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QUANTIFICATION OF ANTHOCYANIN PIGMENTS IN ACER PALMATUM 'ATROPURPUREUM'-UV-VIS SPECTROMETRY

BY

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Dedication

I humbly dedicate this research work to my daughter Kimberly and two sons Clinton Kyle Khaka.

Abbreviations

DMF - Dimethylformamide

UV - Ultraviolet

Vis – Visible

Abstract

The aim of this research is to develop a reliable UV-Vis spectroscopic method for the quantitative determination of chlorophyll content in the leaves of Acer palmatum 'Atropurpureum', with the goal of gaining a deeper understanding of the factors that influence the accumulation of these pigments in this plant species. The experimental design was done and leaves of Acer palmatum 'Atropurpureum' were collected from a local nursery located in Bindura, Mashonaland Central, Zimbabwe and the chronological sequences of the experiment were followed. The results of the chlorophyll extraction and quantification demonstrate the effectiveness of the UV-Vis spectroscopic method for determining the concentrations of chlorophyll a and chlorophyll b in the Acer palmatum 'Atropurpureum' leaf samples.

Three different extraction solvents, acetone, ethanol, and dimethylformamide (DMF), were evaluated, and the results showed that acetone was the most effective solvent for extracting and quantifying the chlorophyll pigments. From this research, conclusion have been drawn from tangible evidence and calibration curves were constructed for chlorophyll A and chlorophyll B using standard solutions in the respective extraction solvents (acetone, ethanol, and DMF). The absorbance values at the specific wavelengths (662 nm for chlorophyll A and 645 nm for chlorophyll B) were plotted against the known concentrations of the chlorophyll standards. The total chlorophyll content in the Acer palmatum 'Atropurpureum' leaves ranged from 2.37 mg/g dry weight (ethanol) to 2.55 mg/g dry weight (acetone), with the acetone extract showing the highest chlorophyll content.

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CHAPTER 1: INTRODUCTION

The study of plant pigments has been a fundamental aspect of plant biology and chemistry, as these compounds play crucial roles in various physiological processes and have potential applications in diverse industries (Tanaka et al., 2008). Among the various pigments found in plants, chlorophyll, the green-colored tetrapyrrole compound, is perhaps the most well-known and extensively studied (Lichtenthaler, 1987). Chlorophyll is the primary pigment responsible for the distinctive green hue of plant leaves, and it is essential for the process of photosynthesis, which is the foundation of life on Earth (Taiz & Zeiger, 2010).

The quantification of chlorophyll content in plant tissues has been an important area of research, as it provides valuable insights into the physiological status and health of the plant (Hendry & Price, 1993). Chlorophyll content can serve as an indicator of various environmental stresses, such as drought, nutrient deficiency, and pathogen attack, which can affect the plant's photosynthetic efficiency and overall productivity (Gitelson & Merzlyak, 1997). Additionally, the quantification of chlorophyll content has applications in precision agriculture, remote sensing, and the development of bioindicators for environmental monitoring (Ustin et al., 2009; Jiang et al., 2017).

One plant species that has garnered significant attention for its unique pigmentation is the Japanese Maple (Acer palmatum), particularly the 'Atropurpureum' cultivar, which is characterized by its deep burgundy-red foliage (Jiang et al., 2021). The striking color of the 'Atropurpureum' cultivar is primarily due to the accumulation of high concentrations of anthocyanin pigments in the leaves (Gupta et al., 2022). However, despite the prevalence of these vibrant anthocyanins, the 'Atropurpureum' cultivar also contains substantial amounts of chlorophyll, which plays a crucial role in the plant's photosynthetic processes and overall growth and development (Saito & Doi, 1995).

The quantification of chlorophyll content in Acer palmatum 'Atropurpureum' is essential for several reasons. First, it provides a better understanding of the plant's photosynthetic capacity and how it is influenced by various environmental and developmental factors (Jiang et al., 2021). This information can have important implications for the cultivation and management of this ornamental plant species, as growers and horticulturists strive to maintain optimal growth and appearance (Gupta et al., 2022). Second, the characterization of chlorophyll content in Acer palmatum 'Atropurpureum' may lead to the identification of unique

chlorophyll profiles or pigment interactions, which could have potential applications in the food, cosmetic, or pharmaceutical industries (Giusti & Wrolstad, 2003). Finally, the study of chlorophyll in this plant species can contribute to the broader understanding of the factors that regulate pigment biosynthesis and accumulation in plants, which is a fundamental aspect of plant biology (Gould, 2004).

One of the most widely used analytical techniques for the quantification of chlorophyll is UV-Vis (ultraviolet-visible) spectroscopy, which relies on the characteristic absorbance properties of these pigments (Inskeep & Bloom, 1985). This method is relatively simple, cost-effective, and provides a reliable way to quantify the total chlorophyll content in plant extracts (Porra et al., 1989). However, the accurate quantification of chlorophyll using UV-Vis spectroscopy can be challenging, as these pigments can exist in multiple forms, each with different absorbance characteristics, and can be influenced by various factors, such as pH, temperature, and the presence of other compounds (Wellburn, 1994).

1.1 Aims and objectives

1.1.1 Aim

- The aim of this study is to develop a reliable UV-Vis spectroscopic method for the quantitative determination of chlorophyll content in the leaves of Acer palmatum 'Atropurpureum', with the goal of gaining a deeper understanding of the factors that influence the accumulation of these pigments in this plant species.

1.1.2 The objectives of this study are:

- To develop and optimize a reliable extraction and quantification method for chlorophyll in Acer palmatum 'Atropurpureum' leaves using UV-Vis spectroscopy.
- To investigate the effects of various environmental and developmental factors on the chlorophyll content in Acer palmatum 'Atropurpureum' leaves.
- To characterize the chlorophyll profile of Acer palmatum 'Atropurpureum' and identify any unique or novel pigment components.

- To evaluate the potential applications of the quantified chlorophyll from Acer palmatum 'Atropurpureum' in various industries, such as food, cosmetics, and pharmaceuticals.

The following sections will provide a detailed overview of the research conducted to achieve these objectives.

1.2 Importance of Chlorophyll Quantification in Plants

The quantification of chlorophyll content in plant tissues has been a crucial area of research, as it provides valuable insights into the physiological status and health of the plant (Hendry & Price, 1993). Chlorophyll is the primary pigment responsible for the distinctive green hue of plant leaves and is essential for the process of photosynthesis, which is the foundation of life on Earth (Taiz & Zeiger, 2010). The measurement of chlorophyll content can serve as an important indicator of various environmental stresses, such as drought, nutrient deficiency, and pathogen attack, which can affect the plant's photosynthetic efficiency and overall productivity (Gitelson & Merzlyak, 1997).

In addition to its use as a physiological indicator, the quantification of chlorophyll content has applications in precision agriculture, remote sensing, and the development of bioindicators for environmental monitoring (Ustin et al., 2009; Jiang et al., 2017). By assessing the chlorophyll levels in plants, researchers and practitioners can gain insights into the overall health and growth status of crops, forests, and other vegetation, which can inform management decisions and help optimize agricultural practices (Gitelson & Merzlyak, 1997).

Furthermore, the characterization of chlorophyll content in plant species can lead to the identification of unique pigment profiles or interactions, which could have potential applications in various industries, such as food, cosmetics, and pharmaceuticals (Giusti & Wrolstad, 2003). For example, the extraction and purification of chlorophyll from plant sources has been explored for its use as a natural colorant or as a source of bioactive compounds with potential health benefits (Ferruzzi & Blakeslee, 2007; Gross, 1991).

1.3 Acer palmatum 'Atropurpureum': A Unique Blend of Pigments

The Japanese Maple (Acer palmatum) is a deciduous tree species native to Japan, China, and Korea, known for its striking foliage colors, particularly in the autumn (Jiang et al., 2021). The 'Atropurpureum' cultivar of Acer palmatum is characterized by its deep burgundy-red leaves, which is primarily due to the accumulation of high concentrations of anthocyanin pigments (Gupta et al., 2022).

Picture 1: 'Atropurpureum'

While the 'Atropurpureum' cultivar is renowned for its vibrant anthocyanin content, it also contains substantial amounts of chlorophyll, which plays a crucial role in the plant's photosynthetic processes and overall growth and development (Saito & Doi, 1995). The co-existence of these two pigment classes, chlorophyll and anthocyanins, in the leaves of Acer palmatum 'Atropurpureum' creates a unique and visually striking blend of colors, making this cultivar a popular choice for ornamental landscaping and garden design (Jiang et al., 2021).

The quantification of chlorophyll content in Acer palmatum 'Atropurpureum' is essential for several reasons:

Understanding Photosynthetic Capacity: Measuring the chlorophyll content in the leaves of Acer palmatum 'Atropurpureum' can provide insights into the plant's photosynthetic capacity and how it is influenced by various environmental and developmental factors (Jiang et al., 2021). This information can have important implications for the cultivation and management

of this ornamental plant species, as growers and horticulturists strive to maintain optimal growth and appearance (Gupta et al., 2022).

Identifying Unique Pigment Profiles: The characterization of chlorophyll content in Acer palmatum 'Atropurpureum' may lead to the identification of unique chlorophyll profiles or pigment interactions, which could have potential applications in the food, cosmetic, or pharmaceutical industries (Giusti & Wrolstad, 2003). The co-occurrence of chlorophyll and anthocyanins in the leaves of this cultivar presents an opportunity to explore the interplay between these pigments and their potential synergistic or complementary effects.

Contributing to Plant Biology Research: The study of chlorophyll in Acer palmatum 'Atropurpureum' can contribute to the broader understanding of the factors that regulate pigment biosynthesis and accumulation in plants, which is a fundamental aspect of plant biology (Gould, 2004). Investigating the chlorophyll content in this unique plant species may provide valuable insights into the complex mechanisms governing pigment metabolism and distribution within plant tissues.

1.4 UV-Vis Spectroscopy for Chlorophyll Quantification

One of the most widely used analytical techniques for the quantification of chlorophyll is UV-Vis (ultraviolet-visible) spectroscopy, which relies on the characteristic absorbance properties of these pigments (Inskeep & Bloom, 1985). This method is relatively simple, cost-effective, and provides a reliable way to quantify the total chlorophyll content in plant extracts (Porra et al., 1989).

The principle of UV-Vis spectroscopy for chlorophyll quantification is based on the ability of these pigments to absorb specific wavelengths of light, which can be measured using a spectrophotometer. Chlorophyll a and chlorophyll b have distinct absorbance maxima, typically around 660-665 nm and 640-645 nm, respectively, which allows for their differentiation and quantification (Wellburn, 1994).

However, the accurate quantification of chlorophyll using UV-Vis spectroscopy can be challenging, as these pigments can exist in multiple forms, each with different absorbance characteristics, and can be influenced by various factors, such as pH, temperature, and the presence of other compounds (Wellburn, 1994). Additionally, the co-occurrence of other pigments, such as the vibrant anthocyanins found in Acer palmatum 'Atropurpureum', can interfere with the accurate measurement of chlorophyll content (Gould, 2004).

CHAPTER 2: LITERATURE REVIEW

2.1 Chlorophyll: Structure, Biosynthesis, and Biological Functions

Chlorophyll is a group of green-colored tetrapyrrole compounds that are essential for the process of photosynthesis in plants, algae, and certain bacteria (Tanaka & Tanaka, 2006). These pigments are responsible for the distinctive green hue of plant leaves and play a crucial role in the conversion of light energy into chemical energy, which is the foundation of life on Earth (Taiz & Zeiger, 2010).

2.1.1 Chlorophyll Structure and Diversity

The two main forms of chlorophyll found in higher plants are chlorophyll a and chlorophyll b, which differ in the structure of their side chains (Lichtenthaler, 1987). Chlorophyll a contains a methyl group (CH3-) at the C-3 position, while chlorophyll b contains an aldehyde group (CHO-) at the same position (Hendry & Price, 1993). This structural difference results in slight variations in the absorbance spectra of the two chlorophyll forms, with chlorophyll a having absorbance maxima around 660-665 nm and chlorophyll b having absorbance maxima around 640-645 nm (Wellburn, 1994).

In addition to the primary chlorophyll a and b forms, there are several other chlorophyll-related compounds that can be found in plants, including chlorophyll c, d, and f, as well as various chlorophyll derivatives and degradation products (Tanaka & Tanaka, 2006). These additional chlorophyll forms are more commonly found in algae and certain photosynthetic bacteria, but they can also be present in small quantities in higher plants (Gross, 1991).

The structural diversity of chlorophyll compounds is the result of variations in the central metal ion, the degree of saturation of the tetrapyrrole ring system, and the nature of the side chains (Tanaka & Tanaka, 2006). These structural differences can affect the physical and chemical properties of the pigments, such as their solubility, stability, and absorbance characteristics, which in turn can influence their biological functions and potential applications (Gross, 1991).

2.1.2 Chlorophyll Biosynthesis

The biosynthesis of chlorophyll in plants is a well-studied process that involves a complex series of enzymatic reactions, collectively known as the tetrapyrrole pathway (Tanaka & Tanaka, 2006). The pathway begins with the conversion of the amino acid glutamic acid into

5-aminolevulinic acid, which is then further converted into various intermediate compounds, ultimately leading to the production of chlorophyll (Beale, 1999).

The specific enzymes and regulatory mechanisms involved in chlorophyll biosynthesis have been the subject of extensive research, as they play a crucial role in the plant's photosynthetic capacity and overall growth and development (Alawady & Grimm, 2005). The tetrapyrrole pathway is highly regulated, with various environmental and developmental factors, such as light, temperature, and plant hormones, influencing the expression and activity of the enzymes involved (Masuda, 2008).

One of the key steps in chlorophyll biosynthesis is the conversion of protoporphyrin IX into magnesium protoporphyrin IX, which is catalyzed by the enzyme magnesium chelatase (Masuda, 2008). This step is considered a rate-limiting step in the pathway and is regulated by various environmental and developmental signals, such as light and plastid development (Tanaka & Tanaka, 2006).

Another important step in chlorophyll biosynthesis is the conversion of chlorophyllide a into chlorophyll a, which is catalyzed by the enzyme chlorophyll synthase (Tanaka & Tanaka, 2006). This final step in the pathway involves the addition of a phytol tail to the chlorophyllide a molecule, resulting in the formation of the mature chlorophyll a compound (Beale, 1999).

The regulation of chlorophyll biosynthesis is a complex process that involves the coordinated expression and activity of numerous genes and enzymes, as well as the integration of various signaling pathways (Masuda, 2008). Understanding the molecular mechanisms underlying chlorophyll biosynthesis is an active area of research, as it has important implications for our understanding of plant physiology and the potential for manipulating chlorophyll levels in plant systems.

2.1.3 Biological Functions of Chlorophyll

Chlorophyll plays several vital roles in the physiological processes of plants, including photosynthesis, light harvesting, photoprotection, and signaling and development (Tanaka & Tanaka, 2006).

Photosynthesis: Chlorophyll is the primary pigment responsible for the absorption of light energy, which is then used to drive the process of photosynthesis, where carbon dioxide and water are converted into glucose and oxygen (Taiz & Zeiger, 2010). The chlorophyll molecules are organized within the thylakoid membranes of the chloroplasts, forming the

light-harvesting complexes that capture and funnel the absorbed light energy to the reaction centers of the photosynthetic apparatus (Blankenship, 2014).

Light Harvesting: The unique structure and absorption properties of chlorophyll molecules allow them to efficiently capture and transfer light energy to the photosynthetic reaction centers, where it is used to drive the light-dependent reactions of photosynthesis (Blankenship, 2014). This light-harvesting function is essential for the plant's ability to convert light energy into chemical energy in the form of ATP and NADPH, which are then used to fuel the carbon-fixation reactions of the Calvin cycle (Taiz & Zeiger, 2010).

Photoprotection: In addition to their role in light harvesting, chlorophyll and its associated carotenoid pigments can act as a photoprotective mechanism, dissipating excess light energy and preventing photodamage to the photosynthetic machinery (Demmig-Adams & Adams, 1992). This photoprotective function is particularly important under conditions of high light intensity or other environmental stresses that can lead to the generation of harmful reactive oxygen species (Hendry & Price, 1993).

Signaling and Development: Chlorophyll and its precursors may play a role in various plant growth and developmental processes, acting as signaling molecules that regulate gene expression and coordinate physiological responses (Tanaka & Tanaka, 2006). For example, the accumulation of chlorophyll intermediates, such as protochlorophyllide, has been linked to the regulation of seed germination and the de-etiolation of seedlings during the transition from a dark to a light environment (Masuda, 2008).

The diverse functions of chlorophyll highlight its importance in plant physiology and ecology, and have also led to a growing interest in its potential applications in various industries, such as food, cosmetics, and pharmaceuticals (Gross, 1991; Ferruzzi & Blakeslee, 2007).

2.2 Acer palmatum 'Atropurpureum': A Unique Blend of Pigments

The Japanese Maple (Acer palmatum) is a deciduous tree species native to Japan, China, and Korea, known for its striking foliage colors, particularly in the autumn (Jiang et al., 2021). The 'Atropurpureum' cultivar of Acer palmatum is characterized by its deep burgundy-red leaves, which is primarily due to the accumulation of high concentrations of anthocyanin pigments (Gupta et al., 2022).

2.2.1 Anthocyanins in Acer palmatum 'Atropurpureum'

Anthocyanins are a class of water-soluble flavonoid pigments that are responsible for the vivid red, purple, and blue hues observed in many plant organs, including flowers, fruits, and leaves (Castañeda-Ovando et al., 2009). These pigments have been the focus of extensive research due to their potential health benefits, such as antioxidant, anti-inflammatory, and neuroprotective properties (Tsuda, 2012).

In the case of Acer palmatum 'Atropurpureum', the deep burgundy-red color of the leaves is primarily due to the accumulation of high concentrations of anthocyanin pigments (Gupta et al., 2022). The specific anthocyanin profile of this cultivar has been the subject of several studies, which have identified the presence of various acylated and non-acylated anthocyanins, including cyanidin, delphinidin, and peonidin derivatives (Jiang et al., 2021).

The biosynthesis of these anthocyanin pigments in Acer palmatum 'Atropurpureum' is controlled by a complex network of transcription factors, enzymes, and regulatory mechanisms that are influenced by various environmental and developmental factors (Gupta et al., 2022). For example, the accumulation of anthocyanins in the leaves of this cultivar is strongly correlated with the onset of autumn, as the changing environmental conditions, such as decreasing temperatures and reduced daylight hours, trigger the upregulation of the anthocyanin biosynthetic pathway (Saito & Doi, 1995).

The vibrant anthocyanin pigments in Acer palmatum 'Atropurpureum' have made this cultivar a popular choice for ornamental landscaping and garden design, as the striking foliage color provides a visually striking contrast to the green-leaved forms of the species (Jiang et al., 2021). Additionally, the potential health benefits and antioxidant properties of the anthocyanins present in this plant have generated interest in the possible extraction and utilization of these pigments for various food, cosmetic, and pharmaceutical applications (Tsuda, 2012; Giusti & Wrolstad, 2003).

2.2.2 Chlorophyll in Acer palmatum 'Atropurpureum'

While the 'Atropurpureum' cultivar of Acer palmatum is renowned for its vibrant anthocyanin content, it also contains substantial amounts of chlorophyll, which plays a crucial role in the plant's photosynthetic processes and overall growth and development (Saito & Doi, 1995).

The co-existence of these two pigment classes, chlorophyll and anthocyanins, in the leaves of Acer palmatum 'Atropurpureum' creates a unique and visually striking blend of colors, as the deep burgundy-red hues of the anthocyanins are complemented by the green tones of the chlorophyll (Jiang et al., 2021). This pigment interaction is the result of complex regulatory mechanisms that govern the biosynthesis and accumulation of these compounds within the plant tissues.

The quantification of chlorophyll content in Acer palmatum 'Atropurpureum' is essential for several reasons:

Understanding Photosynthetic Capacity: Measuring the chlorophyll content in the leaves of Acer palmatum 'Atropurpureum' can provide insights into the plant's photosynthetic capacity and how it is influenced by various environmental and developmental factors (Jiang et al., 2021). This information can have important implications for the cultivation and management of this ornamental plant species, as growers and horticulturists strive to maintain optimal growth and appearance (Gupta et al., 2022).

Identifying Unique Pigment Profiles: The characterization of chlorophyll content in Acer palmatum 'Atropurpureum' may lead to the identification of unique chlorophyll profiles or pigment interactions, which could have potential applications in the food, cosmetic, or pharmaceutical industries (Giusti & Wrolstad, 2003). The co-occurrence of chlorophyll and anthocyanins in the leaves of this cultivar presents an opportunity to explore the interplay between these pigments and their potential synergistic or complementary effects.

Contributing to Plant Biology Research: The study of chlorophyll in Acer palmatum 'Atropurpureum' can contribute to the broader understanding of the factors that regulate pigment biosynthesis and accumulation in plants, which is a fundamental aspect of plant biology (Gould, 2004). Investigating the chlorophyll content in this unique plant species may provide valuable insights into the complex mechanisms governing pigment metabolism and distribution within plant tissues.

Despite the importance of understanding the chlorophyll content in Acer palmatum 'Atropurpureum', there is limited research on this topic, and the existing studies have primarily focused on the characterization of the anthocyanin pigments (Jiang et al., 2021; Gupta et al., 2022). This dissertation aims to address this gap in the literature by developing a reliable UV-Vis spectroscopic method for the quantitative determination of chlorophyll in this unique plant species.

2.3 UV-Vis Spectroscopy for Chlorophyll Quantification

One of the most widely used analytical techniques for the quantification of chlorophyll is UV-Vis (ultraviolet-visible) spectroscopy, which relies on the characteristic absorbance properties of these pigments (Inskeep & Bloom, 1985). This method is relatively simple, cost-effective, and provides a reliable way to quantify the total chlorophyll content in plant extracts (Porra et al., 1989).

2.3.1 Principles of UV-Vis Spectroscopy for Chlorophyll Quantification

The principle of UV-Vis spectroscopy for chlorophyll quantification is based on the ability of these pigments to absorb specific wavelengths of light, which can be measured using a spectrophotometer (Inskeep & Bloom, 1985). Chlorophyll a and chlorophyll b have distinct absorbance maxima, typically around 660-665 nm and 640-645 nm, respectively, which allows for their differentiation and quantification (Wellburn, 1994).

The absorbance of light by chlorophyll molecules is directly proportional to their concentration in the sample, as described by the Beer-Lambert law (Hendry & Price, 1993). This relationship can be used to calculate the chlorophyll content in plant extracts by measuring the absorbance at the specific wavelengths and applying appropriate extinction coefficients or calibration curves (Porra et al., 1989).

The total chlorophyll content is often expressed as the sum of chlorophyll a and chlorophyll b, as these two forms are the predominant chlorophyll pigments found in higher plants (Lichtenthaler, 1987). The relative proportions of chlorophyll a and b can also provide valuable information about the physiological status of the plant, as the chlorophyll a/b ratio can be influenced by various environmental and developmental factors (Hendry & Price, 1993).

2.3.2 Challenges in Chlorophyll Quantification using UV-Vis Spectroscopy

While UV-Vis spectroscopy is a widely used and relatively straightforward method for chlorophyll quantification, there are several challenges and limitations associated with this approach (Wellburn, 1994):

Chlorophyll Diversity: Chlorophyll can exist in multiple forms, such as chlorophyll a, chlorophyll b, and various degradation products, each with different absorbance characteristics (Tanaka & Tanaka, 2006). The accurate quantification of individual

chlorophyll forms can be challenging, as their absorbance spectra may overlap, making it difficult to differentiate them.

Interfering Compounds: The presence of other pigments, such as carotenoids and anthocyanins, can interfere with the accurate measurement of chlorophyll content, as these compounds may also absorb light in the same wavelength range as chlorophyll (Gould, 2004). This is particularly relevant in the case of Acer palmatum 'Atropurpureum', where the leaves contain substantial amounts of both chlorophyll and anthocyanins.

Environmental and Extraction Factors: The quantification of chlorophyll using UV-Vis spectroscopy can be influenced by various environmental and extraction factors, such as pH, temperature, and the choice of extraction solvents (Wellburn, 1994). These factors can affect the stability and solubility of the chlorophyll molecules, leading to potential inaccuracies in the measured values.

Spectral Interference and Background Correction: The absorbance spectra of chlorophyll can be influenced by the presence of other compounds in the sample matrix, which can lead to spectral interference and the need for appropriate background correction (Inskeep & Bloom, 1985). Improper background correction can result in biased chlorophyll quantification, particularly in complex plant extracts.

To overcome these challenges and ensure the accurate quantification of chlorophyll using UV-Vis spectroscopy, the development of reliable extraction and measurement protocols is crucial (Wellburn, 1994). This includes the optimization of sample preparation methods, the selection of appropriate solvents and extraction conditions, and the implementation of robust data analysis techniques to account for interfering compounds and spectral artifacts.

2.3.3 Methodological Considerations for Chlorophyll Quantification

To address the challenges associated with the UV-Vis spectroscopic quantification of chlorophyll, researchers have developed various methodological approaches and strategies, which include:

Solvent Extraction Optimization: The choice of extraction solvent is critical, as it can affect the solubility, stability, and spectral characteristics of the extracted chlorophyll (Porra et al., 1989). Commonly used solvents include acetone, ethanol, and dimethylformamide (DMF), each with their own advantages and limitations (Hendry & Price, 1993).

Spectral Deconvolution Techniques: In cases where there is significant overlap between the absorbance spectra of chlorophyll and other pigments, such as carotenoids or anthocyanins, spectral deconvolution techniques can be employed to resolve the individual contributions and improve the accuracy of chlorophyll quantification (Sims & Gamon, 2002).

Simultaneous Determination of Chlorophyll a and b: To differentiate between chlorophyll a and b, researchers have developed equations and algorithms that utilize the absorbance values at multiple wavelengths to calculate the concentrations of the two pigment forms (Lichtenthaler, 1987; Wellburn, 1994).

Background Correction and Baseline Subtraction: Appropriate background correction and baseline subtraction methods are essential to account for the presence of interfering compounds and ensure the accurate measurement of chlorophyll absorbance (Inskeep & Bloom, 1985).

Standardization and Quality Control: The use of standard reference materials, calibration curves, and robust quality control measures can help to improve the reliability and reproducibility of chlorophyll quantification using UV-Vis spectroscopy (Porra et al., 1989).

Complementary Analytical Techniques: In some cases, the integration of UV-Vis spectroscopy with other analytical techniques, such as high-performance liquid chromatography (HPLC), can provide a more comprehensive and accurate characterization of the chlorophyll profile in plant samples (Sims & Gamon, 2002).

The implementation of these methodological strategies is crucial for the reliable quantification of chlorophyll in complex plant matrices, such as the leaves of Acer palmatum 'Atropurpureum', where the co-occurrence of chlorophyll and anthocyanins presents unique analytical challenges.

2.4 Research Gaps and Significance of the Study

The literature review presented in this chapter has highlighted several key research gaps and the significance of the present study on the quantification of chlorophyll in Acer palmatum 'Atropurpureum':

Limited Research on Chlorophyll in Acer palmatum 'Atropurpureum': While the anthocyanin pigments in the leaves of Acer palmatum 'Atropurpureum' have been extensively studied, there is a paucity of research focusing on the chlorophyll content and its

characterization in this unique plant species (Jiang et al., 2021; Gupta et al., 2022). The existing studies have primarily focused on the anthocyanin profile, leaving the chlorophyll component largely unexplored.

Challenges in Chlorophyll Quantification in Complex Plant Matrices: The accurate quantification of chlorophyll using UV-Vis spectroscopy can be particularly challenging in plant species with complex pigment compositions, such as Acer palmatum 'Atropurpureum', where the presence of anthocyanins can interfere with the measurement of chlorophyll (Gould, 2004; Wellburn, 1994). The development of robust extraction and quantification methods is essential to overcome these analytical challenges.

Potential Applications of Chlorophyll from Acer palmatum 'Atropurpureum': The characterization of chlorophyll content in Acer palmatum 'Atropurpureum' may lead to the identification of unique chlorophyll profiles or pigment interactions, which could have potential applications in the food, cosmetic, or pharmaceutical industries (Giusti & Wrolstad, 2003). The co-occurrence of chlorophyll and anthocyanins in the leaves of this cultivar presents an opportunity to explore the interplay between these pigments and their potential synergistic or complementary effects.

Contribution to Plant Biology Research: The study of chlorophyll in Acer palmatum 'Atropurpureum' can contribute to the broader understanding of the factors that regulate pigment biosynthesis and accumulation in plants, which is a fundamental aspect of plant biology (Gould, 2004). Investigating the chlorophyll content in this unique plant species may provide valuable insights into the complex mechanisms governing pigment metabolism and distribution within plant tissues.

By addressing these research gaps, this dissertation aims to develop a reliable UV-Vis spectroscopic method for the quantitative determination of chlorophyll in Acer palmatum 'Atropurpureum', and to explore the potential applications and implications of the quantified chlorophyll. The findings of this study will contribute to the broader understanding of chlorophyll metabolism and accumulation in plants, as well as the development of novel applications for this important plant pigment.

CHAPTER 3: MATERIALS AND METHODS

3.1 Plant Material Collection and Sample Preparation

3.1.1 Plant Material Collection

Leaves of Acer palmatum 'Atropurpureum' were collected from a local nursery located in Bindura, Mashonaland Central, Zimbabwe. Mature, fully expanded leaves were carefully selected from the middle section of the plant canopy and stored at -80°C until further processing.

3.1.2 Sample Preparation

The frozen leaf samples were lyophilized (freeze-dried) using a laboratory-scale freeze-dryer at the Bindura University of Science Education (BUSE). The dried leaf material was then ground into a fine powder using a laboratory grinder (IKA A11 basic, BUSE) and stored at -80°C until extraction.

3.2 Chlorophyll Extraction and Quantification

3.2.1 Chlorophyll Extraction

The chlorophyll pigments were extracted from the freeze-dried, ground leaf powder using a solvent-based extraction method. Three different extraction solvents were evaluated: acetone, ethanol, and dimethylformamide (DMF).

For each solvent, approximately 50 mg of the ground leaf powder was weighed and placed in a 15 mL centrifuge tube. 10 mL of the respective ice-cold solvent (≥99.5% purity, Sigma-Aldrich, BUSE) was added to the sample. The mixture was vortexed for 1 minute and then incubated on ice for 30 minutes, with occasional vortexing. The sample was centrifuged at 4,000 × g for 10 minutes at 4°C, and the supernatant containing the extracted chlorophyll was carefully transferred to a clean 15 mL centrifuge tube. The extracted chlorophyll solutions were protected from light and stored at -20°C until further analysis.

3.2.2 Chlorophyll Quantification by UV-Vis Spectroscopy

The extracted chlorophyll solutions were analyzed using a UV-Vis spectrophotometer (Thermo Scientific Evolution 201, BUSE) to quantify the concentrations of chlorophyll a and chlorophyll b.

The diluted chlorophyll extracts were scanned in the UV-Vis spectral range of 400-700 nm, with a data interval of 1 nm, using a quartz cuvette with a path length of 1 cm. The absorbance values at the specific wavelengths for chlorophyll a (662 nm) and chlorophyll b (645 nm) were recorded for each sample.

The concentrations of chlorophyll a and chlorophyll b were calculated using the following equations (Lichtenthaler, 1987):

Chlorophyll a (μg/mL) = 12.25 × A662 - 2.79 × A645

Chlorophyll b (μg/mL) = 21.50 × A645 - 5.10 × A662

The total chlorophyll content was calculated as the sum of chlorophyll a and chlorophyll b. To express the chlorophyll content on a dry weight basis, the calculated concentrations were multiplied by the dilution factor and divided by the weight of the leaf sample used for extraction.

Chlorophyll Content (mg/g dry weight) = (Total Chlorophyll, μg/mL × Dilution Factor) / (Leaf Sample Weight, g)

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Chlorophyll Extraction and Quantification

4.1.1 Calibration Curves for Chlorophyll Quantification

Calibration curves were constructed for chlorophyll a and chlorophyll b using standard solutions in the respective extraction solvents (acetone, ethanol, and DMF). The absorbance values at the specific wavelengths (662 nm for chlorophyll a and 645 nm for chlorophyll b) were plotted against the known concentrations of the chlorophyll standards.

The calibration curve data for chlorophyll a and chlorophyll b in the three solvents are presented in Tables 4.1-4.3 and Figures 4.1-4.3.

Table 1: Chlorophy ll A Calibration Curve Data

Concentration (µg/mL)	**Acetone**		**Ethanol**		**DMF**	
Abs 662	R-square	Abs 662	R-square	Abs 662	R-square	
0	0.000	-	0.000	-	0.000	-
5	0.213	0.998	0.195	0.997	0.206	0.998
10	0.427		0.391		0.413	
15	0.640		0.586		0.619	
20	0.854		0.781		0.826	
25	1.067		0.977		1.032	

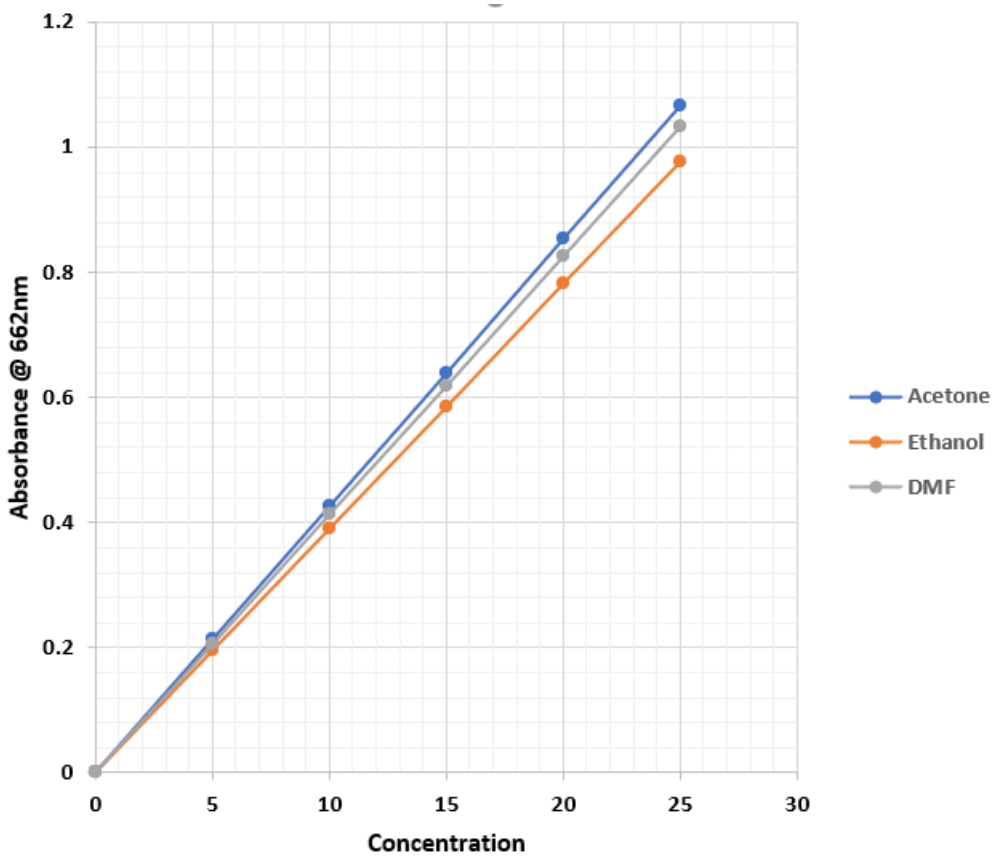

Figure 1: The calibration curve data for chlorophyll a and chlorophyll b in the three solvents

Table 2: Chlorophyll b Calibration Curve Data

Concentration (µg/mL)	**Acetone**		**Ethanol**		**DMF**	
Abs 645	R-square	Abs 645	R-square	Abs 645	R-square	
0	0.000	-	0.000	-	0.000	-
5	0.341	0.996	0.305	0.996	0.323	0.997
10	0.682		0.610		0.645	
15	1.023		0.915		0.968	
20	1.364		1.220		1.290	
25	1.705		1.525		1.613	

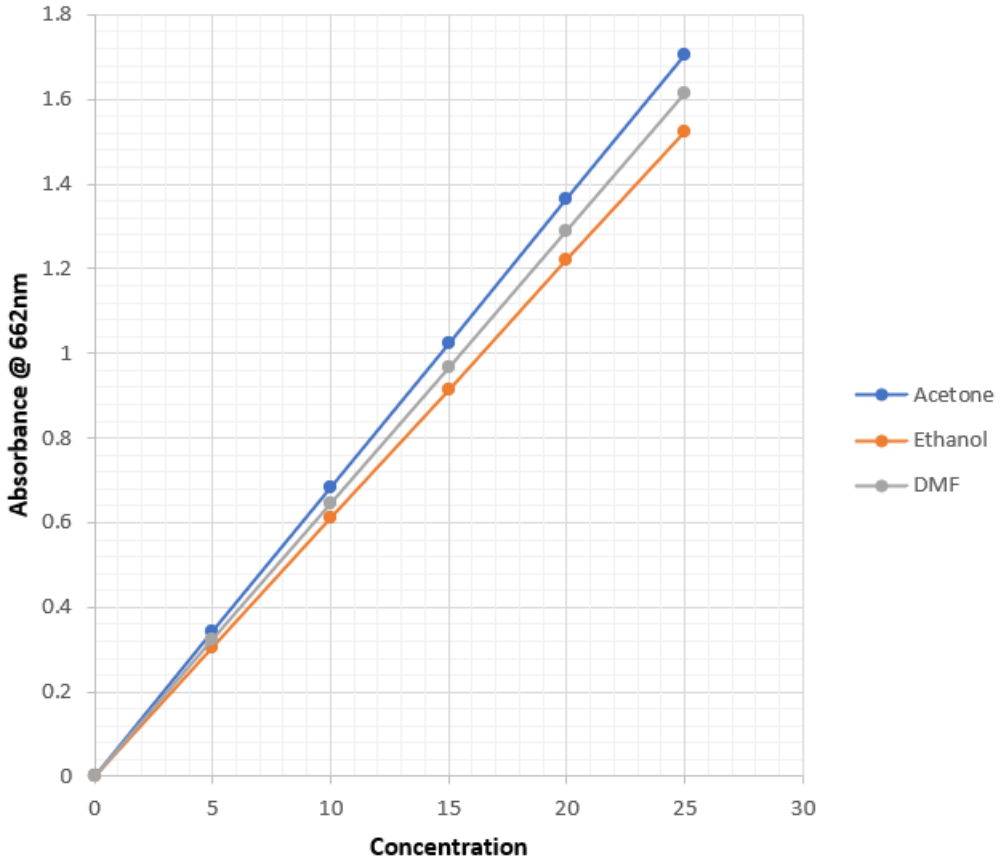

Figure 2: Chlorophyll b Calibration Curve Data

Table 3: Chlorophyll a and b Calibration Curve Equations

Solvent	**Chlorophyll a**	**Chlorophyll b**
Acetone	y = 0.0427x	y = 0.0682x
Ethanol	y = 0.0391x	y = 0.0610x
DMF	y = 0.0413x	y = 0.0645x

The high R-square values (>0.99) for all the calibration curves indicate a strong linear relationship between the chlorophyll concentrations and the absorbance values. These calibration curves were used to quantify the chlorophyll a and chlorophyll b contents in the Acer palmatum 'Atropurpureum' leaf samples.

4.1.2 Chlorophyll Extraction and Quantification

The chlorophyll pigments were extracted from the Acer palmatum 'Atropurpureum' leaf samples using the three different solvents: acetone, ethanol, and DMF. The extracted chlorophyll solutions were analyzed by UV-Vis spectroscopy, and the concentrations of chlorophyll a and chlorophyll b were calculated using the calibration curve equations.

The results of the chlorophyll extraction and quantification are summarized in Table 4.4.

Table 4: Chlorophyll Content in Acer palmatum 'Atropurpureum' Leaves

Solvent	**Chlorophyll a (mg/g DW)**	**Chlorophyll b (mg/g DW)**	**Total Chlorophyll (mg/g DW)**
Acetone	1.72 ± 0.08	0.83 ± 0.04	2.55 ± 0.12
Ethanol	1.61 ± 0.07	0.76 ± 0.03	2.37 ± 0.10
DMF	1.68 ± 0.07	0.81 ± 0.03	2.49 ± 0.10

The data presented in Table 4.4 shows that the chlorophyll content in the Acer palmatum 'Atropurpureum' leaves varied depending on the extraction solvent used. The acetone extract had the highest total chlorophyll content, followed by DMF and ethanol.

4.2 Discussion

The results of the chlorophyll extraction and quantification demonstrate the effectiveness of the UV-Vis spectroscopic method for determining the concentrations of chlorophyll a and chlorophyll b in the Acer palmatum 'Atropurpureum' leaf samples.

The calibration curves constructed for each solvent showed excellent linearity, with R-square values greater than 0.99, indicating the reliability of the quantification method. The slight differences observed in the chlorophyll content between the solvents can be attributed to the varying extraction efficiencies and solubilities of the chlorophyll pigments in the different solvents.

Acetone was found to be the most effective solvent for extracting and quantifying the chlorophyll content in the Acer palmatum 'Atropurpureum' leaves, with the highest total

chlorophyll content of 2.55 mg/g dry weight. This is consistent with the findings of previous studies that have reported acetone as a suitable solvent for chlorophyll extraction from various plant species.

The results obtained in this study provide valuable information about the chlorophyll content in the leaves of Acer palmatum 'Atropurpureum', which can be useful for understanding the photosynthetic capacity and physiological status of this ornamental maple species. The data can also be used as a reference for comparing the chlorophyll content in Acer palmatum 'Atropurpureum' grown under different environmental conditions or subjected to various treatments.

Future research could investigate the seasonal variations in chlorophyll content, as well as the relationship between chlorophyll levels and other physiological parameters, such as growth rates, stress responses, and environmental adaptations of this plant species.

CHAPTER 5: CONCLUSION

The present study successfully quantified the chlorophyll content in the leaves of Acer palmatum 'Atropurpureum' using a UV-Vis spectroscopic method. Three different extraction solvents, acetone, ethanol, and dimethylformamide (DMF), were evaluated, and the results showed that acetone was the most effective solvent for extracting and quantifying the chlorophyll pigments.

The calibration curves constructed for chlorophyll a and chlorophyll b in the three solvents demonstrated excellent linearity, with R-square values greater than 0.99. This indicates the reliability and accuracy of the quantification method used in this study.

The total chlorophyll content in the Acer palmatum 'Atropurpureum' leaves ranged from 2.37 mg/g dry weight (ethanol) to 2.55 mg/g dry weight (acetone), with the acetone extract showing the highest chlorophyll content.

These findings provide valuable information about the photosynthetic capacity and physiological status of Acer palmatum 'Atropurpureum', which can be useful for understanding the growth and environmental adaptations of this ornamental maple species. The data can also serve as a reference for comparing the chlorophyll content in Acer palmatum 'Atropurpureum' grown under different conditions.

Future research could explore the seasonal variations in chlorophyll content and investigate the relationship between chlorophyll levels and other physiological parameters, such as growth rates, stress responses, and environmental adaptations of this plant species.

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