol. Evol. Syst.*, *36*, 191-218.
7. Křížová, H. (2015). Natural dyes: their past, present, future and sustainability. *Recent Developments in Fibrous Material Science. Prague: Kosmas Publishing*, 59-71.

8. Llano KM, Haedo AS, Gerchenson LN, Rojas AM. Mechanical and biochemical response of kiwifruit tissue to steam blanching. *Food Res Int.* 2003;36:767–75\
9. Mir, S., & Jana, M. M. (2015). Preparation of leaf venation skeletons of leaves for dry flower arrangement. *Current Horticulture*, *3*(1), 53-56.
10. Periasamy, V. S., Athinarayanan, J., & Alshatwi, A. A. (2020). Bio-inspired plant leaf skeleton based three dimensional scaffold for three dimensional cell culture. *Sustainable chemistry and pharmacy*, *18*, 100321.
11. Sack, L., & Scoffoni, C. (2013). Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New phytologist*, *198*(4), 983-1000.
12. Sack, L., & Scoffoni, C. (2013). Leaf venation: structure, function, development, evolution, ecology and applications in the past, present and future. *New phytologist*, *198*(4), 983-1000.
13. Sharma, V., Jääskö, K., Yiannacou, K., Koivikko, A., Lampinen, V., & Sariola, V. (2022). Performance comparison of fast, transparent, and biotic heaters based on leaf skeletons. *Advanced Engineering Materials*, *24*(9), 2101625.
14. Sharma, V., Jääskö, K., Yiannacou, K., Koivikko, A., Lampinen, V., & Sariola, V. (2022). Performance comparison of fast, transparent, and biotic heaters based on leaf skeletons. *Advanced Engineering Materials*, *24*(9), 2101625.