

**Can Plant Extracts Enhance Growth and Development, Yield and Simultaneously Sustaining the Post-harvest Quality and Shelf-life of Potatoes (*Solanum tuberosum* L.)?**

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Submitted in Partial Fulfilment of the Academic Requirements for

**MASTER OF SCIENCE IN AGRICULTURE**

**(HORTICULTURAL SCIENCE)**

**in the**

College of Agriculture, Engineering and Science

School of Agricultural, Earth and Environmental Sciences

Discipline of Horticultural Science

Pietermaritzburg campus

South Africa




December 2023

## DECLARATION

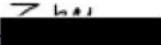
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The research work presented in this thesis was a result of the experiments carried out in the School of Agriculture, Earth and Environmental Science, University of KwaZulu-Natal, Pietermaritzburg from March 2022 to June 2023.

By submitting this dissertation, I, **Siphokuhle Mbuyisa**, hereby declare that the results presented in this research were findings of my own experiments and written up in my own words, unless otherwise stated and referenced accordingly.


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## DECLARATION 2 - PUBLICATIONS

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Mbuyisa, S., Bertling, I. and Ngcobo, B.L., 2023. Foliar Application with Plant-Derived Extracts Enhances Growth, Physiological Parameters, and Yield of Potatoes (*Solanum tuberosum* L.). In MDPI: *Biology and Life Sciences Forum*, 27(1), 34. Published: 27 October 2023. <https://doi.org/10.3390/IECAG2023-15385>

Mbuyisa, S., Bertling, I. and Ngcobo, B.L., 2023. Impact of Foliar-applied Plant Extracts on Growth, Physiological and Yield Attributes of Potato (*Solanum tuberosum* L.). Accepted for publication by *Agronomy*, Journal by MDPI, 21 December 2023.

## ACKNOWLEDGEMENTS

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Firstly, I would like to extend my sincere gratitude to God Almighty for his wisdom, strength, courage and fortitude, which kept me running throughout this research.

I would also like express my deep appreciation and gratitude to the following:

- Prof. Bertling – I just want to thank her supervision, guidance and financial assistance, as well as willingness to assist and availability, which was the key throughout the duration of this study.
- Dr Ngcobo – I extend my deep appreciation to him for helping me with reviewing and editing throughout preparation and writing of this dissertation. I'd also like to pass my sincere thankfulness to him for being a good life and academic mentor in my entire MSc career.
- My mom, Thandazile Ntimbane, for her spiritual and emotional support. I thank her for praying for my protection, mental well-being and good health. With this stated, I pray that God may continue blessing her with more years of living.
- Mr Thokozani Nkosi - Lab technician is also well- appreciated for his technical support in the lab, especially during biochemical analysis of potatoes.
- Special appreciation to my friends, family and colleagues of Horticultural Science Disciplines for their input and technical support in the laboratory.



## DEDICATION

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I, Siphokuhle Mbuyisa, dedicated this dissertation/thesis to **Thandazile Ntimbane**, my dearest mom

## GENERAL ABSTRACT

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With an exponentially growing global population, the demand for healthy and nutritious food has increased exponentially to meet human dietary needs and sustain healthy people. The agricultural sector is, therefore, facing a major challenge of having to increase food production and to reduce post-harvest losses regardless of the impacts of climate change. The alteration in the global environment contribute to biotic and abiotic crop stress, including drought, salinity, weed infestations, as well as pests and diseases, thus, threatening agricultural crop productivity and sustainability. To achieve high food availability and low post-harvest losses, the use of inorganic fertilizers and synthetic pesticides, has become vital; however, their continuous, excessive usage has caused harm to consumer's health and has been detrimental to the environment. Due to these adversities, it is necessary to reduce the employment of such chemicals, despite their effectiveness. Hence, to mitigate these challenges, the use of natural plant extracts, as a promising, innovative and eco-friendly approach, has gained popularity, as they can enhance plant growth and development, as well as yields, and simultaneously improve post-harvest quality and prolong the shelf-life of agricultural commodities. The general aim of this study was to determine, if certain plant extracts are able to enhance growth and development, yield, post-harvest quality and shelf-life of potato (*Solanum tuberosum* L.).

This study consists of two experiments, one conducted in the glasshouse and another one in the post-harvest laboratory at the University of KwaZulu-Natal in 2022-23. The first experiment was carried out to investigate the effect of pre-harvest foliar application of various plant extracts on growth and development, as well as certain physiological and yield attributes of potato (cv. Sifra). This study was laid out following a completely randomized design (CRD) with five replications. The experiment comprised four treatments (different plant extracts), namely *Ascophyllum nodosum* extract (ANE), aloe vera leaf extract (AVE), garlic bulb extract (GBE) and moringa leaf extract (MLE), plus control (no application). The first foliar application of treatments was performed four weeks after planting and repeated weekly until tuber harvesting. The obtained results demonstrate that foliar application of plant extracts significantly improves ( $p \leq 0.05$ ) growth, development and yield parameters of potato plants (viz. plant height, number of leaves, number of stems, leaf area, chlorophyll content index, fresh and dry above-ground biomass, tuber mass and total number of tubers per plant). Among all treatments, ANE and MLE showed the best growth and yield response by producing the highest values of all parameters measured compared to AVE, GBE and the control.

The second experiment was conducted to evaluate the effect of post-harvest applications of various plant extracts on the nutritional quality and shelf-life of potatoes (cv. Sifra). The post-harvest dipping of tubers into different treatments was performed immediately after harvesting. Treated tubers were stored on benchtops in the post-harvest laboratory for 28 days. The results obtained demonstrate that dipping of tubers into various plant extracts has a significant influence ( $p \leq 0.05$ ) on the percentage mass loss of potatoes during storage, with AVE showing the least mass loss of all treatments, including the control. The application of treatments also had a significant effect ( $p \leq 0.05$ ) on the nutritional components of potatoes, with ANE and MLE characterized by a better retention of tuber mineral content, total soluble solids, total carbohydrate and protein concentration compared with AVE, GBE and the control. ANE- and MLE-treated tubers were further preserving potato vitamin C (ascorbic acid), total phenolic and flavonoid concentration best, as well as the antioxidant activity during storage; hence, producing the highest overall antioxidant properties in comparison with AVE and GBE the control. Based on the results obtained in this study, application of plant extracts, especially of ANE and MLE could, therefore, be possibly used as an alternative to achieve highly nutritious and healthy food (fruit and vegetables) with enhanced post-harvest quality, without negatively impacting ecosystems and compromising consumers' health.

**Keywords:** antioxidant activity, growth response, post-harvest quality, natural plant extracts, yield

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## LIST OF ACRONYMS

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**AAE:** Ascorbic acid equivalent

**ANE:** *Ascophyllum nodosum* extract

**AVE:** Aloe vera leaf extract

**BSA:** Bovine serum albumin

**CEF:** Controlled environmental facilities

**GAE:** Gallic acid equivalent

**GBE:** Garlic bulb extract

**ICP-MS:** Inductively coupled plasma mass spectrometry

**MLE:** Moring leaf extract

**MLP:** Moringa leaf powder

**ML (%):** Mass loss percentage

**QE:** Quercetin equivalent

**TFC:** Total flavonoid concentration

**TPC:** Total phenolic concentration

**TSS:** Total soluble solids

### General introduction

#### 1.1 Background

In the last century, the consumption of fruit and vegetables, as a part of a well-balanced diet, has come into the spotlight, because the ingestion of such food commodities plays a pivotal role in satisfying human dietary needs. Fruit and vegetables are regarded as good sources of health-promoting phytonutrients, such as carbohydrates, proteins, fibre, fats, minerals and certain antioxidant compounds, which are essential in sustaining a healthy body (Randhawa *et al.*, 2015). The Solanaceae are commonly known as the nightshade family, one of the most important vegetable families, alongside Apiaceae, Brassicaceae, Alliaceae, Cucurbitaceae and Asteraceae (Knapp *et al.*, 2004; Samuels, 2015). The Solanaceae is also ranked as the third-most economically important food crop family for humans, after Poaceae and Fabaceae (Rabara *et al.*, 2015). As an important human food source, the Solanaceae comprise four major vegetable crops: potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*) and eggplant/brinjal (*Solanum melongena*), with potato being, economically, the leading species (Athira *et al.*, 2021).

Potato (*Solanum tuberosum* L.) is popularly known as the ‘King of Vegetables’ (Athira *et al.*, 2021). In South Africa, potato is regarded as a vegetable crop, while in many parts of the world, it is considered a staple food crop due to its frequent consumption and high starch concentration (Hussain, 2016). In terms of production, potato is ranked as the fourth most-consumed food crop worldwide, after wheat (*Triticum aestivum*), rice (*Oryza sativa*) and maize (*Zea mays*) (Rigano *et al.*, 2013). According to FAOSTAT (2023) estimates, the global annual potato production exceeded 376 million tons in 2021, with China remaining the world's largest producer (94.36 million tons). Approximately one-third of the world's potato production originates in China and India (FAO, 2023). As a result, potato is the only non-grain food crop with immense potential for global food security (Potato Genome Sequencing Consortium, 2011). In South Africa, the *per capita* consumption of potatoes reached 32.5 kg in 2020 (FAO, 2023); this could be due to their high nutritional value and the health benefits associated with its consumption (Rigano *et al.*, 2013). Potatoes are not only an excellent source of carbohydrates, but also contain several health-promoting phytonutrients, including proteins, vitamins, fibre and mineral nutrients (*viz.* potassium, phosphorus, magnesium, calcium,



manganese, iron, zinc and copper) (Zaheer and Akhtar, 2015). Potatoes are also antioxidant-rich commodities, containing numerous antioxidant compounds, including phenolics and carotenoids, which are vital phytochemicals sustaining human health (King and Slavin, 2013; Lovat *et al.*, 2016).

## **1.2. Rationale**

By 2050, the global population is expected to have risen to 9.7 billion people (Searchinger *et al.*, 2013). Due to this constantly growing world population, food demand is also anticipated to increase by 50-70%; already there is not sufficient food for approximately 1 billion malnourished people worldwide (Bond *et al.*, 2013). Farmers are, therefore, facing a major challenge having to increase global food production under unpredicted weather patterns due to climate change (Wheeler and von Braun, 2013). In addition, Parajuli *et al.* (2019) also indicated that salinity, drought, weed infestations, as well as pests and diseases, are major abiotic and biotic factors severely limiting horticultural production. These adversities could potentially increase in the future, resulting in approximately 45% of the world's annual food production being lost, either pre- or post-harvest (WWF, 2017). In South Africa, 70% produce loss of fruit, vegetables and cereals, has been reported to occur during and after harvest, resulting in price instability, negatively affecting farmers' and traders' income as well as food security (WWF, 2017).

With agricultural expansion, synthetic pesticides and inorganic fertilizers have become vital for crop production and protection against abiotic and biotic stress (Sharma *et al.*, 2019). Over time, usage of such chemical-based inputs dramatically increased because of their effectiveness in improving crop yields and reducing pre- and post-harvest losses (Sharma *et al.*, 2019). Despite their efficacy, the reliance on chemical-based inputs to enhance agricultural production can also impart negative effects on human health and can simultaneously harm various ecosystems (Alewu and Nosiri, 2021). In addition, continuous misuse of these pesticides to improve agricultural yields may result in the development of new, resistant strains that could become difficult to control (Sharma *et al.*, 2019). Popp *et al.* (2013) reported that higher costs of such inputs offer an economic motivation to minimize the utilization of such chemical inputs; thus, horticultural crop farming could be made simpler, while offering healthy, safe, and sustainable food that is highly valued by consumers. While a reduction in pesticide application is important to human and environmental health, it is necessary to explore and develop alternative horticultural production approaches for long-term viability and to meet the worldwide food demand (Zulfiqar *et al.*, 2020).

Given the need to develop ecological strategies to achieve high yields of high-quality agricultural commodities, the use of plant extracts is a newly developed, promising, eco-friendly and inexpensive approach that various authors have tested on several crops (Spinelli *et al.*, 2009; Colavita *et al.*, 2010). Extracts obtained from various plants contain certain growth-enhancing, yield-promoting, quality-sustaining and stress-reducing phytochemicals; these compounds include certain vitamins, terpenoids, alkaloids, phenolics, phytohormones, polysaccharides and minerals (Zulfiqar *et al.*, 2020). These chemical compounds found in plant extracts can induce various beneficial mechanisms in plants, conveying biofertilization and bio-stimulatory effects pre-harvest, as well as quality-maintaining effects post-harvest (Shrestha *et al.*, 2019). Nasir *et al.* (2016) observed that foliar application of MLE, together with potassium and zinc fertilizer, improved yield and fruit quality of ‘Kinnow’ mandarins (*Citrus reticulata* L.). Additionally, Shrestha *et al.* (2019) demonstrated that the application of different plant extracts significantly improved the post-harvest life and quality of mango fruit (*Mangifera indica* L.).

### **1.3. Aim and objectives**

#### **1.3.1. Aim:**

The general aim of this study was to determine, if certain plant extracts are able to enhance growth and development, as well as certain physiological and yield parameters and concurrently maintain post-harvest quality of potatoes during storage.

#### **1.3.2. Specific objectives were:**

- ✓ To determine the effect of various plant extracts on *S. tuberosum* growth and development, as well as on certain physiological and yield attributes.
- ✓ To determine, if certain plant extracts can preserve post-harvest quality of *S. tuberosum* tubers.

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### **Natural Bioactive Compounds in Agriculture, their Extraction Methods and Mechanism of Action within Plants - A Review**

#### **2.1. Overview**

Due to the exponentially increasing world population, agriculture is facing major challenges, threatening its mandate to provide sufficient and high-quality nutritious food (Povero *et al.*, 2016; Colla *et al.*, 2017; Zulfiqar *et al.*, 2020). Secure food production can only be achieved by improving crop yield and protecting crops, considering that the agricultural arable land area is gradually decreasing, and the genetic potential of crops is also approaching its limit (Povero *et al.*, 2016). Kumar and Alope (2020) reported that it is, therefore, crucial to produce food of high nutritional value, which could play a critical role in protecting the human population against malnutrition and hunger. Thus, adopting sustainable agricultural land management practices is necessary to reduce natural resource depletion and ecosystem degradation, threatened by the growing demand for sustainable food, feed, fibre and fuel.

Among several strategies to sustainably enhance agricultural production, the use of plant extracts has been widely investigated. It has emerged as a promising approach that could be employed in agriculture to improve crop production (Godlewska *et al.*, 2021). Plant extracts are “natural products that are recognized as an excellent source of bioactive compounds, which can be extracted from various plant materials” (Abdullahi *et al.*, 2022). These compounds are also called secondary metabolites, which ‘coexist’ with other primary biochemical compounds in plants (Gurjar *et al.*, 2012). These secondary compounds fulfil a wide array of functions. Barrajon-Catalan *et al.* (2014) reported that secondary plant metabolites can enhance important biological activities, thereby influencing various plant physiological responses and modifying plant phenotypes. Plant extracts could, therefore, be used as natural bio-stimulants to improve crop production, as they often enhance plant growth and reduce stress (Lucini *et al.*, 2018; Abou Chehade *et al.*, 2018).

Qualitative and quantitative studies on extraction techniques of bioactive compounds from plant material have been conducted by various authors, who documented that the extraction efficiency primarily relies on the selection of suitable extraction methods appropriate for the specific tissues and compounds (Smith, 2003; Hayat *et al.*, 2009; Sasidharan *et al.*, 2011).

Additionally, Mustafa and Turner (2011) revealed that extraction efficiency is substantially affected by the conditions at which the extraction process occurs, such as temperature, pressure, and duration, as well as the solvent used. Majors (1999) revealed that sample preparation is one of, if not the, most critical steps during any analytical study. Contemporary spectrophotometric and chromatographic methods have, however, simplified bioactive compound analysis. Poole *et al.* (1990) revealed that the success of bioactive compound analysis still relies on the extraction processes, the conditions under which extraction occurs, and the nature of plant material used. Over the last decades, understanding the dynamic chemical nature of various bioactive compounds has increased the efficacy of bioactive compound analysis (Torssell, 1997). Therefore, industries, such as the pharmaceutical, therapeutical, medicinal, food additive or even the natural pesticide sector, have increasingly shown interest in bioactive compound isolation from natural sources (Anklam *et al.*, 1998; Ambrosino *et al.*, 1999).

The isolation of bioactive compounds from various plant materials can be accomplished through multiple extraction techniques; these techniques are classified into two groups: conventional and non-conventional methods (Soquetta *et al.*, 2018). Conventional extraction methods, such as Soxhlet, maceration and distillation extraction techniques, are still considered as reference methods to compare the success of these newly developed methodologies (Rasul, 2019). Various authors, therefore, extensively reviewed and documented the efficacy of non-conventional extraction technologies in extracting phytochemicals from a range of plant materials (Smith, 2003). Over the last 50 years, non-conventional approaches, which are eco-friendly, owing to the lower amount of synthetic and organic chemicals used, or have better time efficiency and higher phytochemical yield and extract quality, have been developed. Microwave-assisted, ultrasound-assisted, pulsed-electric field, supercritical fluid, enzyme-assisted, and pressurized liquid extraction have been extensively studied as non-conventional approaches and have been demonstrated to positively affect the extraction of targeted bioactive compounds from plant material (Ghafoor *et al.*, 2011). Therefore, the present review aims to discuss the advantages and disadvantages of various bioactive compound extraction techniques, while highlighting the importance of natural bioactive compounds and their mechanism of action in crops.



## 2.2. History of natural bioactive compounds

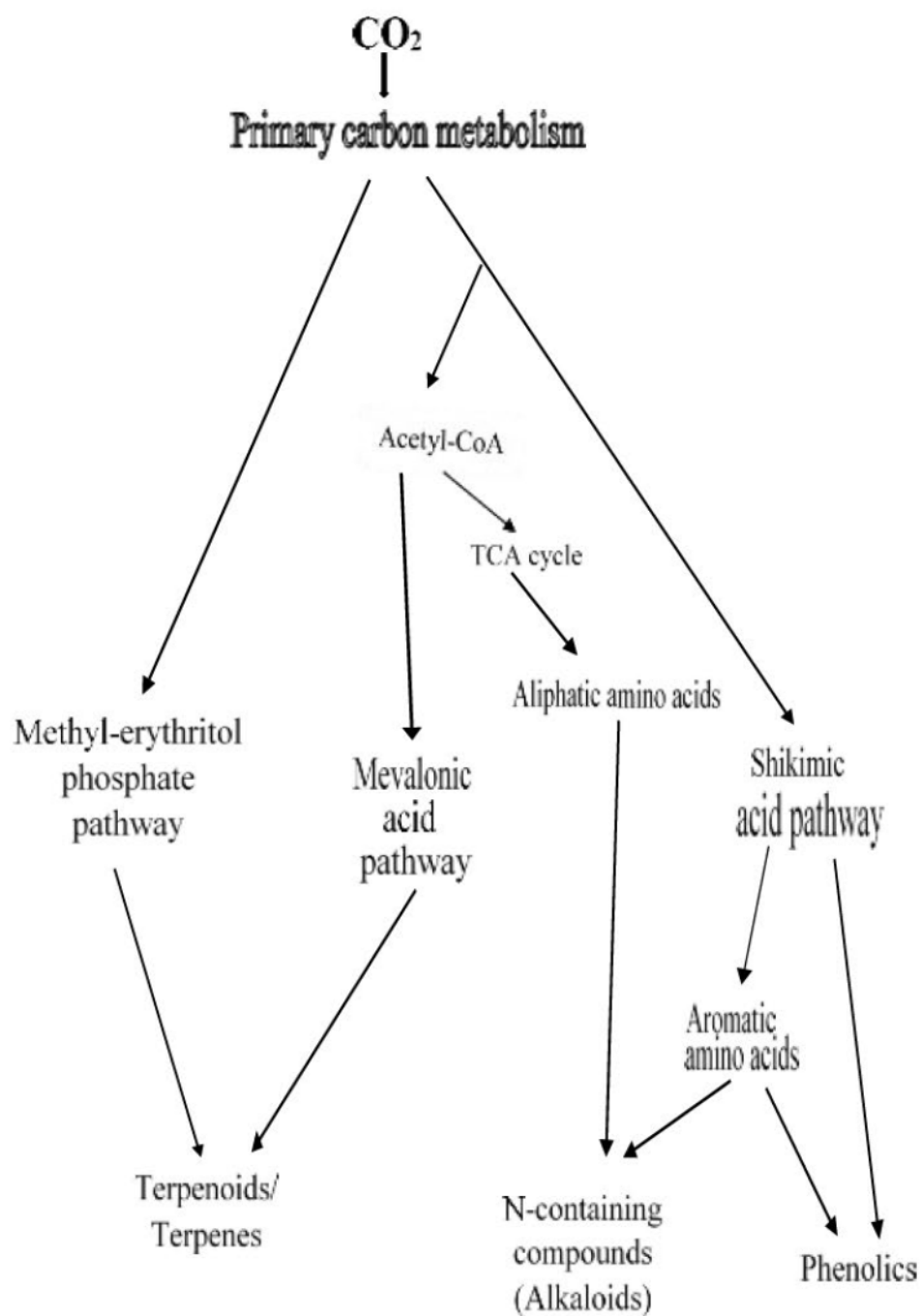
From the dawn of humankind, humans have employed plants for various purposes based on their nutritional and medicinal properties. Azmir *et al.* (2013) revealed that, across various human communities, plants are valuable, natural sources of disease cure and health improvement. Vinatoru (2001) indicated that thousands of recipes, written on Egyptian papyri, demonstrated that coriander and castor oil yielded excellent extracts that were used for multiple purposes, including medicinal, cosmetic, and preservation use. Azmir *et al.* (2013) revealed that during the Greek and Roman periods, ‘plants’ were employed based on their multitude of therapeutic properties. The Romans have long been recognized for their usage of and reporting on therapeutic plants. For instance, Herodotus’ (5<sup>th</sup> century BC) writings revealed that *Leonurus cardiaca* (motherwort) was utilized by people living north of the Danube River as a medicine to stop or prevent bleeding. In the 19<sup>th</sup> century, Romanian pharmacopoeia (a book comprising the list of pharmaceutical substances, their formulae, descriptions and standards) introduced herbal products, and the first institute of medicinal plants was established in Cluj-Napoca, Romania, in 1904 (Vinatoru, 2001).

Pandey *et al.* (2021) defined “bioactive” or “biologically active” compounds as “extra-nutritional constituents that are present in every living organism, particularly microbes, algae and plants, in small quantities”. For these living organisms to survive and thrive, a wide range of phytochemicals, which directly or indirectly contribute towards the organism’s survival, are produced (Azmir *et al.*, 2013). These natural bioactive compounds can be classified into two major categories: primary and secondary plant constituents. Primary metabolites are biomolecules primarily responsible for plant growth and development; these compounds include commonly present carbohydrates, amino acids, proteins, lipids, nucleic acids and hormones (Dhaniaputri *et al.*, 2022). Other than primary metabolites, other types of bioactive compounds are secondary metabolites, subdivided into three major classes: terpenoids, phenolics and N-containing compounds (González Mera *et al.*, 2019; Eljounaidi and Lachman, 2020). Plants in the post-juvenile phase usually produce these compounds to promote plant survival and improve coping mechanisms to overcome stress brought on by the surrounding environment and biotic factors (Azmir *et al.*, 2013). Secondary metabolite production in various species is chiefly determined by the evolution process and the species’ particular demands. Floral species, for example, generate scent to attract insects for pollination and fertilization, whereas toxic compounds are released to inhibit diseases and repel herbivores, as

well as suppress growth of neighbouring plants (weeds); thus, these compounds are now used as natural biostimulants and pesticides (Dudareva and Pichersky, 2000). Some of these secondary metabolites are termed ‘bioactive’ because they affect the biological processes, especially growth and development of living organisms. Consequently, Bernhoft (2010) defined natural bioactive compounds as “secondary metabolites that have toxic or medical effects on other plants, humans and animals”.

### **2.3. Synthesis and classification of bioactive plant compounds**

Based on distinct structural characteristics, functions and the pathways followed during their biosynthesis, secondary metabolites can be classified into several families. According to Dhaniaputri *et al.* (2022), plant secondary metabolites are produced in three major biosynthetic pathways: the shikimic acid, the mevalonic acid and the methyl-erythritol phosphate pathway (MEP). From these pathways natural bioactive compounds can be placed into three major groups: phenolics, terpenoids and N-containing compounds (Bernards, 2010; González Mera *et al.*, 2019; Eljounaidi and Lachman, 2020). The shikimic acid pathway plays a vital role in phenolic compound biosynthesis in plants. In addition, the shikimic acid pathway is also important in the synthesis of N-containing compounds, especially the alkaloids, which are formed from aromatic amino acids produced via this pathway and aliphatic amino acids produced via the tricarboxylic acid cycle (TCA) (Taiz and Zeiger, 2006; Dhaniaputri *et al.*, 2022). Terpenoids are produced via the mevalonic acid or the MEP pathway (Fig. 2.1).

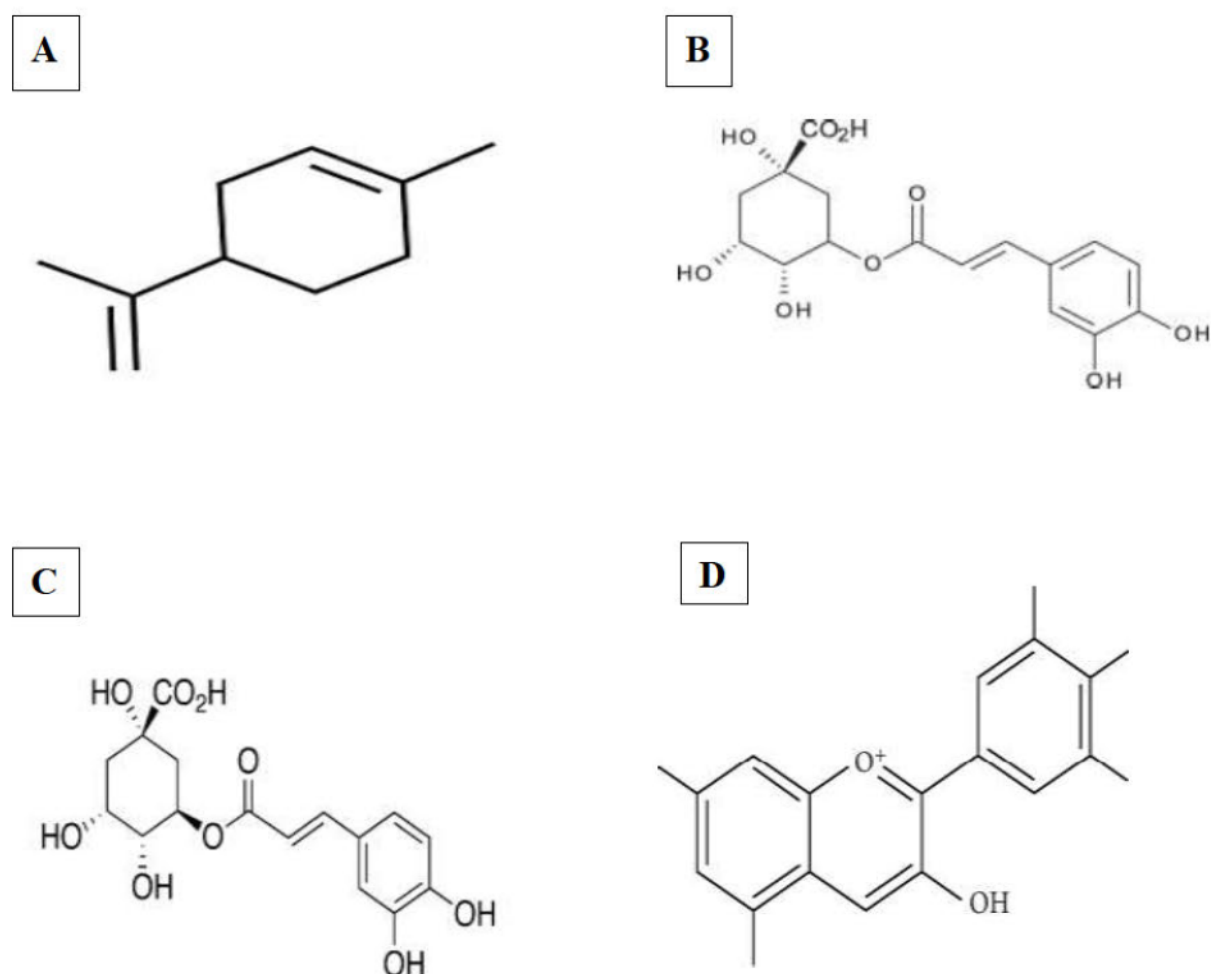


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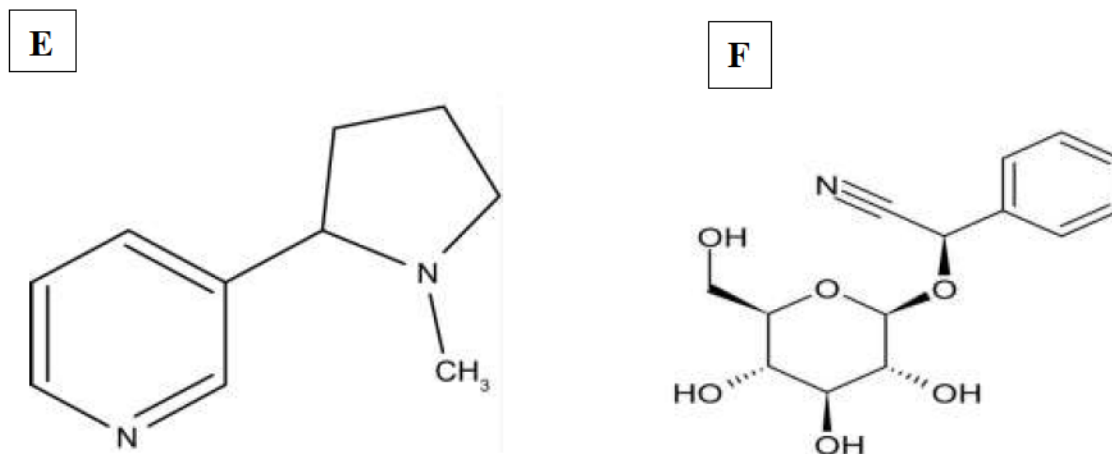
**Figure 2.1:** Primary metabolism pathways involved in the biosynthesis of plant secondary metabolites (adopted from: Dhaniaputri *et al.*, 2022)

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Bioactive plant compounds vary in nature and molecular structure; thus, they can be classified into various categories and subcategories, although there is no consistency in their classification; instead, classification of these compounds relies on a specific objective (Eljounaidi and Lachman, 2020). For instance, a pharmacological classification, which categorizes compounds according to their potential use in drugs, will never match a biosynthetic classification, which provides a simpler way of describing bioactive compounds according to the biosynthetic pathways employed in their manufacture. Therefore, González-Mera *et al.* (2019) indicated that plant secondary metabolites should be divided into three major groups: (a) terpenoids (*i.e.*, monoterpenoids, sesquiterpenoids, diterpenoids, triterpenes, tetraterpenes and polyterpenes), (b) phenolic compounds (*i.e.*, phenolic acids, flavonoids and tannins), and (c) N-containing compounds (*i.e.*, alkaloids, cyanogenic glycosides and non-protein amino acids) (Fig. 2.2).








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**Figure 2.2:** Examples of plant secondary metabolites with their respective structures: Terpenoids: monoterpene (A), diterpene (B), Phenolics: phenolic acid (C), anthocyanidin (D), N-containing compound (alkaloid) (E) and cyanogenic glycoside (F) (adopted from: Wink, 2003)

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## 2.4. Extraction of bioactive compounds

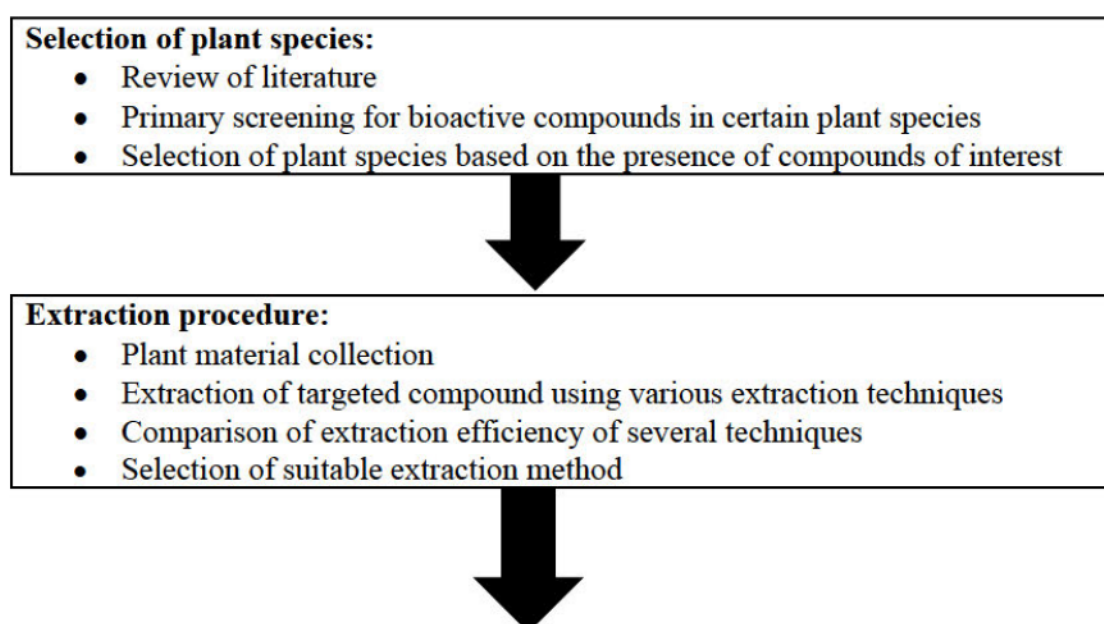
Natural bioactive compounds can be structurally diverse, and some possess several biochemical and physiological properties; therefore, various plant materials, including plant roots, leaves, flowers, fruit and seeds, as well as seaweeds, can be used to manufacture plant extracts, with a high concentration of biologically active constituents (Ali *et al.*, 2019). Thus, these bioactive compounds can be isolated from various plant materials through various extraction procedures, which are also referred to as "extract preparation processes" (Sasidharan *et al.*, 2011). In extract preparation, a sequence of crucial steps is involved in the process, starting from the selection of plant species to the commercialization of the product (extract) (Fig. 2.3). Considering the enormous variation among bioactive compounds, building up a standard and integrated approach to screen bioactive compounds that affect plant growth and development, so that these compounds can be used to sustainably alter plant growth and/ or productivity is necessary.

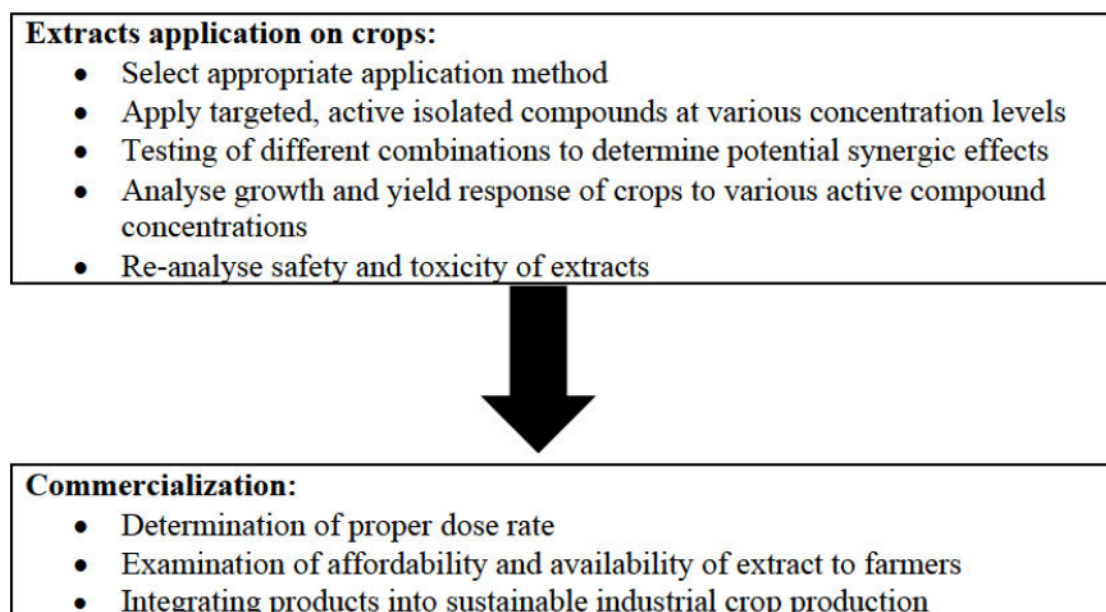
Extraction of such compounds from natural sources as part of sample preparation is the primary step (Azmir *et al.*, 2013). After extraction, further separation of these compounds, their identification and characterization, it is essential to allow screening for the targeted bioactive plant compounds (Sasidharan *et al.*, 2011). Tiwari (2015), however, indicated that the



screening of active constituents during the sample preparation process depends on several factors, including the extraction method, the nature of plant material, the extraction solvent used and the duration of the extraction (time). During the extraction of bioactive compounds from plant materials, it is vital to assure that the active ingredients of the extract are not distorted, destroyed or even lost. Fabricant and Farnworth (2001) revealed that selection of plant material and the extraction solvent used to screen out active constituents largely depends on the nature of the targeted bioactive compounds.

Extraction of targeted bioactive constituents from different plant materials can be accomplished using various extraction methods or conditions. Many extraction protocols have remained very similar over hundreds of years; interestingly, Smith (2003) revealed that these methods share the same objectives: (a) to extract compounds of interest from complex plant samples, (b) to improve the selectivity of the extraction, (c) to increase the sensitivity with which a compound can be detected, (d) to transform the biologically active compounds into a form suitable for its isolation and classification, and (e) to generate a comprehensive and feasible approach of extraction that is not dependent on the plant material. The techniques can be classified into conventional/traditional/classical *versus* non-conventional methods. In conventional methods, specific organic solvents, as well as specific temperature and agitation regimes, are required (Rasul, 2018). In contrast, Rodriguez-Perez *et al.* (2015) reported that non-conventional or novel methods are “green” or “clean” approaches that require less energy for the extraction and no organic solvents are required, reducing harmful effects on the ecosystem.





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**Figure 2.3:** Flow diagram of processes followed in the extraction of naturally bioactive compounds (adopted from Farnworth *et al.*, 1985).

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## 2.5. Extraction solvent selection

The screening of biologically active compounds from various plant materials is carried out using certain solvents used for the extraction procedure (Cowan, 1999). As aforementioned, it is, therefore, crucial to carefully select an appropriate extracting solvent. The selection of such a solvent also depends on the nature of the targeted bioactive compounds (Smith, 2003; Sasidharan *et al.*, 2011). Factors, such as molecular affinity between solvent and solute, mass transfer, co-solvent utilization, cost-efficiency, eco-friendly nature and hazardous to humans, should also be critically considered when selecting a solvent for extraction of biologically active constituents (Azmir *et al.*, 2013). Therefore, properties including low toxicity, high evaporation rate at low temperatures, low boiling point, together with rapid mass transfer, preservative action and the inability to make the extract dissociate, are good assets of suitable extracting solvent (Gurjar *et al.*, 2012). Thus, solvents must be non-toxic and should not interfere with the bioassay because the extraction end-product will otherwise contain residues of the solvent (Ncube *et al.*, 2008).



In most cases, the initial screening of bioactive constituents begins with a crude alcohol extraction, and then various organic solvent extraction techniques follow. Thus, the most commonly used organic solvents for the extraction of bioactive compounds from plant materials are methanol and ethanol. Besides these two solvents, researchers have studied other extraction solvents, including water, acetone, chloroform, dichloro-methanol and ether (Gurjar *et al.*, 2012). Water is a universal solvent used to extract plant biologically active compounds. Despite water being cheap and versatile, the extraction of phytochemicals using organic solvents has proven to be more useful than water extraction (Parekh *et al.*, 2005). Phytochemicals, such as flavonoids, particularly anthocyanins and some phenolic acids, are water-soluble; thus, water is a proper solvent to extract these compounds. Some studies have revealed that certain phenolics and tannins are better extracted using aqueous acetone than methanol (Gurjar *et al.*, 2012). In another study, Harmala *et al.* (1992) demonstrated that chloroform was an appropriate solvent to extract non-polar bioactive constituents among twenty varieties of solvents investigated. Examples of secondary plant constituents extracted using different solvents are given in Table 2.1.

**Table 2.1:** Various solvents used to extract bioactive compounds (adopted from: Cowan, 1999).

Acetone	Chloroform	Dichloro-methanol	Ethanol	Ether	Methanol	Water
Flavonoids	Flavonoids	Terpenoids	Alkaloids	Alkaloids	Anthocyanins	Anthocyanins
Polyphenols	Terpenoids		Flavonoids	Terpenoids	Polyphenols	Saponins
			Polyphenols		Saponins	Tannins
			Tannins		Tannins	Terpenoids
			Terpenoids		Terpenoids	

## 2.6. Mechanism of action of natural bioactive compounds in crops

As a crop grower, it is important to understand the mechanism of action of various plant extracts, familiarizing oneself with anatomical, cellular, molecular and functional alterations the extract can perform to enhance or suppress plant physiological processes, when it is applied to plants. Various plant extracts obtained from different plant materials contain bioactive compounds that exert numerous growth-enhancing, yield-promoting and quality-sustaining, as well as stress-eliciting effects. Such benefits are primarily due to the stimulatory effect of the

compounds contained in the extracts, resulting in a series of reactions within plants, enhancing overall growth, yield and tolerance to biotic and abiotic stress (Yasmeen *et al.*, 2013). Enhanced growth and higher yields of horticultural crops following extract application may be attributed to improved key biological, biochemical, molecular, and physiological processes (Zulfiqar *et al.*, 2020). These alterations are brought about by a multitude of growth-promoting substances, such as phytohormones, vitamins, sugars, phenolics and minerals, therefore, work in several ways. The effect on plant growth is commonly achieved by enhancing plant nutrient uptake, thereby increasing yield and often, additionally, postharvest quality of the commodity.

Various authors have revealed that the foliar extract application or application by soil drench improves nutrient acquisition resulting in enhanced nutrient use efficiency (NUE) and enhanced water use efficiency (WUE) due to the presence of mineral nutrients, proteins, peptides, and peptones in these plant extracts (Lucini *et al.*, 2018; Yasmeen *et al.*, 2013). In addition, Colla *et al.* (2017) revealed that the microbial activity in the soil or the medium was also enhanced due to the presence of nutrients in the extract, thereby increasing soil organic matter, hence, improving soil structure and air movement between soil particles. On the other hand, Elzaawely *et al.* (2017) indicated that plant growth and total yield improvements following plant extract application have also been attributed to the presence of bioactive peptides in the extracts, which trigger the expression of genes involved in phytohormone biosynthesis, such as of auxins, gibberellins and cytokinins (Stirk *et al.*, 2004), further promoting cell division and elongation, as well as flowering and fruit set.

Furthermore, improvements in growth and yield were closely linked to the sugars present in these extracts, fuelling plant growth and providing energy to stimulate the carbon assimilation process (Kumar *et al.*, 2020). Many bioactive compounds, including phytohormones, vitamins and other antioxidants, can induce crop tolerance to various biotic (pathogens) and abiotic stressors, such as drought, salinity, and frost. The application of plant extracts under stressful conditions induces the modulation of phytohormone concentrations, such as salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA) and ethylene (ET), all playing a pivotal role in defence signalling pathways (Khan *et al.*, 2009). As a result, plant extract applications also trigger the expression of antioxidant-producing genes, secondary metabolites, and disease-resistance genes, significantly increasing crop tolerance to biotic and abiotic stress (Ertani *et al.*, 2018). Lucini *et al.* (2018) further noted that conveyed stress tolerance was not only due to phytohormone and certain antioxidants, such as carotenoids, phenolics, ascorbic acid and flavonoids, but the extract also modulated other factors resulting in delayed photo-inhibition

and increased sterol concentrations, thereby enhancing stress tolerance through improved membrane stability. In addition, plant extracts directly induce stress-related gene transcription; thus, they reduce the severity of stress, improving plant growth and productivity (Lucini *et al.* 2018).

Furthermore, Hayat *et al.* (2009) indicated that the application of plant extracts induces antioxidant-related gene transcription, resulting in an increased production of antioxidant compounds that counteract stress by changing the redox state of the cell. Thus, an increased in reactive oxygen species results, activating synthesis of specific antioxidant compounds to balance the redox state. Plant extract application, therefore, induces a priming effect on the plant defence system, triggering stress tolerance. As a result, plants become healthier, producing high-quality, nutritious fruit with enhanced external quality parameters, such as fruit mass and fruit firmness, flesh thickness, as well as, internally higher vitamin C, total phenolic and carotenoid concentrations, as well as more total soluble solids (TSS), thereby extending fruit shelf-life.

## **2.7. Conventional extraction techniques: Advantages and drawbacks**

Screening of plant bioactive compounds from plant material requires the application of several techniques to separate and isolate the compounds of interest. Thus, choosing a proper extraction method is of high importance. Conventional extraction methods, such as Soxhlet, maceration and hydro-distillation, have been previously applied to extract targeted molecules from sample matrices (Ashraf *et al.*, 2020). These techniques are usually environmentally unfriendly because large volumes of organic solvents and amounts of energy are required for the sample preparation procedure to be successful (Dao *et al.*, 2021). As a result, consumers have raised serious concerns, as these techniques are harmful to the environment, due to their non-sustainability and, therefore, are not “green” extraction strategies, that use smaller volumes of organic solvents and more energy (Garcia-Vaquero *et al.*, 2020). Besides the large volume of solvents and energy required, some of these traditional extraction techniques are also time-consuming, if a high extraction efficiency of the selected bioactive compounds is to be achieved. These techniques also have common drawbacks, such as high energy usage due to the extended time equipment must run to ensure high extraction efficiency; thus, energy required for heating and cooling the extract makes them energy-inefficient (Azmir *et al.*, 2013).



Furthermore, tools necessary for all conventional extraction techniques are usually not as affordable, so that more innovative, sustainable extraction approaches, which extract faster and are less energy-inefficient, are required. As aforementioned, the long-established conventional methods seem to be slowly substituted by novel means, resulting in Soxhlet extraction and manual maceration being substituted by ultrasound or microwave-assisted extraction methodologies in response to the current need for eco-friendly and sustainable technologies. These newer methods are currently used more frequently, because they allow a more cost-effective extraction of biologically active constituents with simpler equipment than the conventional methods, or they achieve better stability of the selected compounds. (Garcia-Vaquero *et al.*, 2020a).

### **2.7.1. Soxhlet extraction method**

The Soxhlet extraction technique was first carried out by the German chemist ‘Franz Soxhlet’ in 1879 and used a type of condenser known as the Soxhlet apparatus (Ashraf *et al.*, 2020). The traditional Soxhlet extractor comprises a thimble holder and a distillation flask, into which sample and solvent are added. When the solvent reaches its boiling point, it enters the sample as vapour, solubilizing compatible compounds. Thereafter, the solvent condenses on the cooling tubes and travels back into the initial flask with the extracted compounds. This operation is repeated until the extraction is deemed complete (Ashraf *et al.*, 2020; Garcia-Vaquero *et al.*, 2020a). Like any other extraction, Soxhlet extraction has advantages and drawbacks. Advantages of the Soxhlet method are: (i) the solvent is recycled and then re-used, allowing a better extraction of compounds from the sample, it also increases the extraction efficiency of targeted compounds, (ii) the temperature of the sample is maintained throughout the process, (iii) no filtration or centrifugation is required to separate the final extracts from the original biomass, (iv) the extraction is possible at relatively low cost with easy operational processes, as the basic equipment is affordable and simple (Garcia-Vaquero *et al.*, 2020; Lopez-Bascon and De Castro, 2020). Nonetheless, the Soxhlet extraction technique also has drawbacks: (i) it uses large volumes of organic solvents, such as ethanol, methanol, acetone, hexane and ethyl acetate (ii) long periods of extraction are needed until the number of cycles that ensure sufficient extraction are completed (Garcia-Vaquero *et al.*, 2020); (iii) elevated temperature is employed to boil the solvents, potentially affecting the active ingredients of the extract (Ashraf *et al.*, 2020); (iv) additional agitation does not improve the efficiency of this technique (Manousi *et al.*, 2019; Lopez-Bascon and De Castro, 2020).

Nevertheless, the Soxhlet extraction method has been improved through automating the process, aiming to shorten the extraction time. Recently, Soxhlet extraction has been coupled with innovative technologies, resulting in high-pressure Soxhlet extraction, supercritical fluid-Soxhlet extraction, and automated Soxhlet extraction. Applying auxiliary energies, such as ultrasound or microwaves, can also improve the efficiency than the conventional Soxhlet extraction (Lopez-Bascon and De Castro, 2020).

### **2.7.2. Maceration method**

Abubakar and Haque (2020) defined maceration as “an extraction protocol in which coarsely powdered material, either from leaves, stem bark or root bark, is placed inside a container; the solvent is poured on top until the plant material is completely covered”. It is one of the most popular and inexpensive solid-liquid extraction techniques extensively used to isolate various biologically active compounds from complex plant materials (Manousi *et al.*, 2019). This simple extraction method has been employed to extract essential oils and other, especially thermolabile, compounds (Azmir *et al.*, 2013). The success of maceration is primarily influenced by extraction temperature, extraction duration, agitation and solvent used (De Castro and Priego-Capote, 2010). Solvent selection depends on the polarity and nature of targeted compounds (Patel *et al.*, 2019). Generally, the maceration process involves a series of steps: (i) increasing the surface area of the plant material by grinding it into small particles, thus promoting proper mixing with an appropriate solvent, (ii) leaving the sample in the solvent for a certain period to allow the movement of targeted compounds from the plant material into the solvent, (iii) filtering the extract to obtain a residue-free supernatant (Azmir *et al.*, 2013).

Like any other extraction method, maceration also has advantages and limitations. The maceration procedure is simple and inexpensive; variables, such as solvent, temperature, agitation intensity and time, can easily be manipulated or changed to ensure that the extraction of targeted compounds is optimized (Poole *et al.*, 1990). Due to the ease with which this method can be scaled up, it is still extensively used in many laboratories and industries. In comparison with other extraction methods, however, maceration requires large volumes of organic solvents and has long extraction times, resulting in major limitations of this method (Sasidharan *et al.*, 2011). In addition, this method is associated with several centrifugation and filtration steps to separate supernatant and solid residues, thus, increasing the energy required for the process (Gallo *et al.*, 2017; Özkan and Özcan, 2017).

### **2.7.3. Hydro-distillation extraction method**

Hydro-distillation has been used as a classical technique for extracting natural bioactive compounds from various plant materials for over 5000 years (Kockmann, 2014). Grosso *et al.* (2015) revealed that this method is commonly used to extract polar compounds of volatile and non-volatile nature from plant material. Unlike other conventional extraction techniques, hydro-distillation uses distilled water instead of organic solvents (Soquetta *et al.*, 2018). Three physicochemical processes (decomposition, hydro-diffusion and hydrolysis) are involved in hydro-distillation extraction. When first established, this method was primarily applied to generate distilled water from various plant materials (Kockmann, 2014). The practicality of this technique is, however, impacted negatively by several factors, such as the required high temperature, long extraction time and agitation (Silva *et al.*, 2005; Petigny *et al.*, 2014; Garcia-Vaquero *et al.*, 2020). Hydro-distillation is a simple technique, easy to scale up and, therefore, commonly used to extract and purify compounds (Prado *et al.*, 2021). The major drawback of hydro-distillation is its high operational temperature, 100°C that can destroy heat-sensitive compounds during the process, although thermostable compounds can be effectively isolated from plant material using this technique (Wu *et al.*, 2015).

### **2.8. Non-conventional extraction methods**

Organic solvents are often hazardous, need long extract periods, while providing little selectivity of the extracted compounds; therefore, low yields of the desired compounds are common and thermal decomposition of thermolabile compounds may occur (De Castro and Garcia-Ayuso, 1998). Thus, alternative techniques are currently investigated to overcome limitations associated with conventional extraction methods (Garcia-Vaquero *et al.*, 2020b). These newer techniques are referred to as ‘green’, non-conventional techniques because they are less time-consuming, require a smaller volume of solvent and less energy than the conventional methods to complete the extraction; thus, they are regarded as ‘sustainable’. In addition, Picot-Allain *et al.* (2022) revealed that in the production of natural compounds that are recognized as safe and preferred by consumers, ‘green’, environmentally friendly solvents are vital. ‘Green’, non-conventional techniques must have a straightforward extraction protocol, be time-efficient and must use low volumes of organic solvents and have a decreased water- and energy-use (Khoddami *et al.*, 2013). In line with this ‘green chemistry’, Chemat *et*



*al.* (2020) defined ‘green extraction’ as a process based on the discovery and design of extraction procedures, which will reduce energy consumption, allow the use of alternative solvents and renewable products to achieve safe and high-quality extracts. It is, therefore, unsurprising that alternative solvents and the use of renewable plant materials for extraction, combined with lower energy uses in the production of such extracts are in demand. ‘Green chemistry’ is the concept of using naturally produced compounds as an alternative to minimize or eliminate the use and generation of hazardous substances (De la Guardia and Armenta, 2011). These ‘green’, non-conventional extraction techniques include ultrasound-assisted extraction, enzyme-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, pressurized liquid extraction and pulsed-electric field-assisted extraction (Kadam *et al.*, 2013; Hrnčić *et al.*, 2018).

### **2.8.1. Enzyme-assisted extraction (EAE)**

Plant material contains numerous phytochemicals; some are ubiquitous in the cell cytoplasm, while others are tightly bound to the cell wall or the polysaccharide-lignin frame by hydrophobic or hydrophilic bonds, rendering these compounds inaccessible when using standard solvent extraction procedures. Rosenthal *et al.* (1996) considered enzyme-assisted extraction (EAE) a practical approach, suitable for extracting lignin-bound polysaccharides. Enzymes, such as cellulases,  $\alpha$ -amylases and pectinases, play a crucial role in breaking down the cell wall in the EAE procedure, hydrolysing structural polysaccharides and lipids, thereby allowing a wider spectrum of chemicals to be extracted with high efficiency (Singh *et al.*, 1999). Latif and Anwar (2009) revealed that the EAE method comprises two extraction procedures, namely, enzyme-assisted aqueous extraction (EAAE) and enzyme-assisted cold pressing (EACP).

Various authors, including Rosenthal *et al.* (1996; 2001) and Sharma *et al.* (2002), reported that EAAE was developed mainly for the extraction of oils and certain water-soluble compounds from a wide variety of crops. Like in other extraction methods, several features affect the EAAE procedure; these include plant material particle size, hydrolysis time, solid-to-water ratio, enzyme composition and concentration (Hanmoungjai *et al.*, 2001). Lastly, the moisture content of the plant material has also been reported to be a critical factor in enzymatic hydrolysis (Dominguez *et al.*, 1995). Concha *et al.* (2004) further noted that, in the EACP method, enzymes are substantially used to hydrolyze cell walls. Due to non-toxic and non-

inflammable nature of EACP, Bhattacharjee *et al.* (2006) recognized this method as a safe alternative for extracting bioactive constituents from oilseeds.

Since EAAE uses water instead of organic solvents used in the conventional methods, Puri *et al.* (2012) described this technique as environmentally friendly for oil and aqueous bioactive compounds extraction. A comparative study by Dominguez *et al.* (1995) demonstrated a significant increase in free fatty acid and phosphorus concentrations in oil extracted using the EAAE method rather than conventional oil extraction with hexane. In addition, Meyer *et al.* (1998) tested EAAE to extract phenolic compounds from grape (*Vitis x vinifera*) pomace and observed a correlation between the yield in total phenolics and the degree to which plant cells were broken down through the enzymes' action. Landbo and Meyer (2001) compared the efficacy of various commercial pectinolytic enzymes, including Grindamyl™ pectinase, Macer8® FJ, Macer8® R, Pectinex® BE and Novozym® 89 protease, to extract phenolic compounds from blackcurrant (*Ribes nigrum*) pomace. These authors found that each of the tested enzymes, except Grindamyl™ pectinase, significantly increased the amount of total phenolic compounds extracted, compared with the extraction without these pectinolytic enzymes. These authors also observed that Macer8® FJ and Macer8® R enzymes decreased the concentration of anthocyanins.

Moreover, Li *et al.* (2006) tested a wide variety of enzymes using the EAE technique and found the enzyme “Celluzyme MX” to increase total phenolic compound extraction from the peel of five citrus peels, ‘Yen Ben’ and ‘Meyer’ lemon (*Citrus limon*), grapefruit (*Citrus x paradisi*), mandarin (*Citrus reticulata*) and orange (*Citrus sinensis*). Maier *et al.* (2008) used a 2:1 ratio of pectinolytic to cellulolytic enzymes for extracting bioactive compounds, such as phenolic acids, non-anthocyanin flavonoids and anthocyanins, from grape pomace and produced a positive effect on overall extract yield efficiency. Laroze *et al.* (2010) added twelve different commercial enzymes, such as Cellubrix®, Olivex®, Pectinex® Ultra SPL, Ultrazym® 100G, Viscozym®, Rohapect® 10L, Rohapect® DA6L, Rohapect® Max, Rohavin® L, Granozyme®, Grindamyl™ CA 150 and Maxoliva®, to a hydro-alcoholic extraction; these authors found that Grindamyl and Maxoliva extracted more phenolic compounds from raspberry (*Rubus idaeus*) solid waste than other enzymes used and extraction without enzymes. Various enzymes, including Celluclast®, Pectinex® and Novoferm®, were used by Gomez-Garcia *et al.* (2012) in determining the phytochemical composition of grape waste extracted using EAE. The authors observed that Novoferm® extracted the phenolic compounds from grape waste,

confirming that the EAE technology is powerful in extracting phenolics from agro-industrial by-products.

### **2.8.2. Ultrasound-assisted extraction (UAE)**

Ultrasound is a soundwave with a frequency above human hearing, ranging from 20 kHz to 100 MHz Azmir *et al.* (2013) revealed that ultrasound-assisted extraction (UAE) results in cavitation, which is the formation, growth and subsequent collapse of vapour bubbles in water by using ultrasonic irradiation. In UAE, an ultrasound device is placed in an appropriate position in a solvent extraction unit to enhance the extraction efficiency (Vinatoru *et al.*, 2001). Only liquid and liquid-containing solid materials display, however, cavitation. In UAE a significant amount of energy is produced when kinetic energy is converted into heat bubbles, rendering UAE an energy-saving method that is fast and efficient. Successful UAE is accomplished, when heat bubbles have a temperature of about 5000°C, under a pressure of 1000 atm and a heating and cooling rate above  $10^{10}$  K/s is achieved (Suslick, 1990). A wide range of phytochemicals from complex plant material can be extracted with this technology. As ultrasound waves disrupt plant cell walls, they accelerate tissue temperatures, and, thereby, mass transfer, resulting in an improved extraction of compounds of interest (Rosello-Soto *et al.*, 2015). Diffusion across the cell wall and rinsing the cell content with distilled water after breaking the cell walls, are two critical steps in UAE extraction (Mason *et al.*, 1996). Factors, such as sample moisture content, particle size of the plant material, and the chosen extraction solvent, significantly affect UAE extraction efficiency. In addition, other factors, such as temperature, pressure, wave frequency and sonication time, also affect UAE efficiency (Rajha *et al.*, 2015). Generally, UAE uses lower solvent volumes, is easier applicable, more time-efficient and financially more financially viable compared with conventional methods, especially maceration (Briones-Labarca *et al.*, 2015).

Riera *et al.* (2004) considered the UAE technique a promising alternative method to the standard maceration extraction, as UAE speeds up the rate at which these compounds are extracted and enhances the final concentration of the compounds present in the extract. Besides the relatively small volumes of solvent required, its ease of usage, time-efficiency and financial feasibility, its primary advantages are: facilitation of organic and inorganic compound isolation from complex plant material with little energy input (Herrera and De Castro, 2004), ultrasound energy is used to promote effective mixing of sample and solvent,



while reducing the extraction temperature, resulting in selective extraction, reduced equipment size, quick set-up of the extraction facility and reduced extraction time (Chemat *et al.*, 2008).

Ultrasound extraction has long been used to extract phytochemicals, such as polysaccharides, essential oils, proteins, peptides, pigments, and secondary plant metabolites from raw plant material (Tiwari, 2015). Xu *et al.* (2015) used water as a solvent in UAE and observed that the lengthened exposure of the material-solvent solution to ultrasound significantly increased the concentration of polysaccharides and natural antioxidant compounds extracted from *Eucommia ulmoides* (hardy rubber tree) using UAE. D'Alessandro *et al.* (2014) investigated the influence of various factors, including extraction time (0-240 min), solvent composition (ethanol-water ratio) and amount of ultrasound energy (0-100 W) applied during their extraction of bioactive compounds from fresh black chokeberry fruit (*Aronia melanocarpa*). These authors observed that ultrasound extraction is suitable to remove antioxidant compounds, especially anthocyanins and polyphenols from plant tissues.

Sivakumar *et al.* (2014) evaluated the usefulness of UAE in extracting tannins from *Senna auriculata* (Matura tea tree), demonstrating a 160 % increase in tannin concentration extracted when using 100 W ultrasound compared with simple magnetic stirring. In addition, Khan *et al.* (2010) studied the impact of the UAE method on the extraction of polyphenols, particularly flavonoids, from orange peel. The authors found using 150 W power and a 4:1 (ethanol: water) solvent ratio at 40°C to be the best for UAE, since this combination gave the highest flavonoids yield and antioxidant activity compared with standard solvent extraction (except sonification), proving UAE to be a suitable method for extracting phytochemicals from fruit. Various authors have used UAE to isolate biologically active compounds from moringa (*Moringa oleifera*) leaves, concluding that this method is a very good extraction means for this plant material, since higher yields in bioactive compounds were observed in UAE compared with conventional solid-liquid extraction methods (maceration) (Rodriguez-Perez *et al.*, 2015). Chen *et al.* (2015) reported that an ultrasound power of 190 W with a water-to-plant material ratio of 8:5, at 69°C for 75 min was the most-suitable condition for extracting polysaccharides and water-soluble antioxidants from black mulberry fruit (*Morus alba*).

### 2.8.3. Pressurized liquid extraction (PLE)

Pressurized liquid extraction (PLE) is an extraction technique that was first described in 1996 by Richter *et al.* (1996). In due course the PLE technique has been given several names, such as high-pressure solvent extraction (HPSE), enhanced solvent extraction (ESE), accelerated fluid extraction (ASE), pressurized hot solvent extraction (PHSE), pressurized fluid extraction (PFE) and high-pressure high-temperature solvent extraction (HPHTSE) (Nieto *et al.*, 2010; Alvarez-Rivera *et al.*, 2020). In this method, elevated pressure and temperature are exerted onto the sample solution in the extraction cell to fast-forward the extraction of biologically active compounds, allowing the solvent to remain liquid, although its normal boiling point is exceeded due to the high-pressure present (Alvarez-Rivera *et al.*, 2020). The PLE technology was developed to reduce extraction time and solvent volume required for extraction. As a result, Richter *et al.* (1996) reported that PLE uses smaller volumes of solvents (~100-200 mL/g plant material) and a shorter extraction time (~20 minutes) compared with traditional methods, such as Soxhlet extraction. Alvarez-Rivera *et al.* (2020) also revealed that elevated temperatures in PLE methods, especially in the PHSE, increase compound solubility and mass transfer rate, concurrently reducing solvent viscosity and surface tension, thereby improving the extraction rate. Kaufmann and Christen (2002) indicated that the effectiveness of PLE for natural compound extraction makes it the best alternative to supercritical fluid extraction for the removal of polar bioactive constituents from the plant material. Wang and Weller (2006) reported that PLE can also be successfully used to extract organic compounds from plant material that are stable at high temperatures (> 80°C). According to Ibanez *et al.* (2012), PLE has, therefore, gained popularity as one of the ‘green extraction techniques’ due to organic solvent required for the extraction of compounds than in the commonly used Soxhlet extraction and maceration methods.

Rostagno *et al.* (2004) reported that PLE improved the extraction of isoflavones from freeze-dried soybean (*Glycine max*) without degradation of these compounds at temperatures between 60 to 200°C, 100 atm, when applied for 5 min. Shen and Shao (2005) compared PLE, Soxhlet extraction and UAE to demonstrate that PLE outperformed the other two methods, when extracting terpenoids and sterols from tobacco (*Nicotiana tabacum*) leaves, with regard to the yield of the compounds analysed, the extraction duration, the assay’s reproducibility and solvent consumption. Therefore, due to the short extraction duration and the small volume of



solvents used, PLE has been considered as an alternative to classic extraction methods. Howard and Pandjaitan (2008) discovered that using PLE with 70% ethanol as solvent at the same elevated temperature of 50 to 150°C extracted more flavonoids from spinach (*Spinacia oleracea*) than using water as a solvent. The extraction of phenolic compounds from parsley (*Petroselinum crispum*) flakes, using PLE, can be positively enhanced by altering various parameters, including pressure, temperature, flush volume, particle size and solid to solvent ratio (Luthria, 2008). In addition, Erdoğan and Erdemoğlu (2011) observed that PLE, used at 40°C, 1500 psi pressure and for 15 min, effectively extracted total phenolics as well as individual phenolic compounds, such as chlorogenic acid, myricetin, caffeic acid, catechin, epicatechin and gallic acids from apricot (*Prunus armeniaca*). Mroczek and Mazurek (2009) also compared PLE with hot-solvent, microwave-assisted and UAE, discovering PLE to outperform other extraction methods for lycorine and galanthamine (Amaryllidaceae alkaloids) extraction from jonquil (*Narcissus jonquilla*).

#### **2.8.4. Microwave-assisted extraction (MAE)**

Over the past decades, microwave-assisted extraction (MAE) has gained increasing recognition as an innovative, financially feasible, time-efficient and eco-friendly ‘green’ technology to extract a wide range of biologically active compounds, especially from food materials (Alupului, 2012). Microwaves are electromagnetic fields made up of an electric and magnetic field, oscillating perpendicular to one another, with a frequency range of 300 MHz to 300 GHz (Zhi *et al.*, 2017). According to Letellier and Budzinski (1999), the heating effect of microwaves depends on their direct impact on the polar material. Microwaves are a non-contact source of heat that can supplement heating, thereby accelerating the conversion of electromagnetic energy into thermal energy (Azmir *et al.*, 2013). Electromagnetic energy is converted into heat energy primarily due to ionic conduction and dipole rotation (Jain, 2009). Heat generation during ionic conduction may be due to the resistance of a medium to allow the flow of ions; so, the higher the resistance, the more heat generation (Azmir *et al.*, 2013).

Li *et al.* (2013) indicated that the MAE method can efficiently isolate several bioactive compounds, such as essential oils, certain antioxidants, pigments, aroma compounds, and other organic compounds from fresh and dried plant material. In MAE, the extraction duration is reduced compared with traditional extraction methods, while the yield and purity of the compounds extracted are commonly improved (Leadbeater, 2014). In addition, Gomez *et al.*

(2020) revealed that MAE requires smaller solvent volumes than standard liquid extraction, making it another primary advantage of this extraction technique. Not only does MAE not require large volumes of solvent, but it also has a low energy consumption to heat the material, allowing easy extraction of targeted compounds, as well as a reduced extraction period and reduced CO<sub>2</sub> emission; thus, making MAE an excellent alternative extraction technique to extract various bioactive constituents (Gomez *et al.*, 2020).

Simha *et al.* (2016) reported the ability of MAE to recover bioactive compounds from pharmaceutically essential plants, such as lemon grass (*Cymbopogon citratus*) and the Malabar nut tree (*Adhatoda vasica*). A comparative study performed by Grigoris *et al.* (2012) demonstrated that MAE was a better method to extract phenolic compounds, such as gallic acid, catechin, chlorogenic acid, rutin and quercetin from pomace of four apple (*Malus x domestica*) varieties, than maceration, pressurized liquid and ultrasound extraction. Additionally, Krishnan and Rajan (2016) investigated the effect of solvent-to-feed ratio (5-40 ml/g) and temperature (40-100°C) on flavonoid yield, extraction rate and effective diffusion coefficient of Belleric myrobalan (*Terminalia bellerica*) fruit material, when using water as an extraction solvent. These authors discovered that a combination of solvent-to-feed ratio (40 ml/g) and temperature (100°C) produced higher flavonoid yield (25 mg QE/g FM) than other solvent-to-feed ratio and temperature combinations. These authors, therefore, concluded that MAE, when using water as an extraction solvent, is a suitable method for flavonoid extraction.

Simic *et al.* (2016) used different combinations of microwave power (300, 450 and 600 W), ethanol concentrations (25, 50 and 75%) and extraction time (5, 10 and 15 min) to optimize extraction of total phenolic compounds from chokeberries (*Aronia melanocarpa*) using MAE technique. These authors observed that a combination of 600 W, 50% ethanol and 15 min extraction time yielded the highest total phenolic concentration (458.4 mg GAE/100 g FW) compared with other tested combinations. In addition, Seixas *et al.* (2014) revealed that extraction time and microwave power have a considerable effect on pectin yield extracted from passion fruit (*Passiflora edulis*) skin regardless of acid (*viz.* acetic, nitric and tartaric) used during extraction; a combination of 628 W and 9 min produced the highest pectin yield (acetic acid: 13.0%, nitric acid: 12.9% and tartaric acid: 30.3) in comparison with other combinations tested.

### 2.8.5. Pulsed-electric field extraction (PEF)

In 2006, Vorobiev and Lebovka reported the pulsed-electric field extraction (PEF) technology as a valuable tool for accelerating various plant material preparation processes, including the pressing and drying of plant samples and the extraction of targeted compounds from complex plant material. This extraction technology triggers the breaking down of cell membranes and cell walls due to the short, high-voltage pulses applied, thereby increasing the release of targeted compounds, while not destroying them. This short exposure of a living cell to a high-voltage electric field destroys the cell membrane, hence, creating ‘pores’ in the cell structure, ultimately increasing cell wall and membrane permeability, making it possible for the electric potential to travel through the cell (Toepfl *et al.*, 2006, Azmir *et al.*, 2013; Rajha *et al.*, 2015). ‘Pores’ are created by the repulsion of charge-carrying molecules, thus, increasing cell permeability soon after the transmembrane potential exceeds a critical value of nearly 1 V (Bryant and Wolfe, 1987). Soliva-Fortuny *et al.* (2009) investigated the “pumpable fluid treatment system”, which consists of a PEF generation unit comprising two electrodes, a high-voltage generator and a pulse generator.

The PEF extraction method is considered a valuable approach for isolating compounds of interest from a variety of plant samples, mainly due to its ability to weaken and destroy the cell membrane, thereby also allowing the release (and subsequent extraction) of cell membrane-bound compounds (Rosello-Soto *et al.*, 2015). Azmir *et al.* (2013) indicated that the success of the PEF technology entirely depends on plant material, input energy, temperature, electric field strength and pulse frequency. By destroying the cell membrane structure, PEF could increase the extraction efficiency and yield of targeted compounds at reduced extraction time. According to Fincan and Dejmek (2002), the PEF method increased cell membrane degradation when using electric fields ranging from 500 to 1000 V/m and time ( $10^{-4}$  –  $10^{-2}$  s) regardless of other factors. Ade-Omowaye *et al.* (2001), therefore, concluded that the employment of PEF methodology could reduce the deterioration of heat-sensitive phytochemicals. In addition, Lopez *et al.* (2009) reported that PEF application, when used as a pre-treatment to conventional extraction, would positively affect the extraction process by lowering the extraction duration and improving the ease of extraction.

Furthermore, Parniakov *et al.* (2016) revealed that recent scientific studies have documented the PEF technique to be a ‘green extraction technology’ because renewable plant resources and alternative, simple solvents, such as water or typical ‘organic solvents’ (*i.e.*, ethanol, methanol,



chloroform), are used. Fincan *et al.* (2004) used the PEF method at low voltage and low energy (1 kV/cm electric field strength and 7 kJ/kg) to extract bioactive compounds from beetroot (*Beta vulgaris subsp. vulgaris*) and a significant increase in the characteristic red betanin pigment concentration was observed. In addition, Guderjan *et al.* (2005) demonstrated a 32 % increase in phytosterols recovered from freeze-dried maize (*Zea mays* L.) cobs and a 20-21 % increment in isoflavonoids, such as genistein and daidzein, extracted from freeze-dried soybean seeds (*Glycine max* L.), when using the PEF method. Various extraction techniques have removed biologically active compounds, such as anthocyanins, from grape (*Vitis x vinifera* L.) by-products. The PEF technique outperformed other methods in extracting the red pigments, anthocyanin mono-glucosides, from grape skin (Corrales *et al.*, 2008). Lopez *et al.* (2008) also showed that PEF application to grape skin before maceration, during the winemaking (vinification) process, can decrease the maceration duration while increasing the stability of bioactive compounds (e.g., anthocyanin and other polyphenols). Dersart *et al.* (2012) further discovered that PEF treatment increased the permeability of 'Merlot' grape skin, resulting in an increased polyphenol and anthocyanin extraction from the skin.

#### **2.8.6. Supercritical fluid extraction**

Supercritical fluid extraction (SFE) was first discovered in 1880 by Hannay and Hogarth, however, the German chemist "Kurt Zosel" should also be given credit for demonstrating its efficacy and patenting it for coffee decaffeination (Azmir *et al.*, 2013). Since then, supercritical fluid extraction technology has gained widespread scientific attention and has been effectively applied in many industries for environmental, pharmaceutical, polymer and food analysis (Zougagh *et al.*, 2004). This method has been employed in various industries but has become particularly known for the preparation of decaffeinated coffee (Ndiomu and Simpson, 1988). Every material on Earth can exist in three, distinctive phases of matter: liquid, gas and solid. Critical point is defined as the "end point of a phase equilibrium curve, where critical temperature and critical pressure" intersect (Azmir *et al.*, 2013). If a substance is subjected to a certain temperature and pressure beyond its critical point, it reaches a certain distinctive phase, referred to as the "supercritical state". Above the critical point, gas and liquid phases are indistinguishable (Pini *et al.*, 1998). By modifying temperature and pressure, certain gas and/or liquid properties vanish, so the supercritical fluid could no longer be classified as a liquid or gas.



In addition to gas-like properties, supercritical fluids exhibit liquid-like properties, such as diffusion, surface tension, viscosity and density. Due to these characteristics, SFE is recognized as a technology well-suited to extracting biological active constituents quickly and with higher yields than conventional liquid-solid methods (Sihvonen *et al.*, 1999). Convection of molecules in a solvent, which is in supercritical stage, serves as the primary transport mechanism of targeted compounds from plant materials (Silva and Martínez, 2014), as SFE can be performed using small amounts of sample and is quick and selective; additionally, the separated compounds do not need further purification (Oroian and Escriche, 2015). The potential to apply the technology in association with analytical and chromatographic technologies, including gas chromatography (GC) and supercritical fluid chromatography (SFC), is another significant benefit (Da Silva *et al.*, 2016). Supercritical fluids possess advantageous transport characteristics that improve their efficacy in extracting targeted compounds.

Compared with liquid solvents used in conventional extraction processes, supercritical fluids have low viscosity and surface tension, thus, spreading easily within a specific solid matrix, promoting rapid solvent penetration into the solid; as a result, extraction efficiency is increased. Raverchon and Marco (2006) revealed that the success of SFE mostly relies on its tuneable factors, such as temperature and pressure, which can be precisely controlled to maximize SFE efficiency. Other factors, such as sample moisture content and particle size, extraction time, and the solvent-to-solid ratio, are also important variables influencing SFE efficiency (Ibanez *et al.*, 2012). Supercritical fluids, including CO<sub>2</sub>, propane, cooking gas (LPG), ethane, methanol, nitrous oxide, n-butene, n-pentene, sulphur hexafluoride and water, have been used in various studies (Da Silva *et al.*, 2016). Out of all these supercritical fluids, carbon dioxide (CO<sub>2</sub>) is recognized as an ideal solvent for the SFE technique due to two main reasons: (i) it reaches its critical point at temperature not too far from room temperature ( $T = 31\text{ }^{\circ}\text{C}$ ) and low pressure (74 BAR), thereby preserving of active ingredients of extract from being lost or destroyed, and (ii) the extract is preserved from contact with air (oxygen) and light, where oxidation reactions occur more rapidly (Temelli and Güçlü-Üstündağ, 2005). As much as CO<sub>2</sub> is recognized as a suitable supercritical fluid for extracting lipids, fats and non-polar compounds, it is not ideal for most pharmaceuticals and drugs, due to its low polarity. These limitations of low CO<sub>2</sub> polarity have been successfully overcome through chemical modifiers, such as ethanol, methanol and acetone, which are chemical substances added to the sample solution to increase the solubility of targeted compounds (Ghafoor *et al.*, 2010). Chemical modifiers vary, however, with the sample properties and compounds of interest.

Due to the fact that CO<sub>2</sub> is an ideal supercritical fluid used in the SFE technique, this extraction method is primarily used to extract non-polar bioactive compounds, such as carotenoids and other lipids from different plant materials (Herrero *et al.*, 2013). Various authors have reported that CO<sub>2</sub> can be successfully used as a supercritical fluid to extract phenolics and other antioxidant compounds from medicinal plants, namely marcela (*Achyrocline satureioides*) fruit (Vargas *et al.*, 2013), and other fruit, such as mango (*Mangifera indica*) (Maneses *et al.*, 2015), Japanese quince (*Chaenomeles japonica*) (Song *et al.*, 2016) and passion fruit (*Passiflora edulis*) (Vigano *et al.*, 2016) as well as various vegetables (Bagheri *et al.*, 2016). The application of CO<sub>2</sub> as a supercritical fluid for the selective extraction of antioxidants from a bilberry (*Vaccinium myrtillus*) was studied by Babova *et al.* (2016), who found that supercritical fluid treatments positively influenced the extraction of targeted compounds, including anthocyanins and other phenolic compounds. In addition, Malaman *et al.* (2011) used the SFE method at different temperature and pressure regimes for the extraction of chemical constituents from Brazilian cherry (*Eugenia uniflora* L.) and observed a significant increase in total phenolic concentrations at an extraction temperature of 50°C compared with other temperature regimes, independent of pressure.

Furthermore, in order to extract carotenoids and chlorophyll *a*, *b*, and *c* from the microalga *Scenedesmus obliquus* for use in food processing, Guedes *et al.* (2013) employed the SFE techniques at 250 BAR, maximum pigment yields were attained. For chlorophyll, a temperature of 40°C was the best, whereas, for carotenoids, a temperature of 60°C was optimal. At a temperature of 60°C and 24 Pa, red pepper fruit (*Capsicum annum* L.) produced the highest vitamin E (97 %) and provitamin A (68 %) yields. Although CO<sub>2</sub> is a suitable solvent for SFE in extracting bioactive compounds from complex material, it has limited capacity to dissolve compounds of high molar mass, such as carotenoids (Araus *et al.*, 2012). Ashraf-Khorassani and Taylor (2004) used the SFE method to extract polyphenols and procyanidins from grape seeds and observed significant catechin and epicatechin concentrations (approximately 79 %) when 40 % methanol was used as a chemical modifier.

## **9. Conclusion and future prospective**

Due to agricultural expansion, safe, simple, cheap, eco-friendly and sustainable approaches to achieve high-quality yields of agricultural crops must be sought. These approaches should minimize the utilization of synthetic pesticides, as there is growing concern about their impact

on human health and the environment. Therefore, interest in using plant extracts as natural biostimulants has increased significantly as an alternative strategy, not only to enhance crop yields, but also to produce plants rich in health-promoting compounds, making such commodities valuable to consumers. According to the presented literature, various natural plant extracts have the potential to increase the production of crops and their quality in a sustainable manner. Although many scientific studies have offered substantial evidence to encourage the use of natural plant extracts, additional information is required to improve the understanding of the efficacy of the extraction techniques and their mechanism of action in agricultural crops. The demand for bioactive compounds from plant extracts is constantly increasing, driving an ongoing search for more convenient and efficient extraction methods. The advancements in chromatography and the increased environmental awareness are two important factors contributing to the development of new, non-conventional extraction processes. A clear understanding of these non-traditional extraction processes is critical because various mechanisms contribute to their enhanced extraction efficiency. The incorporation and development of hybrid methods should also be investigated in light of plant material characteristics and compound selection. On the other hand, the growing economic importance of bioactive compounds and bioactive-compound-rich commodities may lead to the development of more sophisticated extraction methods in the future.

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## CHAPTER THREE

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### **Impact of Foliar-applied Plant Extracts on Growth, Physiological and Yield Attributes of Potato (*Solanum tuberosum* L.)**

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**Mbuyisa, S., Bertling, I. and Ngcobo, B.L., 2023. Foliar Application with Plant-Derived Extracts Enhances Growth, Physiological Parameters, and Yield of Potatoes (*Solanum tuberosum* L.). In Conference Proceedings: *Biology and Life Sciences Forum*, 27(1), 34. Published: 27 October 2023. <https://doi.org/10.3390/IECAG2023-15385>**

(This chapter has been published in *Biology and Life Sciences Forum*, a Journal by MDPI)

**Mbuyisa, S., Bertling, I. and Ngcobo, B.L., 2023. Impact of Foliar-applied Plant Extracts on Growth, Physiological and Yield Attributes of Potato (*Solanum tuberosum* L.). *Agronomy*, Accepted: 21 December 2023.**

(This chapter has been accepted for publication as a fulltext article paper by *Agronomy*, a Journal by MDPI)

(Fulltext article paper attached after appendix)



## Abstract

The current reliance on pesticides and synthetic fertilizers has been vital to sustain and increase agricultural production. The continuous, excessive use of these traditional practices has negatively affected consumers' health and burdened the ecosystem. The use of plant extracts as a tool to minimize agrochemical inputs has been extensively investigated due to their ability to improve plant growth and agricultural productivity. This study was, therefore, conducted to determine the effect of foliar plant extract application on potato growth, as well as certain physiological and yield attributes. From four weeks after planting onwards, five healthy, equal-sized potato plants per treatment received various foliar plant extract applications. These extracts included brown seaweed *Ascophyllum nodosum* extract (ANE), aloe vera leaf extract (AVE), garlic bulb extract (GBE) and moringa leaf extract (MLE). Application of treatments were repeated weekly until harvesting. Data on growth and physiological parameters were collected weekly. Pre-harvest foliar application of various plant extracts significantly enhanced ( $p \leq 0.05$ ) plant growth, physiological and yield attributes of potatoes. Best growth and yield responses were observed following ANE and MLE application. A positive influence of various foliar plant extract applications on growth and yield of potatoes was demonstrated; however, further validation of the response by other crops is still necessary, in an attempt to popularize this application.

Keywords: biostimulants, food security, plant extracts, potato, sustainable agriculture

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### 3.1. Introduction

Potato (*Solanum tuberosum* L.) is a member of the Solanaceae family, native to South America, particularly in Peru, but is now grown in most parts of the world (Hussain, 2016). Amongst the cash crops, potato is one of the world's most important non-grain food crops with a global production of about 376 million tonnes, with China as the largest producer contributing approximately 94 million tonnes annually (FAO, 2021). Potato is also recognized as a staple food, being the third most-consumed food crop worldwide, following rice and wheat (Hassain,

2016). The worldwide *per capita* potato consumption reached 33.1 kg in 2020; this is possibly due to the health and nutritional benefits potato offers (FAO, 2021). According to Zaheer and Akhtar, (2009), potato is an excellent source of dietary fibre, carbohydrates, high-quality protein, vitamins, minerals and other metabolites. Being rich in health-promoting metabolites, potato possesses high antioxidant activity, which helps to reduce the risk of chronic diseases, including heart disease, diabetes and cancer (Khansari *et al.*, 2009).

In the last couple of decades, there has been a rapid increase in potato demand. For all potato growers, it has, therefore, become of immense importance to produce this crop profitably while keeping input costs at a minimum. Additionally, modern agriculture demands sustainable crop production, searching for alternative methods to sustain plant development with little or no compromise to yield. Both subsistence and commercial farmers are facing a major challenge of biotic and abiotic factors aligned with climate change. These include drought, salinity, weed infestation, pests and diseases, which can all devastatingly affect growth and yield of potatoes (Parajuli *et al.*, 2019). Given these challenges, synthetic pesticides and inorganic fertilizers have become vital for the production of crops and their protection against biotic and abiotic constraints (Sharma *et al.*, 2019). The reliance on industrially based inputs may, however, pose multiple threats to human health and impart harmful effects on the ecosystem (Bulgari *et al.*, 2019; Zulfiqar *et al.*, 2020). In addition, Lucas *et al.* (2015) revealed that the continuous, excessive usage of such chemicals might result in the development of new pathogen strains that could become difficult to control, despite the efficacy of the chemical. The aim of modern plant agriculture is, therefore, to reduce the utilization of these chemicals to a minimum; thus, making crop farming simpler and offering healthier, safer and sustainably produced goods.

Farmers and researchers are, therefore, continuously exploring and developing alternative approaches to crop farming, trying to overcome challenges of long-term production viability (Zulfiqar *et al.*, 2020). Among several proposed strategies, the use of plant extracts has been identified as a promising, innovative, eco-friendly and sustainable approach that could improve crop production and crop protection. Recent studies have tested this method on a broad spectrum of solanaceous crops, such as potato, sweet pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.) (Haider *et al.*, 2012; Dunsin and Odeghe, 2015; Basra and Lovatt, 2016; Ngcobo and Bertling, 2021; Ahmed *et al.*, 2022). The present study, therefore, aims to evaluate the influence of foliar application of plant extracts (*viz.* *Ascophyllum nodosum* extract, garlic bulb extract, aloe vera leaf extract and moringa leaf extract) on growth, certain physiological and yield attributes of potato.

## 3.2. Materials and methods

### 3.2.1. Planting material and growing conditions

A pot experiment was carried out in a glasshouse at the Controlled Environment Facility (CEF) of the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29°37'32.9"S 30°24'18.8" E). Locally obtained baby potatoes, cv. 'Sifra', were planted in June (mid-winter) as seed tubers at a depth of 10 cm into 10 L plastic pots filled with a mixture of sandy soil and Gromor® (Gromor, Cato Ridge, South Africa) potting mix. Chemical and physical attributes of the soil and growing medium used were analysed before planting (Cedara College of Agriculture, Department of Agriculture and Rural Development, KwaZulu-Natal) (Table A1). Poultry manure (Nutri-Green Gwano Pellets, Protek, Heidelberg, Gauteng, South Africa) (Table A3), was then applied to amend soil nutrition, at a rate of 25 g/pot, based on the chemical and physical characteristics of the used medium (Table A2). Thirty days after planting, the same fertilizer was re-applied as a top-dressing at a similar rate (25 g/pot). The environmental conditions inside the glasshouse were maintained at  $25 \pm 2^\circ\text{C}$  and 65% relative humidity (RH) during the day, while temperature and RH were kept constant at  $13 \pm 2^\circ\text{C}$  and 72% at night, respectively. Plants were irrigated using automated drip irrigation, dispensing approximately 500 mL per 10 L pot daily.

### 3.2.2. Experimental design and foliar application

The study was laid out following a completely randomized design (CRD) with five replications. Five healthy, similar-sized 'Sifra' sprouted baby potatoes, randomly selected, were used per treatment, with five seed tubers per replicate, giving 25 experimental units (10 L pots). The experiment consisted of four treatments, namely *Ascophyllum nodosum* extract (ANE), moringa (*Moringa oleifera*) leaf extract (MLE), garlic (*Allium sativum*) bulb extract (GBE) and aloe vera (*Aloe barbadensis* Mill.) leaf extracts (AVE), plus the control (no extract application). The above-mentioned treatments were directly applied to potato leaves using a hand-held pressure sprayer, each plant received 50 mL. The first foliar application of treatments was performed four weeks after planting (vegetative stage) and treatment applications were repeated weekly until harvest (mid-August, early spring), plants received 12 foliar sprays in total until harvesting.



### 3.2.3. Collection and extract preparations

Plant material used for extracts preparation was obtained from various suppliers. Brown algae (*Ascophyllum nodosum*) powder (Nature's Choice) was bought locally (Dis-Chem, Woodburn Mall, Pietermaritzburg, South Africa), whereas healthy aloe vera plants were bought locally from Woodland nursery, (Pietermaritzburg, South Africa). Fresh moringa (*Moringa oleifera*) leaf powder (MLP) was supplied by a commercial supplier (runKZN, Pietermaritzburg, South Africa), while fresh Egyptian white garlic was bought from a local supermarket. The used extracts were prepared following the procedure described by Ngcobo and Bertling (2021) (ANE and MLE), Noor *et al.* (2008) (AVE) and Ting-Ting *et al.* (2011) (GBE), with slight modifications. Exactly 10 g of each plant material was weighed out and homogenized in a glass beaker with 450 mL distilled water. The homogenates were then placed onto a hot plate, continuously agitated with a magnetic stirrer and allowed to boil at 100°C for 30 min. After 30 min, the solutions were allowed to stand for 2 hrs to cool down; then the supernatants were collected and filtered three times through muslin cloth. Then, to make a final volume of 1 L, serial dilutions were made with distilled water. Furthermore, the chemical composition of all the extracts were analysed and the information is presented in **Table 3.1**.

**Table 3.1:** Mineral and phytochemical composition of various plant extracts. GAE (gallic acid equivalent), QE (quercetin equivalent) and AAE (ascorbic acid equivalent).

Composition	ANE	AVE	GBE	MLE	Units
Nitrogen (N)	0.08	0.11	-	0.17	%
Phosphorus (P)	4.2	2.2	16.0	10.4	%
Potassium (K)	102.6	79.4	96.8	106.4	%
Calcium (Ca)	10.4	58.6	-	77.4	%
Magnesium (Mg)	16.6	24.0	2.6	23.2	%
Sodium (Na)	127.2	3.0	2.2	0.8	mg/kg
Manganese (Mn)	-	4.0	-	-	mg/kg
Copper (Cu)	-	-	-	-	mg/kg
Zinc (Zn)	0.9	-	-	1.2	mg/kg
Iron (Fe)	-	-	-	-	mg/kg
Ascorbic acid (Vitamin C)	86.5	78.1	57.1	79.7	mg/g DM
Total phenolic content (TPC)	11.9	2.1	2.4	3.9	mg GAE/g DM
Total flavonoids content (TFC)	28.6	3.3	11.7	51.7	mg QE/g DM



Total protein content	104.7	53.7	61.1	81.5	mg/g DM
Antioxidant activity [DPPH (diphenyl-1-picrylhydrazyl)]	105.3	94.9	90.5	95.4	mg AAE/g DM

\* For mineral composition, plant extracts were analysed using inductively coupled plasma mass spectrometry (ICP-MS), whereas the analyses of phytochemicals were carried out following methods of Boonkasem *et al.* (2005) (ascorbic acid concentration), Wang *et al.* (2010) (total phenolics), Gu *et al.* (2019) (total flavonoids), Bradford (1976) (total protein) and Rocchetti *et al.* (2019) (antioxidant activity-DPPH) using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

### 3.2.4. Determination of vegetative growth and certain physiological and yield parameters

#### 3.2.4.1. Plant height and number of leaves

Plant growth parameters, including plant height, number of fully expanded leaves and number of branches, as well as leaf chlorophyll index, were recorded from the first treatment application until stage 4 (tuber bulking) (Awgchew *et al.*, 2016) of potato growth and development, tuber bulking, at 7-day intervals. Plant height (cm) was measured from the base of the stem to the tip of the terminal bud using a tape measure. The total number of leaves were counted manually.

#### 3.2.4.2. Leaf area

From the first treatment application until the tuber bulking stage, leaf area of the entire potato plant was estimated directly from leaf length and width measurements. Leaf area was then calculated using the formula described by Bhatt and Chanda (2003).

$$LA = 11.98 + 0.06 L \times W;$$

where LA = Leaf area (cm<sup>2</sup>), L = leaf length (cm) and W = leaf width (cm).

#### 3.2.4.3. Leaf chlorophyll content index

Leaf chlorophyll content index was determined using a portable, non-destructive and lightweight instrument (CCM-200plus-Opti-Sciences Inc., Hudson, NH, USA). At tuber bulking stage, the chlorophyll content index was measured from three fully developed functional leaves on each potato plant. A total of four plants, randomly selected from each treatment, were measured.

#### **3.2.4.4. Fresh and dry above-ground biomass**

Both fresh and dry above-ground biomass (g/plant) were determined using a fine balance. Fresh mass was recorded immediately after harvesting (mid-August, early spring), whereas dry mass was measured after four days of oven-drying at 80°C.

#### **3.2.4.5. Yield and fresh tuber mass**

At the mature tuber stage, all tubers were harvested from all replicates. Total tuber yield (tuber number/plant) and tuber mass (g) were recorded immediately after harvesting (mid-August, early spring).

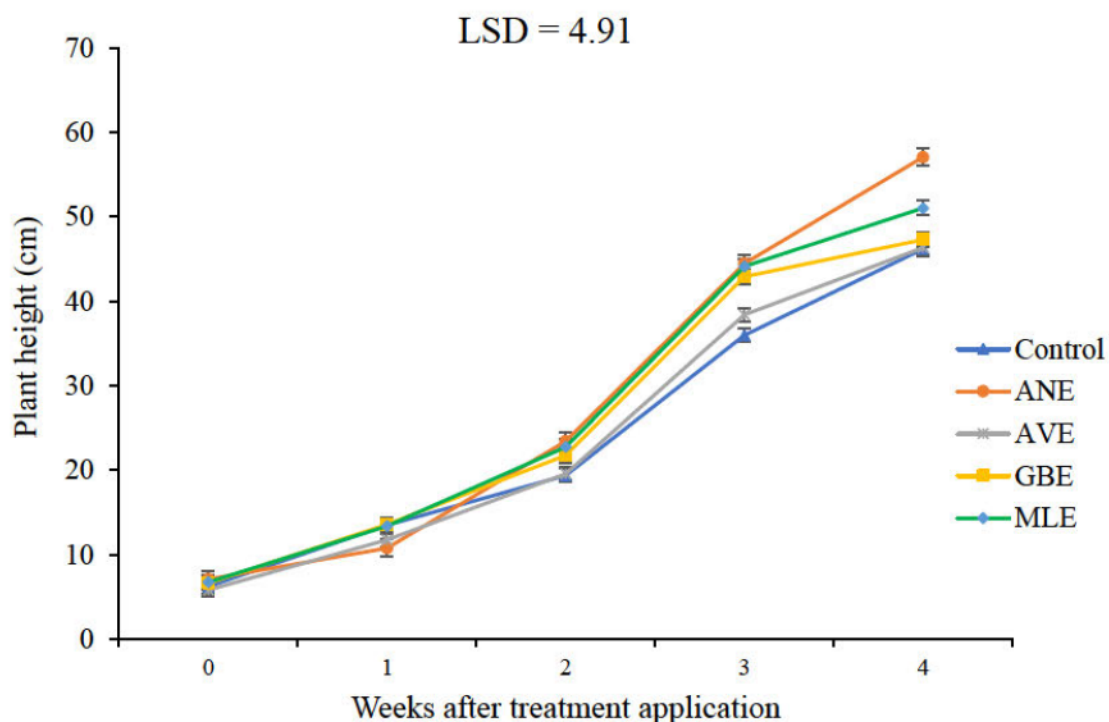
#### **3.2.5. Statistical analysis**

Results obtained were subjected to one-way analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 18th edition, VSN International, UK) and plotted using Microsoft Excel®. Means separation were carried out using Duncan's multiple range test with a difference at  $p \leq 0.05$  considered significant; LSD (Least Significant Difference) values were compared at a 5 % significance level.

### **3.3. Results**

#### **3.3.1. Plant height**

Variations in morphological data revealed a significant effect of plant extract application on the growth of potatoes in terms of plant height. The results indicate that pre-harvest application of ANE at the vegetative stage significantly influenced ( $p \leq 0.05$ ) potato plant height, unlike other treatments, four weeks after treatment application (Fig. 3.1). This treatment, hence, resulted in the tallest plants (57.04 cm), followed by MLE-treated ones, which recorded 51.02 cm on the 4<sup>th</sup> week after treatment application (Fig. 3.1).




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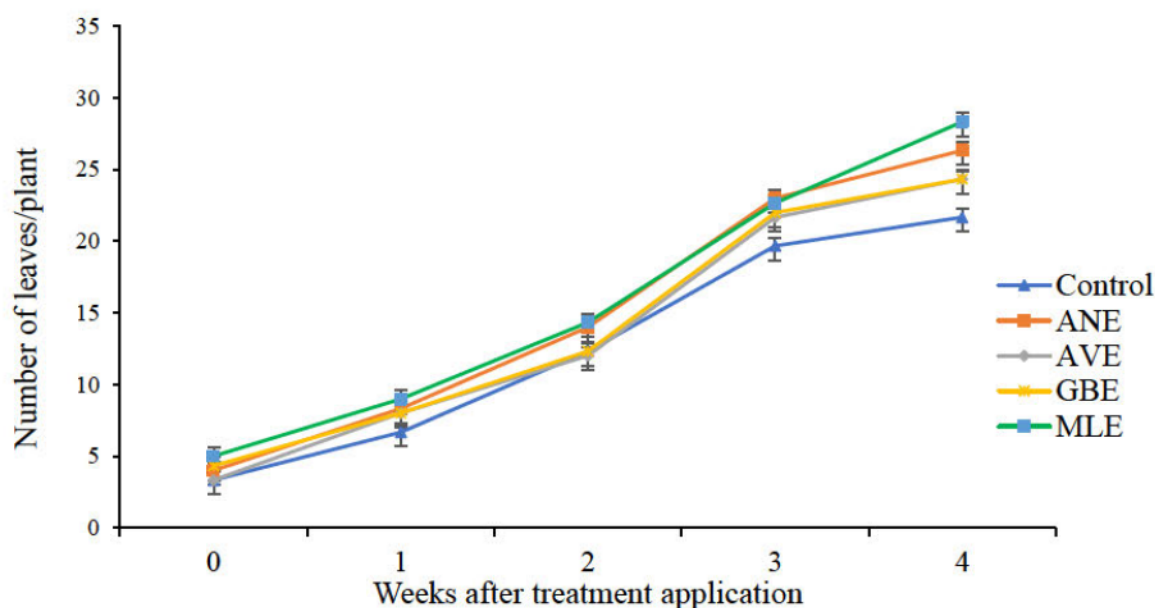
**Figure 3.1:** Effect of foliar-applied plant extracts on plant height of potato at the vegetative stage. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 4.91.

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### 3.3.2. Number of leaves

Four weeks after treatment application, number of leaves in all treatments was significantly higher than in the control ( $p \leq 0.05$ ). The MLE treatment recorded the highest number of leaves (28), followed by ANE (26 leaves) (Fig. 3.2); the number of leaves recorded in the AVE and GBE treatment was less than by ANE and MLE, both bore 24 leaves.

LSD = 1.89

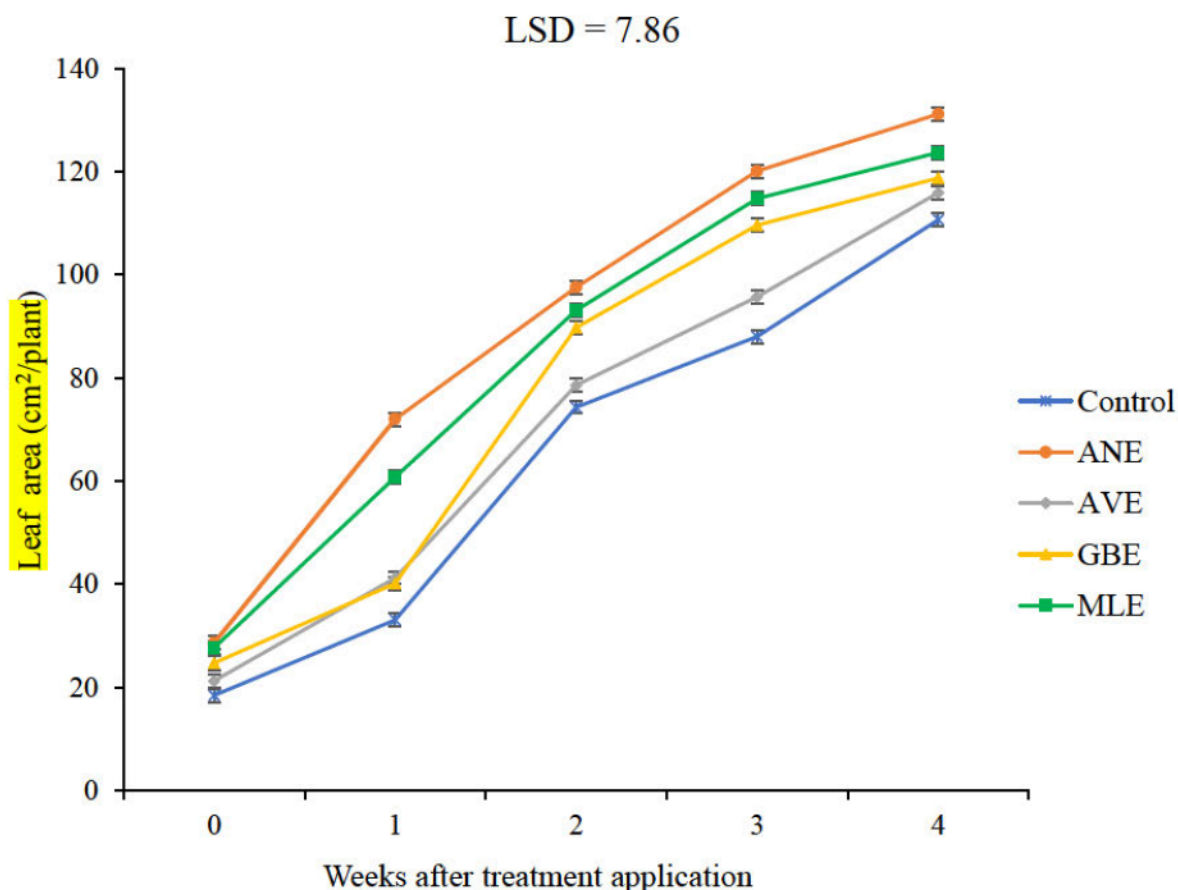


**Figure 3.2:** Effect of weekly applied plant extracts on the number of potato leaves. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 1.89.

### 3.3.3. Leaf area

The treatment of potato plants with various plant extracts positively influenced leaf area development. Four weeks after treatment application, the ANE-treated potato plants produced the largest leaf area (131.13 cm<sup>2</sup>/plant), followed by plants treated with MLE, GBE and AVE (123.67, 118.72 and 115.86 cm<sup>2</sup>/plant, respectively). The smallest leaf area was, however, recorded for the control (110.63 cm<sup>2</sup>/plant). Obtained results were significantly different ( $p \leq 0.05$ ) from each other (Fig. 3.3).

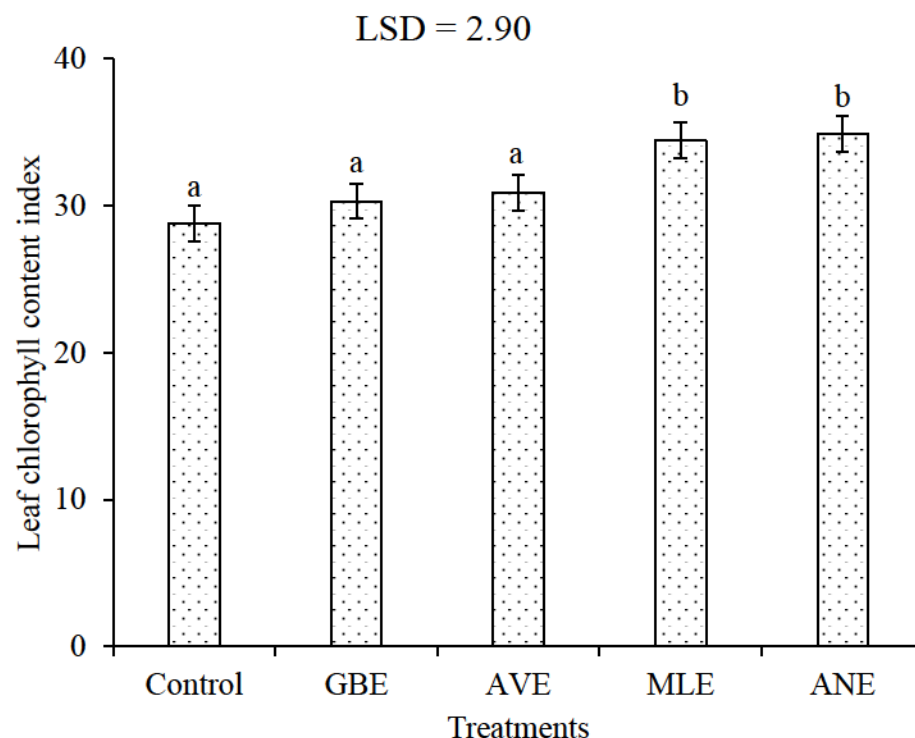




**Figure 3.3:** Effect of foliar-applied plant extracts throughout various stages of plant growth on the leaf area of potatoes. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf). LSD at ( $p \leq 0.05$ ) = 7.86.

### 3.3.4. Leaf chlorophyll content index

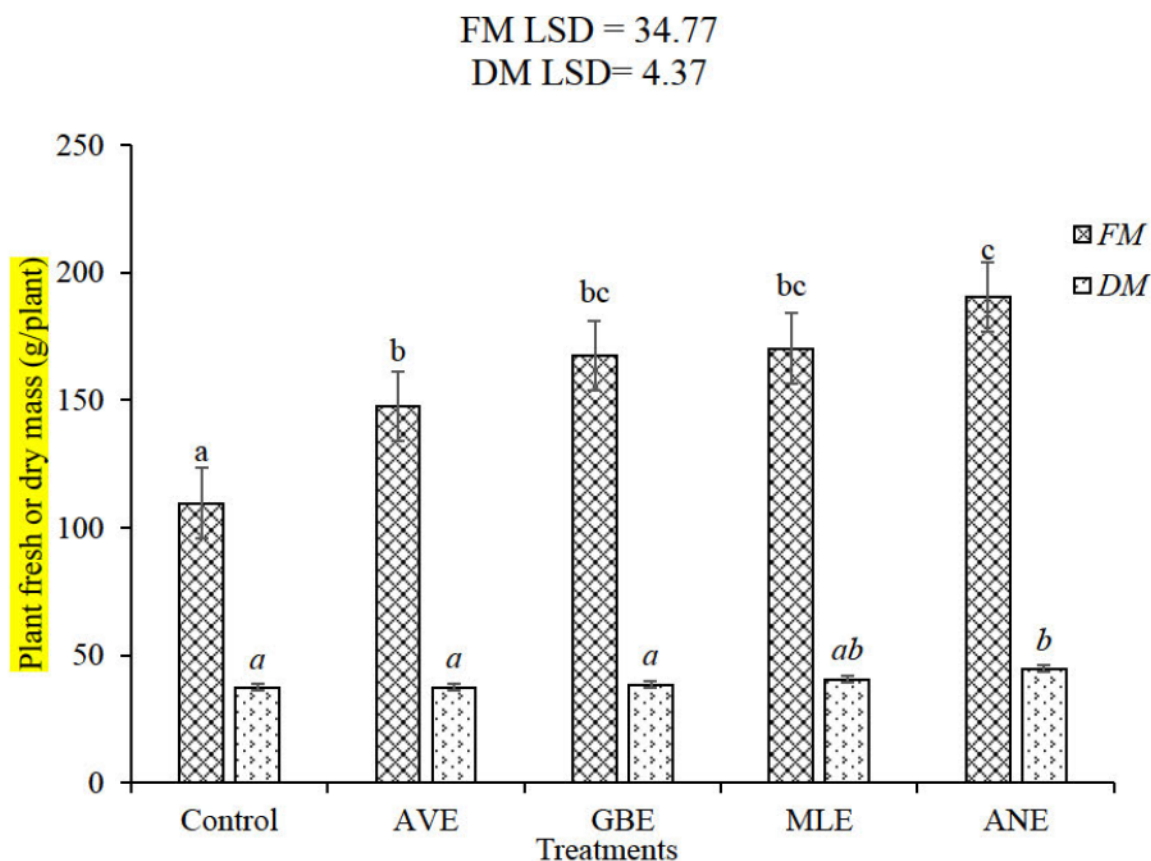
The treatment of potato plants with various plant-derived extracts significantly enhanced the leaf chlorophyll content index ( $p \leq 0.05$ ). The highest leaf chlorophyll content index was obtained in potato plants treated with ANE and MLE (35.89 and 34.45); however, the two latter treatments did not differ significantly from each other. The lowest leaf chlorophyll content index was recorded for the control (28.78) (Fig. 3.4).



**Figure 3.4:** Effects of foliar-applied plant extracts at various stages of plant growth on leaf chlorophyll content in potatoes. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera l leaf extract). Different lowercase letters above each column indicate significant differences between treatments ( $p \leq 0.05$ ) according to Duncan's multiple range test.

### 3.3.5. Fresh and dry above-ground biomass

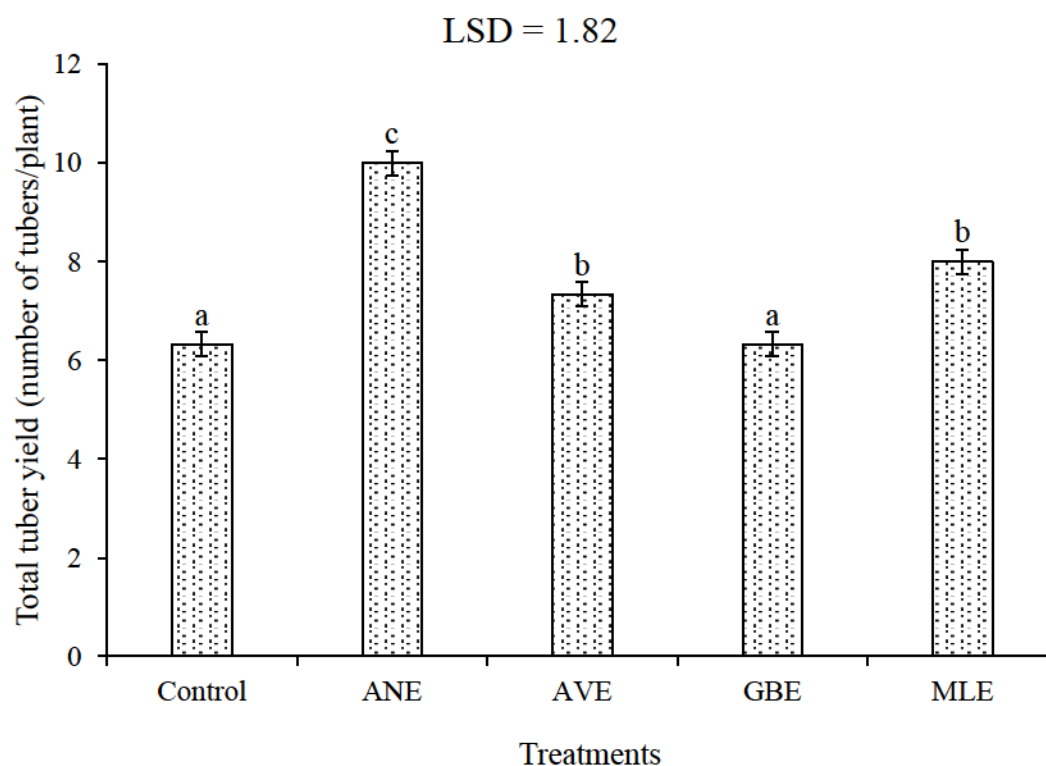
Analysis of variance of the fresh and dry mass of above-ground potato plant biomass indicates a significant difference ( $p \leq 0.05$ ) between treatments (Fig. 3.5). The potato plant treatments showed a pronounced effect on the accumulation of above-ground fresh and dry mass (Fig. 3.5). For fresh mass specifically, ANE-treated plants accumulated significantly higher biomass than the control and AVE, hence, recording (190.3 g/plant). In addition, ANE also accumulated significantly heavier dry biomass than control, AVE and GBE, but significantly not different to MLE. These treatments ANE and MLE, therefore, recorded (44.73 and 40.67 g/plant), respectively.



**Figure 3.5:** Effects of foliar-applied plant extracts on fresh and dry mass of potato above-ground biomass. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract), FM (fresh mass), DM (dry mass). Different lowercase letters above each column indicate significant differences amongst treatments ( $p \leq 0.05$ ) according to Duncan's multiple range test.

### 3.3.6. Tuber number per plant

The obtained results indicate that pre-harvest application of all plant extracts significantly influenced ( $p \leq 0.05$ ) tuber number per potato plant, except for the GBE treatment (Fig. 3.6). While ANE treatment resulted in the most significant tuber number increase, recording 10 tubers, AVE and MLE produced 7 and 8 tubers, respectively, the control and GBE both only recorded 6 tubers per plant.

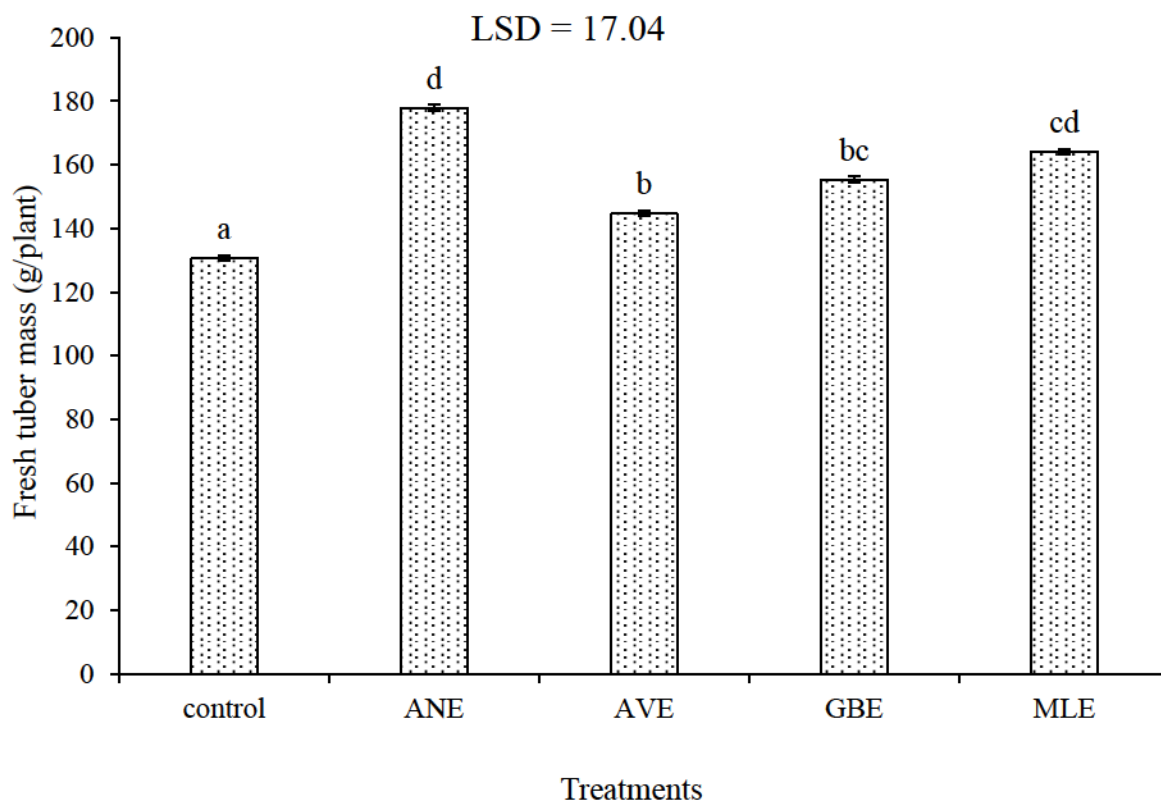


**Figure 3.6:** Effects of foliar-applied plant extracts on tuber number per plant. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). Different lowercase letters above each column indicate significant differences amongst treatments ( $p \leq 0.05$ ) according to Duncan's multiple range test.

### 3.3.7. Tuber fresh mass

The treatment of potato plants with various plant extracts positively affected fresh tuber mass. All treatments were significantly different to the control, with ANE producing the greatest fresh tuber mass ANE (177.9 g), followed by MLE, GBE and AVE treatments (164.1 g, 155.4 g and 144.6 g, respectively). The control recorded the least fresh tuber mass (130.5 g). Overall, the obtained results indicate significant differences ( $p \leq 0.05$ ) in fresh tuber mass between treatments (Fig. 3.7).





**Figure 3.7:** Effects of foliar-applied plant extracts on fresh tuber mass. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extracts). Different lowercase letters above each column indicate significant differences amongst treatments ( $p \leq 0.05$ ) according to Duncan's multiple range test.

### 3.4. Discussion

The use of plant extracts as biofertilizers, biostimulants and soil ameliorants has gained increasing attention owing to the adverse effects of synthetic pesticide and inorganic fertilizer application on human health and the environment (Rajendran *et al.*, 2022). Plant extracts have the ability to increase plant growth and yield attributes by positively interfering with plant physiological processes (Zulfiqar *et al.*, 2020). The present study, therefore, intended to evaluate the growth and yield promotion effects of various plant extracts. The foliar application of the plant ex-tracts made from *Ascophyllum nodosum* (ANE), aloe vera leaves (AVE), garlic bulbs (GBE) and moringa leaves (MLE) had a positive impact on growth and yield parameters of potato.

Increased vegetative growth, leaf chlorophyll concentrations and yield parameters could be attributed to the biofertilization effect of natural plant extracts, especially ANE and MLE (Kumari *et al.*, 2011). This phenomenon has been previously reported on a wide variety of crops, such as soybean (*Glycine max* L.) (Tandon and Dubey, 2015), wheat (*Triticum aestivum* L.) (Khan *et al.*, 2017), sweet pepper (*Capsicum annuum* L.) (Ali *et al.*, 2019) and tomato (Ngcobo and Bertling, 2021). Both ANE and MLE are excellent sources of minerals, including macro- and micro-nutrients (i.e., N, P, K, Ca, Mg, Zn and Na) (Rayorath *et al.*, 2008; Hala *et al.*, 2017). Mineral elements, particularly N and P, have been known for their vegetative growth promotion in plants, hence, exogenous application of plant extracts increased the availability of N and P to the plant, thereby boosting vegetative growth and above-ground biomass (Moore, 2004). In addition, enhanced leaf chlorophyll concentration could be due the presence of the minerals Mg and N, which are vital components of the chlorophyll molecule (Rayorath *et al.*, 2008; Zulfiqar *et al.*, 2020). Magnesium is the central atom in the porphyrin ring of the chlorophyll molecule, while N, is also a structural component of the chlorophyll molecule; this results in a green leaf colour representing a high leaf chlorophyll concentration; both elements are necessary to allow the capture of light energy during photosynthesis (Cakmak *et al.*, 2010; Wen *et al.*, 2019). Minerals, especially K and Zn, are mainly responsible for yield and quality promotion in crops. Cakmak *et al.* (1994) revealed that adequate K nutrition is vital in crops to enhance photosynthate translocation (via the phloem from source to sink). Potassium also plays a critical role in the partitioning of carbohydrates by improving photosynthate translocation and the growth rate of sink and/or source organs (Cakmak *et al.*, 1994). Furthermore, Zn is an essential component in the biosynthesis of tryptophan, which is involved in the synthesis of indole acetic acid (IAA) (Skoog, 1940); this plant hormone is responsible for cell growth and cell elongation; thus, increasing tuber growth substantially.

Besides biofertilization, enhanced vegetative growth, leaf chlorophyll concentration, plant above-ground biomass and yield attributes following plant extract applications could also be ascribed to their bio-stimulatory effect (Jannin *et al.*, 2013). Plant extracts, especially ANE and MLE, are natural sources of several bioactive compounds, including phytohormones, ascorbic acid, polysaccharides, betaines and phenolics (Rayorath *et al.*, 2008; Saini *et al.*, 2016). Exogenous application of such extracts could potentially provide plants with these essential phytochemicals, with many of these compounds working in a synergistic manner (Jannin *et al.*, 2013). Both ANE and MLE contain phytohormones, such as auxins [indole-3-acetic acid (IAA)], gibberellins (GAs) and cytokinin (zeatin) (Arthur *et al.*, 2003; Arif *et al.*, 2022). The presence of such growth-promoting plant hormones in ANE and MLE could possibly induce

cell expansion and cell division, thereby improving vegetative growth, plant biomass and yield attributes, as observed by Rayorath *et al.* (2008) in *Arabidopsis thaliana* (L.). Rioux *et al.* (2007) reported that, besides containing plant hormones, *Ascophyllum nodosum* extract can exhibit a wide range of growth-stimulatory effects because of the polysaccharides present in the extract. Such compounds include laminarin [ $\beta$ -glucan – ( $\beta$ -D-glucose polysaccharide)] and fucoidans (fucose-rich sulphated polysaccharides, consisting primarily of 1,2-linked  $\alpha$ -L-fucose-4-sulfate units with very small amounts of D-xylose, D-galactose, D-mannose, and uronic acid), both exhibiting radical scavenging antioxidant activity (Eluvakkal *et al.*, 2010).

Rayorath *et al.* (2008) also reported significant amounts of betaine (trimethylglycine, a non-protein, methyl-derivative of glycine) present in ANE, which plays a significant role in counteracting metabolic dysfunctions brought on by stress, thereby improving plant growth and survival. In addition to betaines, ANE and MLE contain several antioxidant compounds, including ascorbic acid, tocopherols, flavonoids and polyphenols; their presence triggers antioxidant biosynthesis, thereby reducing stress caused by reactive oxygen species (ROS) (Wang *et al.*, 2009). These ROS can cause cell and membrane degradation, which may fast-forward chlorophyll degradation and senescence; hence, these antioxidant compounds found in ANE and MLE could promote growth and developmental processes and maintain leaf greenness by reducing ROS levels in potato plants (Batool *et al.*, 2020). Increased leaf chlorophyll concentrations due to the foliar application of ANE and MLE could possibly be due to enhanced gene transcripts involved in photosynthesis, cell metabolism and stress response. Application of ANE and MLE suppresses cysteine protease activity (Blunden *et al.*, 1996; Buet *et al.*, 2019), which ultimately results in the inhibition of chlorophyll degradation, thus delaying senescence in plants, as observed by Kałużewicz *et al.* (2017) in broccoli (*Brassica oleracea* var. *italica*) and Bashir *et al.* (2014) in tomato.

The results obtained in the present study confirm findings by Haider *et al.* (2012), who demonstrated a significant improvement in growth attributes of potato plants due to various ANE treatments. Rajendran *et al.* (2022) also demonstrated that growth parameters, such as plant height, number of leaves and branches and leaf area of sweet pepper plants were significantly enhanced by foliar ANE and MLE application; these findings correspond well with the present study. In addition, ANE and MLE applications to tomato plants grown under water-deficit conditions significantly improved plant height, number of leaves and branches, as well as leaf area (Ahmed *et al.*, 2022). Ahmad *et al.* (2019) also noted similar effects on leaf chlorophyll in bulbous cut flowers (*Freesia hybrida*), reporting a significant improvement in



leaf chlorophyll concentration following MLE application. Similar results were also observed by Elzaawely *et al.* (2017) in snap bean (*Phaseolus vulgaris* L.) and Ali *et al.* (2022) in okra (*Abelmoschus esculentus* L.). Various authors noted a considerable improvement in yield attributes of many crop species, such as lima beans (*Phaseolus lunatus* L.) (Reitz *et al.*, 1996), tomato and sweet pepper (Ali *et al.*, 2019), following ANE application, similar to what is observed in this study. These results also coincide with those of Ali *et al.* (2016), who noted that foliar ANE application improved total fruit yield and fruit mass of tomato. Similarly, a significant increase in total tuber yield, following foliar ANE application, was observed by Haider *et al.* (2012); our results confirm the findings of these authors. In addition, Taskos *et al.* (2019) demonstrated a pronounced effect of ANE application on total grape yield; moreover, obtained results concur with Rajendran *et al.* (2022), who noted a positive response in total sweet pepper yield and fruit mass, following ANE foliar application. Therefore, the obtained results demonstrate the usefulness of ANE in potato production.

### 3.5. Conclusion

The present study encourages the use of various plant extracts in the crop farming community. The pre-harvest foliar application of various plant extracts considerably enhanced vegetative growth, physiological and yield attributes of potato. Since modern agriculture necessitates financially feasible and easily accessible organic inputs, use of plant extracts, as biofertilizer, biostimulant and bio-elicitor could effectively be used as an ideal multi-active organic input to improve crop growth and yield potential of agricultural crops. This research has shown that foliar applications of plant extracts, especially of ANE and MLE, have the potential to improve crop productivity and yield. Results presented in this study are, hence, of high significance to commercial as well as small-scale potato growers, as the use of organic plant extracts is an environmentally friendly and a sustainable approach towards increasing crop productivity. Plant extracts have shown beneficial effects on solanaceous crops, but further validations of these effects on other crops is recommended.

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## CHAPTER FOUR

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### **Effects of Pre- plus Post-harvest application of Plant Extracts on the Post-harvest Quality of Potatoes (*Solanum tuberosum* L.) Kept under Ambient Storage Conditions**

#### **Abstract**

Potato is an economically important non-grain, semi-perishable agricultural commodity. After harvesting, potatoes become highly prone to moisture loss and quality deterioration, contributing to post-harvest losses in storage. To combat these issues, synthetic pesticides have been vital; however, their usage can endanger human health and cause environmental problems. Plant extract utilization is a promising, safe, eco-friendly and financially feasible approach that could potentially reduce the usage of hazardous chemicals. The present study, therefore, investigated the efficacy of various plant extracts on the retention of post-harvest quality of potato tubers. Freshly harvested tubers were dipped into plant extracts (10 g/L concentration) and allowed to air-dry for 1 hr on the benchtops before storage at ambient conditions (day:  $25 \pm 2^{\circ}\text{C}$  & 45% RH; night:  $18 \pm 2^{\circ}\text{C}$  and 65% RH). The treatments were: *Ascophyllum nodosum* extract (ANE), aloe vera leaf extract (AVE), garlic bulb extract (GBE) and moringa leaf extract (MLE), as well as the control (water only). Plant extract applications, as post-harvest treatments, significantly ( $p \leq 0.05$ ) influenced post-harvest quality of potato tubers. AVE application reduced physiological mass loss during storage by 60 % compared with the control. Moreover, ANE and MLE treatment application considerably increased potato mineral concentrations and the presence of total soluble solids. These treatments also gave the most promising results as they showed the highest retention potential of tuber

**vitamin C, total carbohydrate, protein, phenolic and flavonoid concentrations, as well as antioxidant activity during storage. Based on these results, the use of natural plant extract, as an alternative to chemicals, is highly advisable and recommended to preserve post-harvest quality of potato tubers, without compromising consumers' health.**

Keywords: Nutritional status, plant extracts, post-harvest quality, shelf-life, storage

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#### **4.1. Introduction**

The world's population is increasing exponentially and is expected to reach 9.7 billion people by 2050 (Falcon *et al.*, 2022). Consequently, the global food demand is also projected to increase by 35-56% between year 2010-2050 (Van Dijk *et al.*, 2021). To achieve sustainable development goals 1 and 2, no poverty and zero hunger respectively, potato (*Solanum tuberosum*), has a high potential to counteract this rising worldwide food demand (Dauvex *et al.*, 2014), as it is a versatile staple food crop, consumed in many parts of the world (Birch *et al.*, 2012; Hussain, 2016). In terms of human consumption, potato is ranked the fourth-most economically important food crop, after rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) (Zhang *et al.*, 2017), with an annual global production exceeding 376 million tons (FAO, 2022). Apart from being an excellent source of carbohydrates, Mahmud *et al.* (2015) noted that potato contains significant amounts of vitamins, minerals, crude protein, crude fats and fibre. In addition, Lovat *et al.* (2016) found a variety of antioxidant compounds present in potatoes, such as flavonoids, carotenoids and polyphenols, all these phytochemicals are vital to human health.

Unlike cereal crops, potato is a semi-perishable commodity; immediately after harvest the commodity becomes highly susceptible to moisture loss, resulting in structural, nutritional and biochemical changes. These post-harvest changes are predominantly induced by the biological processes of sprouting, greening, respiration and transpiration, all contributing to post-harvest disease occurrence such as *Phytophthora infestans*, *Phytophthora erythroseptica* and *Pectobacterium carotovorum*, thus, causing significant post-harvest losses (Paul *et al.*, 2016; Sinha *et al.*, 2018). Worldwide, post-harvest losses are amongst the most-devastating challenges to the potato industry, accounting for 20-25% of annual produce losses. In developing countries, post-harvest losses of potato range from 15-50% (FAO, 2022). Very little information is available on post-harvest losses of potatoes in South Africa (Beretta *et al.*, 2013).



There are, however, several strategies employed, that are found effective in reducing postharvest losses.

The use of certain synthetic chemicals, such as sprout suppressants (e.g., chlorpropham and maleic hydrazide), fungicides or bactericides (e.g., Mancozeb<sup>®</sup>, Ridomil Gold<sup>®</sup> MZ and Metalaxyl<sup>®</sup>, chlorothalonil, copper oxychloride and copper sulfate), is one of the employed strategies to reduce post-harvest losses in potato (Daniels-Lake *et al.*, 2013; Majeed *et al.*, 2017). Due to the possible detrimental effects of these chemicals on human health and the surrounding environment, a reduction in their application is required, despite their efficacy. Given these adversities, new, innovative and eco-friendly alternative approaches that could be employed to reduce post-harvest losses without jeopardizing consumers' health and the environment need to be explored.

The use of natural products, especially of plant-derived extracts, is emerging as a new technology to maintain potato quality, as these products have gained attention as alternative treatments due to their potential in improving food safety and appearance by protecting produce from external factors; thus, they can improve post-harvest quality (Ogbuehi *et al.*, 2016). These compounds have been tested by various authors using many horticultural commodities, including 'Tommy Atkins' mango (*Mangifera indica* L.) (Melo *et al.*, 2018, Shrestha *et al.*, 2018), guava (*Psidium guajava*) (Malik *et al.*, 2015), and blueberry (*Vaccinium corymbosum*) (Jaime-Guerrero *et al.*, 2022). Interestingly, natural extract application has also been tested on vegetables, such as eggplant (*Solanum melongena* L.), (Ogbuehi *et al.*, 2016), kale (*Brassica oleracea* var. *sabellica* L.) (Melo *et al.*, 2018) and tomatoes (*Solanum lycopersicum* L.) (Liamngee *et al.*, 2019). Little information is available on the natural extract application on potatoes postharvest, therefore the current study aimed to evaluate the effect of pre- and post-harvest plant extract applications on the post-harvest quality of potatoes.

## **4.2. Materials and methods**

### **4.2.1. Plant material and environmental conditions**

The study was conducted in the post-harvest laboratory of the University of KwaZulu-Natal, Pietermaritzburg Campus. Potato tubers used in this study were harvested from plants grown in a glasshouse at the Controlled Environment Facility (CEF). Potato tubers used in this experiment were harvested from potato plants treated at pre-harvest using various plant extracts, the same extracts as used as post-harvest treatments in the post-harvest experiment

(Chapter Three). Immediately after harvesting, tubers were treated using various plant extracts, allowed to dry and stored on benchtops. The environmental condition in the laboratory were maintained at  $25 \pm 2^{\circ}\text{C}$  and 45% RH during the day, while at night they were set to  $18 \pm 2^{\circ}\text{C}$  and 60% RH.

#### **4.2.2. Plant extract preparations**

Plant extracts were prepared following the method described in subsection 3.2.3.

#### **4.2.3. Experimental design**

The experiment was laid out following a completely randomized design (CRD) consisting of five treatments replicated three times. After harvest, seventy-five healthy ‘Sifra’ potato tubers were randomly selected for each of the five treatments. The five treatments were: *Ascophyllum nodosum* extract (ANE), moringa leaf extract (MLE), garlic bulb extract (GBE) and aloe vera leaf extract (AVE), as well as the control (distilled water). Potato tubers were immersed in the above-mentioned treatments for 30 sec, then placed on the laboratory bench and allowed to air-dry before storage at ambient conditions ( $25 \pm 2^{\circ}\text{C}$  and 45% RH). The dipping of tubers into these treatments was performed immediately after harvesting (day 0 of storage). Prior to potato immersion, data on percentage mass loss were collected and this recording continued weekly until day 28, the last day of the storage period. Before each day of measuring the nutritional quality parameters (*i.e.*, total carbohydrate, total protein, ascorbic acid, total phenolic, total flavonoid concentration and total antioxidant activity), the periderm of tubers was peeled off prior to freeze-drying on a VirTis benchtop Pro freeze-dryer (SP Scientific Inc, Stone Ridge, NY, USA) and milled using a coffee grinder (Mellerware, China) to produce potato flour.

#### **4.2.4. Determination of nutritional quality parameters**

##### **4.2.4.1. Mass loss (%)**

Tuber mass was recorded immediately after harvest (initial mass,  $M_i$ ), then weekly until day 28 day of storage using a fine analytical balance (AS220.R2. PLUS, Rawdwag Wagi Elelectroniczne, Poland). Mass loss (%) was determined using the following equation:

$$\text{ML (\%)} = \frac{M_i - M_f}{M_i} \times 100;$$

where  $M_i$  (initial tuber mass) and  $M_f$  (tuber mass on day 28 of storage).

#### **4.2.4.2. Determination of mineral elements and total soluble solids (TSS)**

Samples were analyzed for mineral composition using inductively coupled plasma mass spectrometry (ICP-MS) at the Analytical Services Laboratory at Cedara Agricultural College. Total soluble solid percentage (TSS) was determined using a refractometer (RFM340+, Bellingham + Stanley Ltd, Tunbridge Wells, UK) and expressed in °Brix, where 1°Brix = 1g sucrose/100g extract solution.

#### **4.2.4.3. Determination of total carbohydrate concentration**

A traditional phenol-sulfuric acid method (DuBois *et al.*, 1956), with slight modifications, was used to determine total carbohydrates. Freeze-dried potato flour (0.5 g) was weighed out and transferred into test tubes; thereafter, 80% ethanol was added to the test tubes, and the solutions vortexed. Samples were then incubated in a water bath at 85°C for 15 min, and subsequently placed on ice for 5 min, before 10 ml filtrate was obtained using Whatman filter paper No. 1. An aliquot of this clear solution (1 ml) was mixed with 1 ml distilled water, before the such-prepared sample was transferred into thoroughly rinsed test tubes. Following this, 1 ml 5% phenol solution (Sigma-Aldrich, Burlington, MA, USA) and 2.5 ml 75% H<sub>2</sub>SO<sub>4</sub> was added to the test tubes for colour development. After an incubation period of 10 min at 85°C, samples were cooled on ice. Thereafter, absorbance was read at 490 nm on a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). The concentration of each sample was calculated from a standard curve prepared using glucose solution (20 mg/ml) with the resulting equation ( $y = 0.0974x - 0.0467$ ;  $R^2 = 0.9917$ ) and expressed in mg glucose equivalent per g DM.

#### **4.2.4.4. Determination of total protein concentration**

The protein concentration of samples was determined following the procedure developed by Bradford (1976). A mass of 0.5 g freeze-dried, ground potato material was weighed out and transferred into centrifuge tubes; then, 30 ml 100 mM TRIS buffer (pH 8.0) was added and the sample vortexed. Thereafter, samples were centrifuged using an Avanti J-26S XP centrifuge (Beckman Coulter, Brea, CA, USA) at 12096 G for 15 min at 5°C, before 0.1 ml sample supernatant was transferred to test tubes, to which 5 ml Bradford reagent was added. The absorbance of each sample was then determined spectrophotometrically at 590 nm. The protein

concentration of each sample was calculated from a standard curve prepared using Bovine Serum Albumin (BSA) (Sigma-Aldrich, Burlington, MA, USA) (using the equation  $y = 0.0056x + 0.1201$ ;  $R^2 = 0.9842$ ) and expressed in mg BSA per g DM).

#### **4.2.4.5. Determination of tuber ascorbic acid concentration**

Ascorbic acid was quantitatively determined following the 2,6-dichlorophenolindophenol (DCPIP) method developed by Boonkasem *et al.* (2015), with slight modifications. A mass of 0.5 g freeze-dried potato powder was weighed out and transferred into centrifuge tubes. Then, 20 mL of 3% metaphosphoric acid was added to each tube; thereafter, tubes were centrifuged at 12098 G for 10 minutes. The supernatant was collected, and 1 mL was mixed with 3 mL 0.2 mM DCPIP and agitated for 15s. Samples absorbance was read at 515 nm. The ascorbic acid concentration was calculated using the equation  $y = -0.0368x + 0.8879$  ( $R^2 = 0.9942$ ). Results were expressed in mg ascorbic acid equivalent (AAE) per 100 g dry mass (mg AAE / 100 g DM).

#### **4.2.4.6. Determination of total phenolic concentration (TPC)**

The total phenolic concentration of potato tubers was determined with a slightly modified Folin-Ciocalteu assay, as described by Wang *et al.* (2009). Freeze-dried potato flour (0.5 g) was weighed out and transferred into centrifuge tubes. After addition of 25 ml 80% acetone and vortexing, samples were centrifuged at 12298 G for 30 min at 5°C. The supernatant (1ml) was combined with 2.5 ml 0.2 N Folin-Ciocalteu reagent, before samples were allowed to stand for 5 min. Thereafter, 4 ml  $\text{Na}_2\text{CO}_3$  were added, followed by incubation at 80°C for 30 min. After incubation, tubes were cooled on ice for 5 min and absorbance read at 736 nm. The total phenolic concentration was calculated using a gallic acid standard curve ( $y = 0.0229x + 0.1907$ ;  $R^2 = 0.9886$ ) and expressed as mg gallic acid equivalent (GAE) per g sample mass (mg GAE/ g DM).

#### **4.2.4.7. Determination of total flavonoid concentration (TFC)**

The total flavonoid concentration was quantitatively determined with the aluminium chloride ( $\text{AlCl}_3$ ) assay described by Gu *et al.* (2019), with slight modifications. Freeze-dried potato flour (0.5 g) was weighed and transferred into centrifugation tubes. Before vortexing the sample, 10 ml of 95% ethanol were added as extraction solvent. Thereafter, tubes were incubated at 25°C



for 30 min before being filtered through Whatman No. 1 filter paper to remove sample residues and obtain clear supernatants; then, 100 µl supernatant and 100 µl AlCl<sub>3</sub> were simultaneously added to test tubes. Prior to a further incubation at 25°C for 30 min, 200 µl sodium acetate solution was added to the sample. The absorbance of the so-prepared samples was read at 440 nm. A quercetin standard curve ( $y = 0.027x + 0.102$ ;  $R^2 = 0.989$ ) was used to determine TFC, expressed as quercetin equivalents (QE) per gram dry mass (mg QE/g DM).

#### **4.2.4.8. Determination of antioxidant activity [DPPH (2, 2-Diphenyl-1-picrylhydrazyl)]**

Free radical scavenging ability was calculated according to the 2,2-Diphenyl-1-picrylhydrazyl antioxidant (DPPH) assay described by Rocchetti *et al.* (2019), with slight modifications. Freeze-dried potato flour (0.5 g) was placed into test tubes, 10 ml of 95% ethanol was added and the sample vortexed. Before filtering the supernatant through Whatman No. 1 filter paper to remove sample residues, test tubes were incubated at 25°C for 10 min. Then, 100 µl sample and 300 µl 0.1 mM DPPH (0.1 mM in 95% methanol) reagent were added to the test tubes, these were incubated in the dark for 30 min before absorbance was read at 517 nm. The scavenging ability of DPPH was calculated using a linear equation ( $y = -0.0368x + 0.8879$ ;  $R^2 = 0.994$ ) and was expressed as ascorbic acid equivalent per gram sample dry mass (mg AAE/g DM).

#### **4.2.5. Data analysis**

Data collected was subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 21<sup>st</sup> edition, VSN International, UK), where significant differences were observed. For mean separation and comparison, Duncan's multiple range test at 5% level of significance ( $p \leq 0.05$ ) was used.

### **4.3. Results**

#### **4.3.1. Mineral composition**

Statistically, the application of various plant extracts to potato plants had a significant effect ( $p \leq 0.05$ ) on the mineral composition of harvested potato tubers. Most plant-based extract applications resulted in a considerable increase in the mineral content of harvested tubers (Table 4.1), although ANE and MLE showed the best performance in enhancing minerals, such

as Ca, K and Mg; hence, values recorded in both treatments showed no significant difference. In addition, mineral N and P was significantly enhanced by the application of MLE and ANE, and these treatments outperformed all other treatments, hence, recorded the highest N (3%) and P (0.26%), respectively.

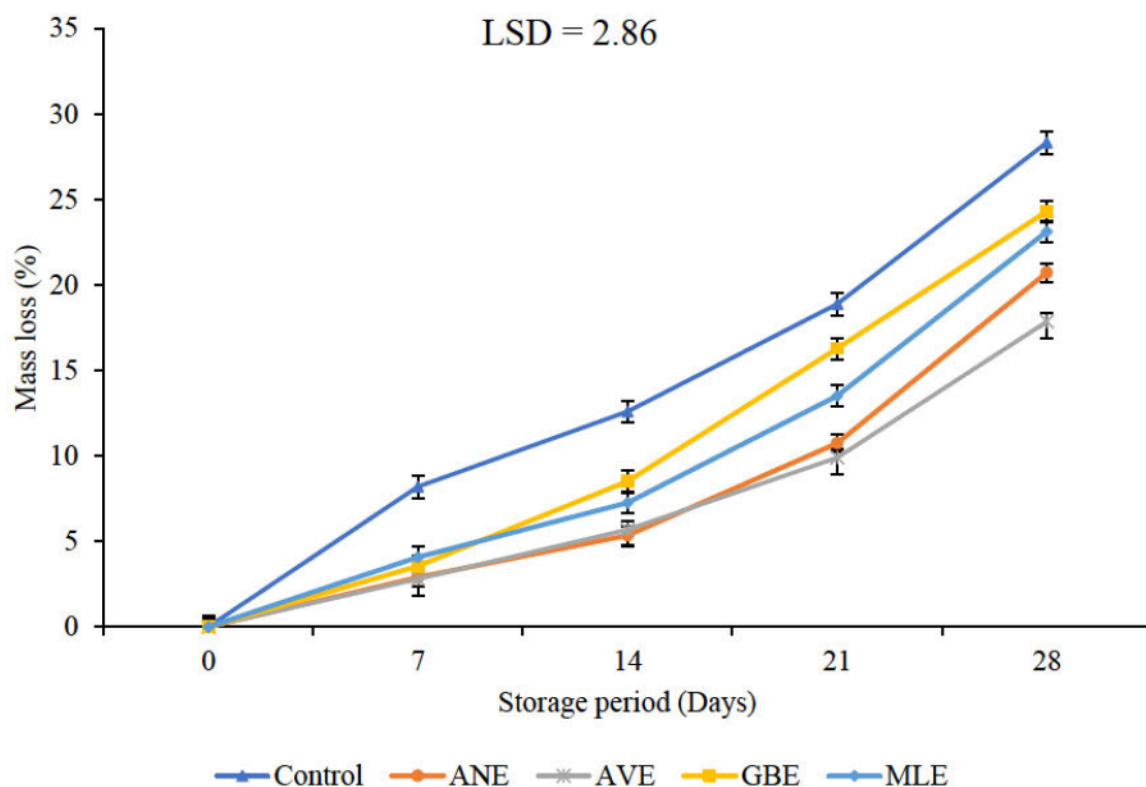
**Table 4.1:** Mineral composition (% DM) of freshly harvested (immediately after harvesting) potato tubers as affected by pre-harvest application of different plant extracts.

Treatments	Ca	Mg	P	N	K
<b>Control</b>	0.03 ± 0.003 a	0.13 ± 0.004 a	0.22 ± 0.006 a	1.75 ± 0.04 a	2.63 ± 0.24 a
<b>ANE</b>	0.05 ± 0.003 c	0.15 ± 0.004 c	0.26 ± 0.006 d	1.97 ± 0.04 c	2.83 ± 0.24 c
<b>AVE</b>	0.04 ± 0.003 b	0.14 ± 0.004 b	0.23 ± 0.006 b	1.86 ± 0.04 b	2.60 ± 0.24 a
<b>GBE</b>	0.03 ± 0.003 a	0.13 ± 0.004 a	0.23 ± 0.006 b	1.93 ± 0.04 bc	2.72 ± 0.24 b
<b>MLE</b>	0.05 ± 0.003 c	0.15 ± 0.004 c	0.25 ± 0.006 c	3.00 ± 0.04 d	2.89 ± 0.24 c
LSD	0.007	0.007	0.008	0.074	0.063

**NB:** Values followed by different lower-case letters in each column are statistically different according to Duncan's multiple range test ( $p \leq 0.05$ ). **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (*moringa* leaf extract), **GBE** (*garlic bulb* extract) and **AVE** (*aloe vera* leaf extract). Values are means ( $n = 3$ ) ± standard error.

#### 4.3.2. Mass loss (%)

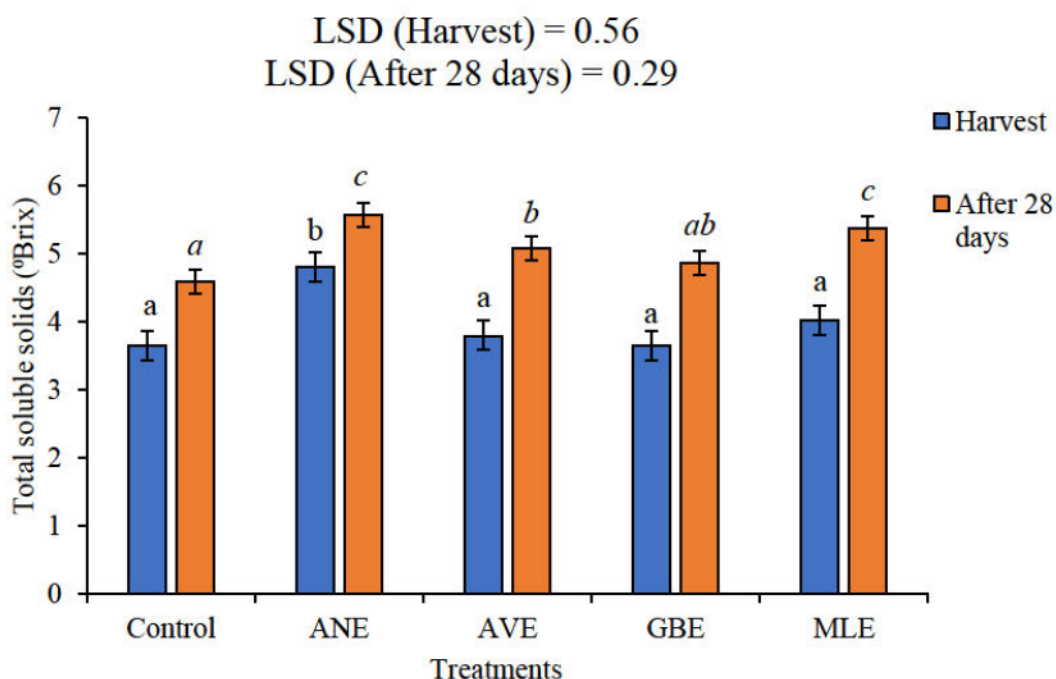
Mass loss of potato tubers increased with storage duration, as expected; however, all post-harvest treatments significantly reduced ( $p \leq 0.05$ ) mass loss more so than the control (Fig 4.1). The mass lost over the 28-day storage by the AVE treatment was significantly less than that of other treatments; hence, AVE recorded the lowest mass loss percentage (17.84%), followed by ANE and MLE, which recorded 20.71 and 23.11% mass loss, respectively (Fig 4.1).



**Figure 4.1:** Influence of pre- plus post-harvest treatments on the rate of mass loss of potatoes during storage. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 2.86.

#### 4.3.3. Total soluble solids (TSS)

Analysis of variance of the total soluble solids (TSS) indicated significant differences ( $p \leq 0.05$ ) between treatments (Fig 4.2). As expected, the TSS of tubers increased with storage duration; hence, after 28 day of storage, TSS values of treated potato tubers were significantly higher than the control (Fig 4.2). Specifically, ANE and MLE application increased TSS significantly, yielding the highest TSS values (5.58 & 5.38 °Brix, respectively) on day 28 of storage compared with other treatments.

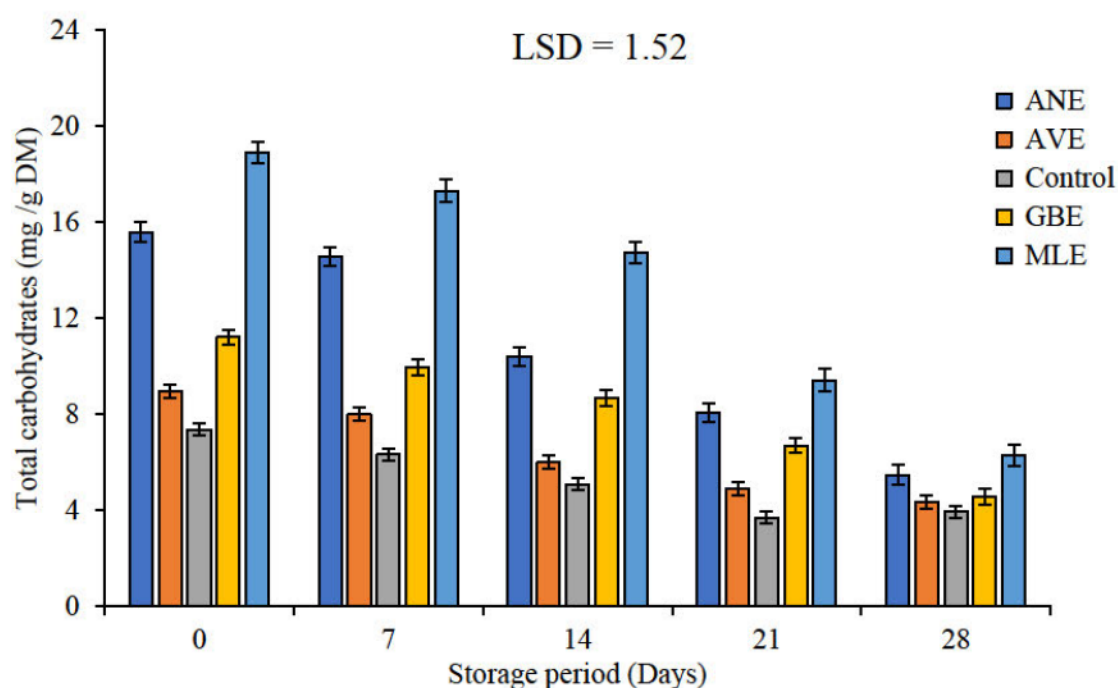


**Figure 4.2:** Effect of pre- plus post-harvest treatments on the total soluble solids (TSS) of potatoes stored for 28 days. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). Different lowercase letters above each column indicate significant differences between treatments ( $p \leq 0.05$ ) according to Duncan's multiple range test. LSD ( $p \leq 0.05$ ) (Harvest) = 0.56 and (After 28 days) = 0.29. Bars indicate means ( $n = 3$ )  $\pm$  standard error.

#### 4.3.4. Total carbohydrate concentration

Significant differences ( $p \leq 0.05$ ) between treatments were observed over storage period. The total carbohydrate concentration decreased with storage duration for all treatments (Fig. 4.3). While total carbohydrates decreased, pre- and post-harvest application of all plant extracts sustained carbohydrates at a higher concentration than the control (Fig. 4.3). On day 28 of storage, MLE and ANE recorded (6.27 and 5.45 mg/g DM, respectively), hence, these two treatments were not significantly different, although MLE had a tendency to outperform ANE. These two treatments resulted in a significantly higher carbohydrate concentration compared to all other treatments.

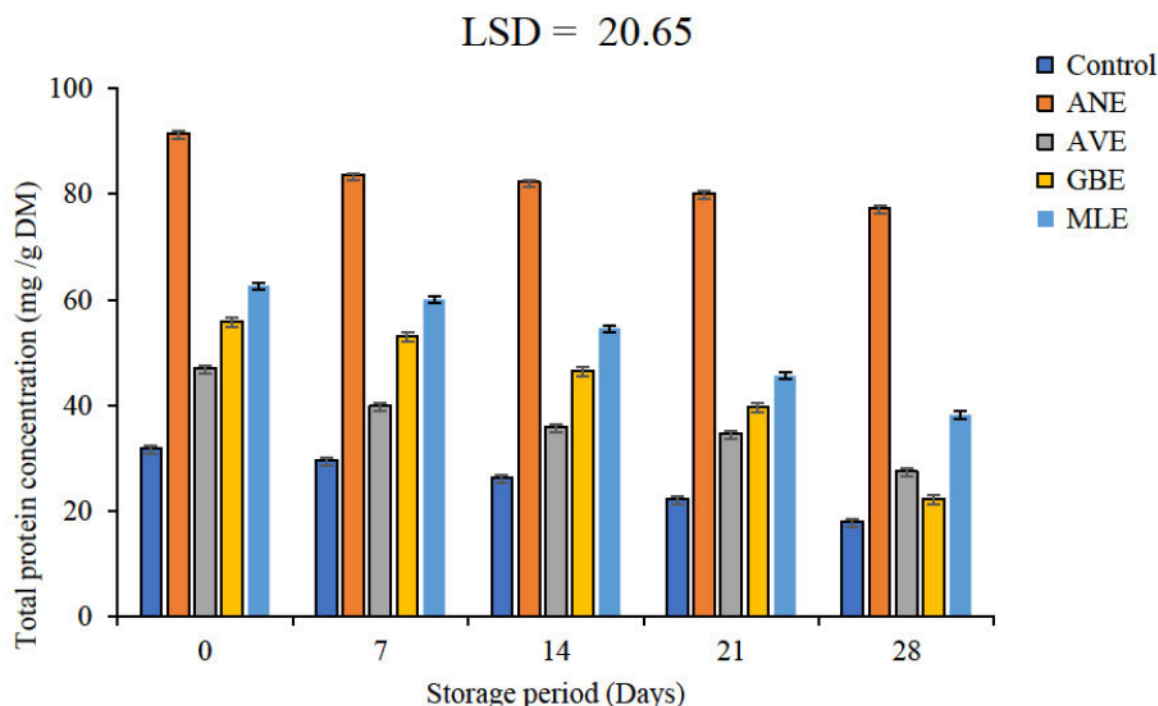




**Figure 4.3:** Influence of pre- plus post-harvest treatments on total carbohydrate concentration of potatoes stored for 28 days. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 1.52. Bars indicate means ( $n = 3$ )  $\pm$  standard error.

#### 4.3.5. Total protein concentration

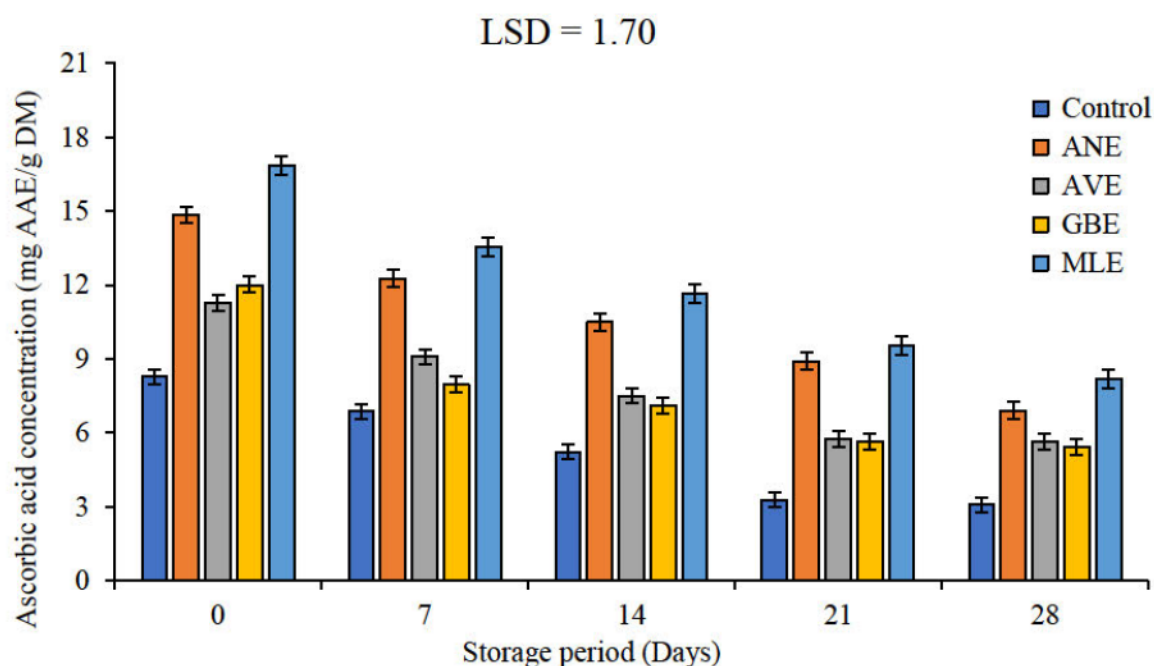
The pre- and post-harvest treatments of potato tubers significantly maintained ( $p \leq 0.05$ ) protein concentrations at levels higher than the control over the storage period. Total protein decreased with storage in all treatments, as expected; however, ANE-treated potatoes maintained the highest total protein concentration over the entire storage period. On day 28 of storage, this treatment, thus, recorded the highest protein concentration (77.4 mg/g DM), followed by MLE with about half of that protein (38.2 mg/g DM) (Fig 4.4).



**Figure 4.4:** Influence of pre- plus post-harvest treatments on total protein concentration of potatoes during storage. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 20.65. Bars indicate means ( $n = 3$ )  $\pm$  standard error.

#### 4.3.6. Vitamin C (Ascorbic acid)

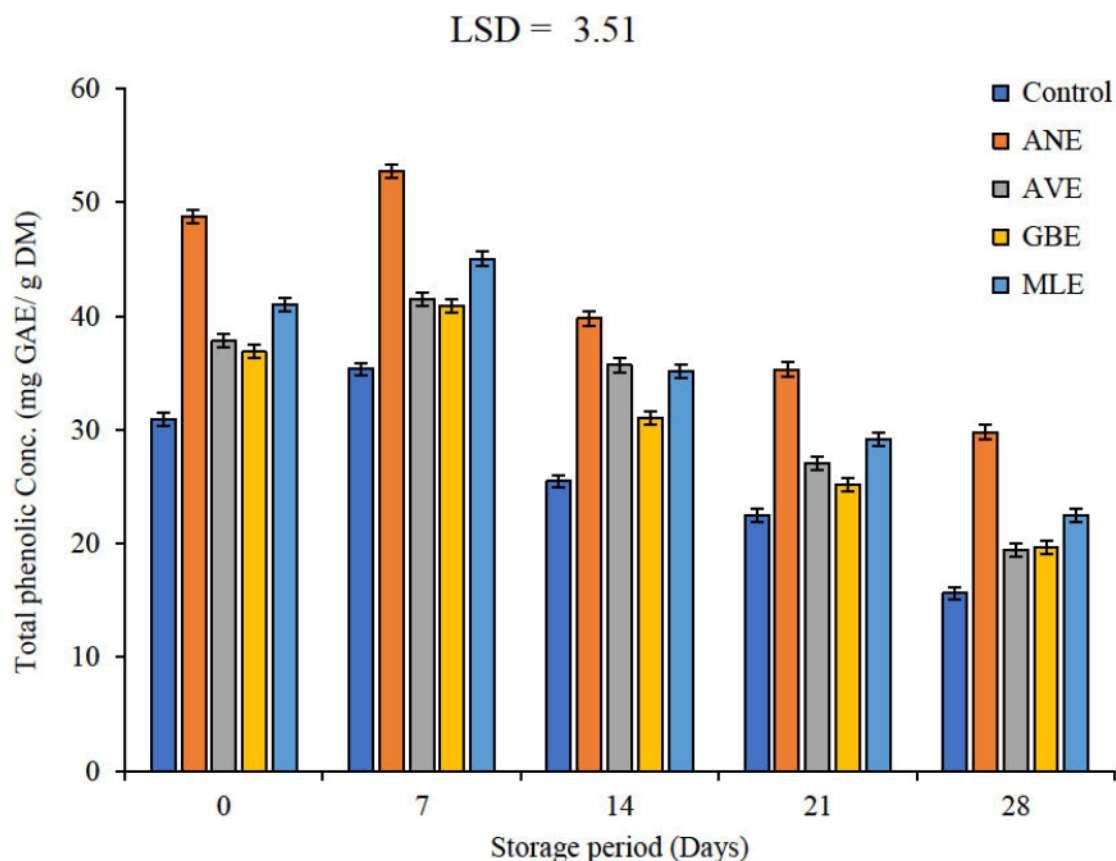
Significant differences ( $p \leq 0.05$ ) in ascorbic acid equivalents (AAE) were determined between potatoes treated pre- and post-harvest with the various plant extracts. The Vit C concentration decreased with the storage period, particularly up to day 21 of storage (Fig 4.5). Potato tubers treated with plant extracts showed a considerable decrease in Vit C concentration lower than a control. On day 28 of storage, MLE treatment had a pronounced effect in Vit C concentration, unlike other treatments, maintain levels higher than other treatments recording 8.19 mg AAE/g DM, with the second-highest concentration being ANE-treated tubers with 6.9 mg AAE/g DM.



**Figure 4.5:** Influence of pre- plus post-harvest treatments on vitamin C concentration of potatoes during storage. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 20.65. Bars indicate means ( $n = 3$ )  $\pm$  standard error.

#### 4.3.7. Total phenolic concentration (TPC)

The total phenolic concentration (TPC) of potato tubers increased from harvest to day 7 of storage, then decreased gradually during further storage (Fig 4.6). The pre- and post-harvest treatment of potato tubers using all extracts positively affected total phenolic concentrations, maintaining TPC higher than the control till day 21 of storage. On day 28 of storage, the highest TPC was recorded in potato tubers treated with ANE (29.8 mg GAE/g DM), followed by MLE (22.47 mg GAE/g DM). The control recorded the lowest TPC (15.62 mg GAE/g DM).

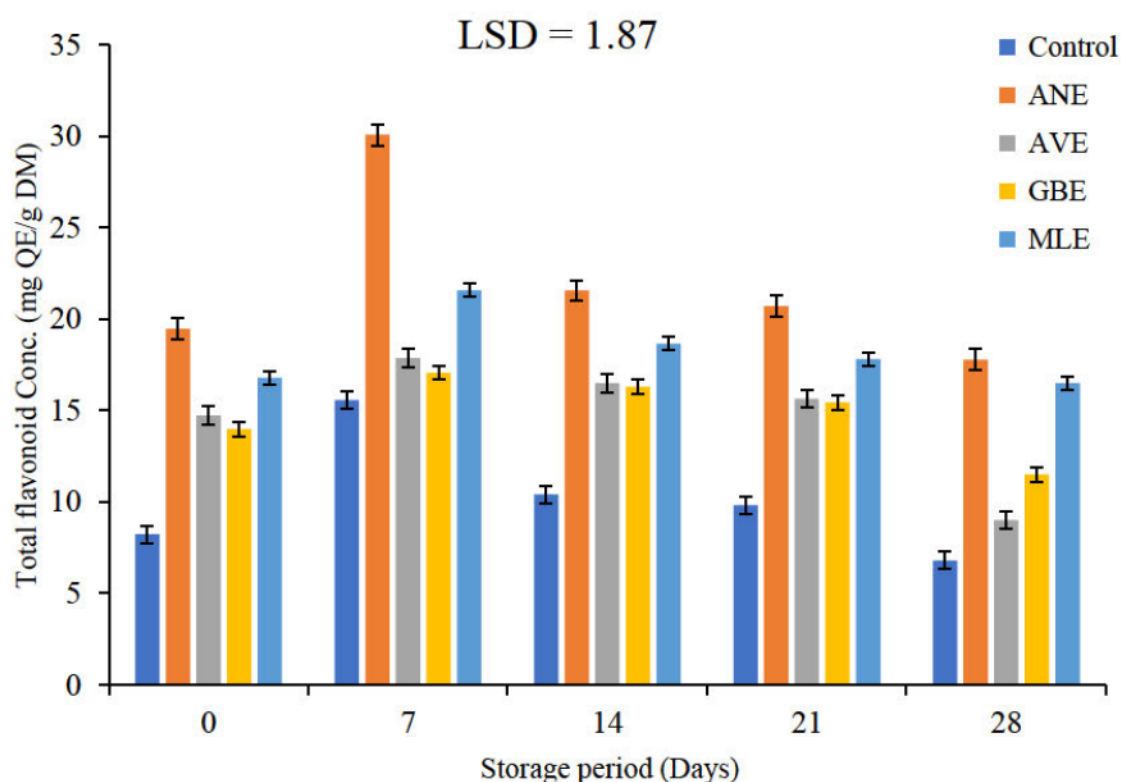


**Figure 4.6:** Influence of pre- plus post-harvest treatments on total phenolic concentration of potatoes during storage. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 3.51. Bars indicate means ( $n = 3$ )  $\pm$  standard error.

#### 4.3.8. Total flavonoid concentration (TFC)

Post-harvest treatment of potato tubers using all treatments significantly influenced ( $p \leq 0.05$ ) TFC during the storage period. An increase in TFC was only observed from day 0 to 7, then decreased gradually with further storage (Fig 4.7). On day 28 of storage, ANE-treated tubers recorded the highest TFC (17.78 mg QE/g DM) compared with other treatments and the control. The second highest flavonoid concentration was found in MLE-treated tubers, which recorded 16.50 mg QE/g DM (Fig 4.7).

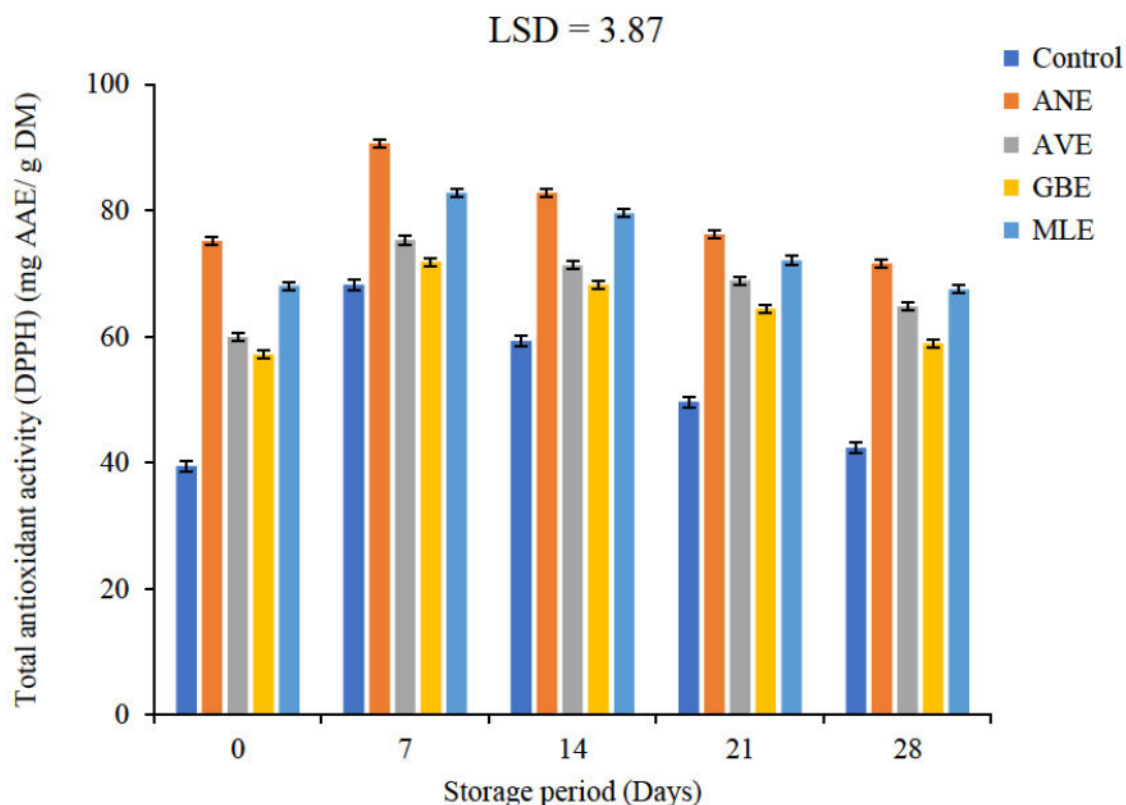




**Figure 4.7:** Influence of pre- plus post-harvest treatments on total flavonoid concentration of potatoes during storage. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 1.87. Bars indicate means ( $n = 3$ )  $\pm$  standard error.

#### 4.3.9. Total antioxidant activity

Analysis of variance of the total antioxidant activity indicated a significant difference ( $p \leq 0.05$ ) between the treatments (Fig 4.8). Total antioxidant activity of potato tubers increased from day 0 to 7 of storage, then decreased gradually with further storage. On day 28 of storage, total antioxidant activity of all treated potato tubers was considerably higher than the control (Fig 4.8). ANE treatment yielded the highest antioxidant activity, thus, recorded (71.59 mg AAE/g DM), followed by MLE (67.47 mg AAE/g DM) and AVE (64.78 mg AAE/g DM) at day 28 of storage.



**Figure 4.8:** Influence of pre- plus post-harvest treatments on total antioxidant activity of potatoes during storage. **Control** (no application), **ANE** (*Ascophyllum nodosum* extract), **MLE** (moringa leaf extract), **GBE** (garlic bulb extract) and **AVE** (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 3.78. Bars indicate means ( $n=3$ )  $\pm$  standard error.

## 4.5. Discussion

Due to the perishable nature of fruit and vegetables, protection and quality preservation of such food commodities have become vital components to achieve sustainable development goals 1 and 2, thereby achieving global food security (Ogunnupebi *et al.*, 2020). Plant extracts application, as pre- and post-harvest treatments, has received large attention due to its positive effect on reduced post-harvest spoilage, extended shelf-life and preserved post-harvest quality, without posing negative effects on consumers' health and the ecosystem as a whole (Ogbuehi *et al.*, 2016). Plant extracts have an immense potential to interfere with physiological processes that are part of post-harvest quality deterioration without causing adverse effects (Zulfiqar *et al.*, 2020). The present study, therefore, intended to evaluate the effects of various plant

extracts on preserving post-harvest quality of potato tubers. The application of extracts prepared from *Ascophyllum nodosum* algae (ANE), aloe vera leaves (AVE), garlic bulbs (GBE) and moringa leaves (MLE) as post-harvest treatments had a positive influence on post-harvest quality of potato (Table 4.1; Fig. 4.1-4.8).

Application of plant extracts significantly increased the nutritional and post-harvest quality of potato. Such positive effects caused by plant extract applications on post-harvest quality have been reported in several crops, such as spinach (*Spinacia oleracea*), tomato and mango (Fan *et al.*, 2014; Tunwari *et al.*, 2019; Hassan, 2022). There is, however, very limited information on post-harvest quality of potato tubers, following the application of plant extracts (Beretta *et al.*, 2013). The results obtained in the present study reveal that pre-harvest foliar application of various plant extracts significantly improved the mineral content of potato tubers (Table 4.1). Such an increase in mineral concentration in potatoes might be attributed to the bio-fertilization effect of these extracts (Kumari *et al.*, 2011), as the applied plant extracts, especially ANE and MLE, are good sources of mineral nutrients, such as N, P, K, Mg, Ca, Zn, Fe and Na (Table 3.1; Rayorath *et al.*, 2010; Kosolo *et al.*, 2010). Exogenous application of MLE has been found to increase the availability and uptake of mineral elements, including N, P, K, Mg, Ca and other micro-nutrients into root and shoots of many plants (Sivakumar and Ponnusami, 2011).

The MLE application could have exerted a positive effect on the nutritional value of potatoes (Table 4.1) simply through the enhanced transpirational pull due to larger biomass produced (Fig 3.6). In general, mineral elements are transported by xylem along the transpiration stream from the soil via roots to the leaves; so, many storage organs, such as fruits and undergrounds storage organs (e.g., tubers, bulbs, corms, rhizome, etc) exhibit very low transpiration rates. Therefore, these tissues receive their minerals primarily through redistribution from the source (leaves) via the phloem (Subramanian *et al.*, 2011). In this case, foliar application of such extracts as a source of minerals could have affected phloem transport; thus, stimulating transportation of mineral nutrients and photo-assimilates from source (leaves) to sink organs (potato tubers). As modified stems, tubers also obtain their minerals via direct uptake from the soil across the periderm, thereby increasing the nutrient concentration of tubers (Subramanian *et al.*, 2011). These results are in accordance with Bertling and Mabaso (2019), who noted a significant increase in mineral content of radish (*Raphanus sativus*) storage root and green bean (*Phaseolus vulgaris*) pods following 50% MLE application. Similarly, Ali *et al.* (2016) reported that application of 0.5% ANE considerably enhanced mineral composition of tomato fruits. In addition, Abdel-Mawgoud *et al.* (2010) observed similar results of higher mineral



contents in cucumber (*Cucumis sativus*), watermelon (*Citrullus lanatus*) and pepper (*Capsicum annuum*).

Mass loss causes deterioration of structural and visual quality characteristics, thus, rendering tubers unmarketable, resulting in significant economic losses. In the present study, it was observed that mass loss increased with a longer storage period; however, dipping of tubers into various plant extracts considerably reduced this mass loss over time (Fig 4.1). These findings correspond with Liamngee *et al.* (2019), who found that dipping of tomato fruit into MLE solutions of different concentrations substantially decreased mass loss during storage. Similarly, Fan *et al.* (2014) reported that an ANE concentration of 1.0 g/L significantly reduced percentage mass loss of spinach during a 35-day storage period. Abidi *et al.* (2023) also demonstrated a positive effect of olive leaf extract (5%), reducing mass loss of peaches (*Prunus persica*) during storage; these results are in line with the present study. Reduction in mass loss could be explained by plant extracts forming a layer around the tuber surface that is difficult to permeate (coating); hence, this treatment reduced the respiration rate by inhibiting gaseous exchange (Mahajan *et al.*, 2018). Such a coating ultimately resulted in reduced moisture loss, thereby suppressing sprout development (Sogvar *et al.*, 2016). In addition to this, plant extracts contain a wide range of compounds, such as vitamin C, phenolics and flavonoids (Table 4.1), which exhibit antimicrobial and antioxidant properties (Tsfay *et al.*, 2016).

Generally, total soluble solids (TSS) are chemical components used to predict fruit maturity and shelf-life (Reboredo-Rodriguez *et al.*, 2014); hence, this parameter helps to determine, if the nutritional status of the produce is acceptable, thereby alluding to its economic value. The present study reveals that potato tuber TSS increases with an increase in the storage period, although application of various plant extracts as post-harvest treatments, especially ANE and MLE, had a profound effect on maintaining tuber TSS levels during storage (Fig 4.2). These findings are similar to Shrestha *et al.* (2018), who observed a significant increase in TSS levels of mango fruit during storage, following the application of various plant extracts. Abidi *et al.* (2023) also reported similar results on peaches, where application of 5% MLE as well as 5% olive leaf extract enhanced TSS during storage. Rasheed *et al.* (2020) revealed that the application of 60% aloe vera extract and 40% neem extract significantly reduced TSS accumulation in 'Kinnow' mandarin (*Citrus reticulata* L.) fruit during storage. An increase in TSS in fruit tissues over time could be attributed to the accumulation of sugars caused by breaking down of complex starch molecules into simple sugars, such as glucose, fructose and sucrose (Brishti *et al.*, 2013). Azene *et al.* (2014) reported that, as the fruit approaches



senescence, stored carbohydrates are converted into simple soluble sugars, thereby increasing TSS, similar trend was seen in our study (Fig 4.3). The increase in TSS level could also be explained by fact that ANE and MLE are polysaccharide-rich natural products (Abdalla, 2014; Chater *et al.*, 2016). Application of such extracts, therefore, could have increased the activity of certain enzymes, including sucrose synthase, invertase and amylase, resulting in the hydrolysing of starch molecules and converting them into simple, soluble sugar molecules (Kumar *et al.*, 2008), thereby increasing tuber TSS.

Carbohydrates are most abundant and widely distributed primary metabolites in horticultural commodities, especially in root vegetables and tubers, which comprise of 15-25% carbohydrates (Yahia and Carrillo-Lopez, 2018). In most agricultural crops, carbohydrates contribute significantly to texture, colour, flavour and nutritive value (Yu *et al.*, 2021). Carbohydrates also fuel and regulate the accumulation of specialized ‘sensory’ compounds that are crucial post-harvest quality of agricultural commodities (Trouvelot *et al.*, 2014). In the present study, it was observed that carbohydrates decrease with an extending storage period (Fig 4.5). Even though carbohydrates overall decline, treatment application, especially MLE, had a pronounced effect, maintaining carbohydrates of tubers during storage at levels higher than the control (Fig 4.3). These findings coincide with Idah and Aderibigbe (2007), who noted that a significant decline in carbohydrate concentration in dried tomatoes as the storage period continues. Idah *et al.* (2010) also demonstrated a considerable decrease in carbohydrate concentration as the storage period of tomato and orange (*Citrus sinensis*) was extended, similar to results found in the present study. Similarly, Famuyini *et al.* (2020) demonstrated a significant decrease in carbohydrate concentration in cherry tomatoes (*Solanum lycopersicum* var. *cerasiforme*) during 18-day refrigerated storage. These carbohydrates decline during storage might be due to the breaking down of complex, insoluble starch molecules into simple, soluble sugar, resulting in an increase in TSS (Fig 4.2). The decrease in carbohydrate concentration could also be due to plant extract applications, which might have increased sucrose synthase, invertase and amylase activity, resulting in rapid conversion of carbohydrates to simple sugars (Kumar *et al.*, 2008), thereby decreasing tuber carbohydrates. Additionally, a decrease in carbohydrate concentration could be closely linked to oxidation processes, due to the available O<sub>2</sub> triggering the conversion of starch into simple sugars (Azene *et al.*, 2014).

Generally, proteins are essential in plants playing important roles in many biological processes, such as fruit development, ripening and senescence (Pedrischi, 2017). Most importantly, proteins play a pivotal role as enzymes (Rasheed *et al.*, 2020) and take part in various metabolic

pathways, producing compounds that induce resistance of fruit and vegetables to abiotic and biotic stimuli (Pedrischi, 2017). Even though potatoes are rich in protein, changes occurring in the protein concentration of potato tubers during storage have not been studied extensively. The present study demonstrates that the protein concentration decreases with an increase in the storage period (Fig 4.4). This has been previously described by Idah *et al.* (2010), who observed a significant decrease in protein concentration with an increase in the storage period of tomato and orange fruit. Similarly, Lee and Cho (2012) reported a decline in protein concentration of black soybean (*Glycine max*) stored at room temperature. The application of various plant extracts, as post-harvest treatments, especially ANE, had, however, a preserving effect on protein concentrations during storage (Fig. 4). The decline in protein during storage could be due to Maillard reactions, which occur between free amino groups of protein and carbonyl groups of reducing sugars, forming complex intermediate compounds by interacting with each other during storage (Rehman, 2006). These complex intermediate compounds, therefore, might have inhibited proteolytic and amylolytic enzyme activities, thus, causing a distinct decrease in protein levels (Lee and Cho, 2012). In addition, proteins also play a crucial role in secondary metabolite production, especially of solanine, a glycoalkaloid compound that could induce protection of tubers against post-harvest stress (Barceloux, 2009). This solanine production could have caused the total tuber protein concentration to decrease with storage. This phenomenon also suggests that potato proteins might have been broken down into simple peptides and amino acids during storage (Shewry *et al.*, 1995). The protein concentration-preserving effect of ANE could be due to the amount of protein this plant extract contains (Table 3.1; Nayak *et al.*, 2020). Potato is an excellent source of protein compared to other staple food crops, thus, reducing the decline in protein content is highly beneficial to sustain its nutritional status.

In recent years, consumption of plant-based foods, particularly fruit and vegetables, has gained immense attention due to their potential positive role in human health (Külen *et al.*, 2013). Intake of plant foods with high concentrations of the analysed phytochemicals has been associated with the reduced risk of chronic disease, including cancer, diabetes and atherosclerosis (Gundgaard *et al.*, 2003), as these foods contain large amounts of phytochemical compounds with antioxidant activities (Krinsky, 2001). Vitamin C, also known as ascorbic acid, phenolic compounds and flavonoids are major antioxidant compounds, present in potato tubers, which exhibit antioxidant activity (Campos *et al.*, 2006). Independent of treatment application, vitamin C decreased greatly with an increasing storage duration (Fig

4.5), as also observed by Abidi *et al.* (2023), who noted a remarkable decline in vitamin C during peach storage. To the contrary, Galani *et al.* (2017) demonstrated a significant increase in vitamin C of potato tubers stored at room temperature (25-32°C). Shrestha *et al.* (2018) also reported that vitamin C concentrations initially increase, then decline during storage. This loss of ascorbic acid might be attributed to the rapid conversion of L-ascorbic acid into dehydro-ascorbic acid with the aid of ascorbic acid oxidase (ascorbinase) (Shrestha *et al.*, 2018). Moringa leaf extract (MLE) treatment had the highest retention of ascorbic acid among all treatments throughout the storage period. This phenomenon might be ascribable to the fact that MLE contains vitamin C (Table 3.1; Abdalla, 2014). The pre-harvest foliar application of MLE, therefore, improved transportation of vitamin C from the source to the sink (tubers), which ultimately resulted in enhanced ascorbic acid concentration even at post-harvest storage. This higher ascorbic acid concentration in MLE-treated tubers could also be due to the MLE application, which created a semi-permeable barrier on the tuber surface, thus, retarding the flow of oxygen into the tuber, in turn lowering tuber respiration rate (Saha *et al.*, 2014).

Total phenolic and flavonoid concentrations, as well as antioxidant activity, initially increased postharvest, then decreased (Fig 4.6, 4.7 and 4.8). Loss of antioxidant compounds, especially phenolics and flavonoids, during storage might be ascribed to their oxidative breakdown (Mditshwa *et al.*, 2017). Galani *et al.* (2017) also reported that the decrease in antioxidant activity during storage could also be associated with the decreasing levels of antioxidant compounds (*viz.* ascorbic acid, phenolics, flavonoids, anthocyanins and carotenoids) (Fig 4.5, 4.6 and 4.7). The best retention of total phenolics, flavonoids and antioxidant activity were observed following ANE application; this could be due to the fact that ANE is an excellent source of such compounds (Table 3.1; Nayak *et al.*, 2020). Exogenous application of ANE, therefore, also increased the internal concentration of antioxidant compounds in tubers; thus, tubers exhibited a high antioxidant activity following ANE treatment. These results are in line with Galani *et al.* (2017), who observed that total phenolic concentrations of potato tubers stored at room temperature (27°C) initially increase, then decrease throughout the storage period. In contrast, Abidi *et al.* (2023) demonstrated a considerable decrease in total phenolic and flavonoid concentrations during peach storage.

#### **4.6. Conclusion**

Various plant-based extracts studied in this investigation had significant impacts on nutrient composition, post-harvest quality of potato tubers. The present study revealed that post-harvest



dipping of potato tubers into plant extracts can improve post-harvest quality and prolong shelf-life. Application of plant extracts, especially of ANE and MLE, showed vast improvements in mineral concentrations, whereas AVE and ANE showed minimal physiological mass loss. In addition, total soluble solids, total carbohydrates, total proteins, vitamin C, total phenolics, total flavonoids and antioxidant activity were highest in ANE- and MLE-treated tubers. The performance of ANE and MLE was, hence, superior among all tested plant extracts, indicating that natural plant extract usage can be an excellent alternative for hazardous, synthetic pesticides. Plant extracts preserved post-harvest quality of potato tubers, while producing safer and healthier food for consumers.

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## General Discussion and Recommendations

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The global issue of population growth is placing enormous pressure on farmers across the world. Consequently, there is a heavy reliance on chemical-based inputs to enhance the yield and productivity of food crops, with the ultimate goal of achieving Sustainable Development Goals 1 and 2. These goals target the eradication of poverty and hunger while improving the overall well-being of humanity. However, these chemical-based inputs are environmentally unfriendly and pose risks to human health. In addition, the utilisation of chemical-based inputs (*i.e.*, inorganic fertilizers and synthetic pesticides) in crop farming does not only harm the surrounding ecosystem and compromise human health, but it also pays a significant contribution towards climate change and its aligned biotic and abiotic factors such as drought, salinity, heavy metals, weed infestation, as well as pest and diseases. Application of these agrochemicals can, hence, result in reduced healthiness of the agricultural products and also increase food and nutrition insecurity. Achieving food security is not limited to agricultural productivity, but post-harvest losses are a further factor that needs to be addressed in order to achieve sufficient food supply for the ever-growing population. As such, pre- and post-harvest application of plant extracts can potentially be used in agriculture to increase crop productivity and yield, while maintaining product quality and extending its shelf-life, without negatively affecting consumers' health and posing threats to the ecosystems.

In sustainable agriculture, there is a clear need to explore and develop novel, financial-feasible and eco-friendly strategies to stimulate plant growth and crop protection. Use of plant-based extracts is an emerging, innovative and natural approach that could be used as natural biostimulant and biopesticide due to high content of several biologically active compounds. Plant extracts contain various growth-enhancing, yield-promoting and quality-sustaining compounds, including minerals, polysaccharides, phytohormones, vitamins and other antioxidant compounds. Most of these compounds exhibit antifungal, antimicrobial and antibacterial properties. This research, therefore, aimed to evaluate the efficacy of various plant extracts on enhancing growth and development, as well as yield, while sustaining post-harvest quality of potatoes because they form part of most people's diet and have various health benefits. The study composed of two experiments, one conducted in a glasshouse and the other one in the post-harvest laboratory at the University of KwaZulu-Natal. The first experiment intended to evaluate the influence of various plant extracts on growth, certain physiological and yield attributes of potatoes, whereas the second experiment determined the impact of



various plant extracts on the post-harvest quality and shelf-life of potatoes kept under ambient conditions.

In the first experimental chapter (chapter three), plant extracts were foliarly sprayed on potato plants (50 mL per plant). The first foliar application of plant extract occurred four weeks after planting, then repeated weekly (7-day interval) until harvesting. From first week of treatment application, data on vegetative growth attributes (i.e., plant height, number of leaves and leaf area) were continuously recorded weekly till harvesting, whereas data on physiological (leaf chlorophyll concentration) and yield attributes (total tuber yield and tuber fresh mass) were only recorded immediately after harvesting. The results obtained in chapter three validates that pre-harvest foliar application of plant extracts to potato plants positively influences growth, physiological and yield parameters, including plant height, number of leaves, number of main stems, leaf area, leaf chlorophyll index, total tuber yield and tuber fresh mass. Amongst treatments, ANE- and MLE-treated potato plants had a profound influence on the measured growth, physiological and yield parameters compared to aloe vera leaf extract (AVE), garlic bulb extract (GBE) and the control. The outstanding performance of these two treatments could be ascribed to the amount of growth-stimulating and yield-promoting phytochemicals present in these extracts. Foliar application of plant extracts has been reported to improve growth and development, as well as yield, of several solanaceous crops, such as tomato (Ahmed *et al.*, 2022), pepper (Rajendran *et al.*, 2022), potato (Haider *et al.*, 2012) and eggplant (Ali *et al.*, 2019).

Apart from plant growth and yield parameters, post-harvest quality of agricultural commodities is significantly important to consumers. Potatoes are consumed as staple food in many parts of the world due to health and nutritional benefits they offer to consumers. Despite being a carbohydrate-rich commodity, potato is also a valuable source of several body-building phytonutrients, including, fibre, protein, vitamins and minerals. As such, the second experimental chapter (chapter four) examined the effect of pre-plus post-harvest application of plant extracts on sustaining the post-harvest quality of potatoes kept under ambient storage conditions. The results obtained in chapter four demonstrates that pre- plus post-harvest application of plant extracts significantly preserved the quality of potatoes during storage. Even though the application of treatments had a positive influence on retaining the quality of potatoes, ANE and MLE were superior in these aspects compared to AVE, GBE and the

control. Similarly, the latter extracts performed well in the pre-harvest chapter (chapter three). Plant extracts have been widely reported to preserve quality of many horticultural commodities, including potato (Haider *et al.*, 2012), eggplant (Ogbuehi *et al.*, 2016), tomato (Liamngee *et al.*, 2019) and kale (Melo *et al.*, 2018).

The results from the present research verify the potential use of plant extracts to enhance plant growth and development, yield, biochemical attributes, as well as shelf-life of agricultural crops. The use of plant extracts could, therefore, be adopted as a tool to improve agricultural productivity, reduce post-harvest losses and sustain the quality of the produce during storage, thus, tackling food insecurity and malnutrition, important goals in light of the constantly rising world population. The results presented in this research are of foremost importance to both, commercial and small-scale farmers, as plant extract utilization is an inexpensive, safe, eco-friendly and sustainable method that could potentially substitute currently employed agrochemicals in crop farming, maintaining food security. In addition, the results presented in this research can help achieve sustainable development goals 1 and 2 which target the eradication of poverty and hunger while improving the overall well-being of humanity. Furthermore, plant extracts, especially ANE and MLE, have shown positive effects on yield and quality parameters of solanaceous crops, but further validation of these extracts on other types of vegetable crops is recommended in order to popularize and commercialize use of such extracts in agriculture.

## **Recommendations**

- ✓ Future research should be conducted to determine impact of individual plant extract and various plant extract combinations on various leafy and fruit vegetable crops.
- ✓ More study should be carried out to compare the efficacy of conventional, non-conventional and aqueous extraction methods in an attempt to identify compounds in the extracts that induce growth-enhancing, yield-promoting and quality-preservative effect in plants.
- ✓ Future research should focus more on the mechanisms of action of plant extracts within plants.
- ✓ Amongst various modes of action, further research studying the effect of plant extracts on enzyme activities and gene expression in treated plants is necessary.

- ✓ Hormonal analysis and activities of plant extracts also needs to be critically studied in future research.

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## Appendix

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**Table A1:** Chemical composition of the soil (Cedara College of Agriculture, Department of Agriculture and Rural Development, KwaZulu-Natal).

Composition	Conc.	Units
Phosphorus (P)	12.5	mg/L
Potassium (K)	93	mg/L
Calcium (Ca)	179	mg/L
Magnesium (Mg)	24	mg/L
Zinc (Zn)	1.2	mg/L
Manganese (Mn)	8.5	mg/L
Copper (Cu)	6.35	mg/L
Nitrogen (N)	0.31	%
Organic C	≥ 6	%
Clay	45	%
Acidity saturation	57.5	%
Exchangeable acidity	1.79	cmol/L
Total cations	3.11	cmol/L
pH (KCl)	4.05	-



**Table A2:** Chemical analysis of growing medium (Cedara College of Agriculture, Department of Agriculture and Rural Development, KwaZulu-Natal).

Composition	Conc.	Units
K	9.84	me/L
Ca	1.54	me/L
Mg	1.80	me/L
Fe	3.36	ppm
Mn	0.19	ppm
Na	5.26	me/L
Al	5.09	ppm
EC	197.77	mS/m
pH	7.44	-
SAR	4.08	me/L
TDS	1 206.38	me/L

**Table A3:** Chemical composition of poultry manure (as per manufacturer).

Composition	Conc.(g/kg)
N	21
P	32
K	27
C	0.35
Zn	2
Cu	0.07
Mo	4.5

Fe	2.2g
B	1.2
Mn	1

# Impact of Foliar-applied Plant Extracts on Growth, Physiological and Yield Attributes of Potato (*Solanum tuberosum* L.)

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**Abstract:** The current reliance on pesticides and synthetic fertilizers has been vital to sustain and increase agricultural production. The continuous, excessive use of these traditional practices has negatively affected consumers' health and burdened the ecosystem. The use of plant extracts as a tool to minimize agrochemical inputs has been extensively investigated; these extracts have the ability to improve plant growth and agricultural productivity. This study was, therefore, conducted to determine the effect of foliar plant extract application on potato growth, as well as certain physiological and yield attributes. From four weeks after planting onwards, five healthy, equal-sized potato plants per treatment received various foliar plant extract applications. These extracts included brown seaweed *Ascophyllum nodosum* extract (ANE), aloe vera leaf extract (AVE), garlic bulb extract (GBE) and moringa leaf extract (MLE). Application of treatments were repeated weekly until harvesting. Application Data on growth and physiological parameters were collected weekly. Pre-harvest foliar application of various plant extracts significantly enhanced ( $p \leq 0.05$ ) plant growth, physiological and yield attributes of potatoes. Best growth and yield responses were observed following ANE and MLE application. Plant extracts have shown beneficial effects on other crops, but further validations of these effects on other crops is still necessary in an attempt to popularize and commercialize such applications.

**Keywords:** biostimulants; food security; plant extracts; potato; sustainable agriculture

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## 1. Introduction

Potato (*Solanum tuberosum* L.) is a member of the Solanaceae family, native to South America, but is now grown in most parts of the world [1]. Amongst the cash crops, potato is one of the world's most important non-grain food crops with a global production of about 376 million tonnes,

with China as the largest producer contributing approximately 94 million tonnes annually [2]. Potato is also recognized as a staple food, being the third most-consumed food crop worldwide, following rice and wheat [1]. The worldwide *per capita* potato consumption reached 33.1 kg in 2020, possibly due to the health and nutritional benefits potato offers [2]. According to Zaheer et al. [3], potato is an excellent source of dietary fibre, carbohydrates, high-quality protein, vitamins, minerals and other metabolites. Being rich in health-promoting metabolites, potato possesses high antioxidant activity, which helps to reduce the risk of chronic diseases, including heart disease, diabetes and cancer [4].

In the last couple of decades, there has been a rapid increase in potato demand. For all potato growers, it has therefore become of immense importance to produce this crop's profitably, while keeping input costs at a minimum. Additionally, modern agriculture demands sustainable crop production, searching for alternative methods to sustain plant development with little or no compromise to yield. Both subsistence and commercial farmers are facing a major challenge of biotic and abiotic factors aligned with climate change. These include drought, salinity, weed infestation, pests and diseases, which can all devastatingly affect growth and yield of potato [5]. Given these challenges, synthetic pesticides and inorganic fertilizers have become vital for the production of crops and their protection against biotic and abiotic constraints [6]. The reliance on industrially based inputs may, however, pose multiple threats to human health and impart harmful effects on the ecosystem [7]. In addition, Lucas et al. [8] revealed that the continuous, excessive usage of such chemicals might result in the development of new pathogen strains that could become difficult to control, despite the efficacy of the chemical. The aim of modern agriculture is, therefore, to reduce the utilization of these chemicals to a minimum; thus, making crop farming simpler and offering healthier, safer and sustainably produced goods.

Farmers and researchers are, therefore, continuously exploring and developing alternative approaches to crop farming, trying to overcome challenges of long-term production viability [7]. Among several proposed strategies, the use of plant extracts has been identified as a promising, innovative, eco-friendly and sustainable approach that could improve crop production and crop protection. Recent studies have tested this method on a broad spectrum of solanaceous crops, such as potato, sweet pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.) [9–12]. The present study, therefore, aims to evaluate the effect of foliar application of plant extracts (*viz.* *Ascophyllum nodosum* extract, garlic bulb extract, aloe vera leaf extract and moringa leaf extract) on growth, including certain physiological and yield attributes of potato.

## 2. Materials and Methods

### 2.1. Plant material and growing conditions

A pot experiment was carried out in a glasshouse at the Controlled Environment Facility (CEF) of the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29°37'32.9"S 30°24'18.8" E). Locally obtained baby potatoes, cv. 'Sifra', were planted in June (mid-winter) as seed tubers at a depth of 10 cm into 10 L plastic pots filled with a mixture of sandy soil and Gromor® (Gromor, Cato Ridge, South Africa) potting mix. Chemical and physical attributes of the soil and growing medium used were analysed before planting (Cedara College of Agriculture,



Department of Agriculture and Rural Development, KwaZulu-Natal). Poultry manure (Nutri-Green Gwano Pellets, Protek, Heidelberg, Gauteng, South Africa), was then applied to amend soil nutrition, at a rate of 25 g/pot, based on the chemical and physical characteristics of the used medium. Thirty days after planting, the same fertilizer was re-applied as a top-dressing at a similar rate (25 g/pot). The environmental conditions inside the glasshouse were maintained at  $25 \pm 2^\circ\text{C}$  and 65% relative humidity (RH) during the day, while temperature and RH were kept constant at  $13 \pm 2^\circ\text{C}$  and 72% at night, respectively. Plants were irrigated using automated drip irrigation, dispensing approximately 50 mL per 10 L pot daily.

## 2.2. Experimental design and foliar application

The study was laid out following a completely randomized design (CRD) with five replications. Five healthy, similar-sized ‘Sifra’ baby potatoes, randomly selected, were used per treatment, with five seed tubers per replicate, giving 25 experimental units (10 L pots). The experiment consisted of four treatments, namely *Ascophyllum nodosum* extract (ANE), moringa (*Moringa oleifera*) leaf extract (MLE), garlic (*Allium sativum*) bulb extract (GBE) and aloe vera (*Aloe barbadensis* Mill.) leaf extracts (AVE), plus the control (no extract application). The above-mentioned treatments were directly applied to potato leaves using a hand-held pressure sprayer, each plant received 50 mL. The first foliar application of treatments was performed four weeks after planting (vegetative stage), and treatment applications were repeated weekly until harvest (mid-August, early spring).

## 2.3. Collection and extract preparations

Plant material used for extracts preparation was obtained from various suppliers. Brown algae (*Ascophyllum nodosum*) powder (Nature’s Choice) was bought locally (Dis-Chem, Woodburn Mall, Pietermaritzburg, South Africa), whereas healthy aloe vera plants were bought locally from Woodland nursery, (Pietermaritzburg, South Africa). Fresh moringa (*Moringa oleifera*) leaf powder (MLP) was supplied by a commercial supplier (runKZN, Pietermaritzburg, South Africa), while fresh Egyptian white garlic was bought from a local supermarket. The used extracts, ANE, AVE, MLE and GBE were prepared following the procedure described by Noor et al. [13], Ting-Ting et al. [14] and Ngcobo and Bertling [11], with slight modifications. Exactly 10 g of each plant material was weighed out and homogenized in a glass beaker with 450 mL distilled water. The homogenates were then placed onto a hot plate, continuously agitated with a magnetic stirrer and allowed to boil at  $100^\circ\text{C}$  for 30 min. After 30 min, the solutions were allowed to stand for 2 hrs to cool down; then the supernatants were collected and filtered three times through muslin cloth. Then, to make a final volume of 1 L, serial dilutions were made with distilled water. Furthermore, the chemical composition of the extracts was analysed and presented in **Table 1**.

**Table 1.** Chemical composition of various plant extracts. GAE (gallic acid equivalent), QE (quercetin equivalent) and AAE (ascorbic acid equivalent). ANE (*Ascophyllum nodosum* extract), AVE (aloe vera leaf extract), GBE (garlic bulb extract) and MLE (moringa leaf extract).

Composition*	ANE	AVE	GBE	MLE	Units
Nitrogen (N)	0.08	0.11	-	0.17	%
Phosphorus (P)	4.2	2.2	16.0	10.4	%
Potassium (K)	102.6	79.4	96.8	106.4	%
Calcium (Ca)	10.4	58.6	-	77.4	%
Magnesium (Mg)	16.6	24.0	2.6	23.2	%
Sodium (Na)	127.2	3.0	2.2	0.8	mg/kg
Manganese (Mn)	-	4.0	-	-	mg/kg
Copper (Cu)	-	-	-	-	mg/kg
Zinc (Zn)	0.9	-	-	1.2	mg/kg
Iron (Fe)	-	-	-	-	mg/kg
Ascorbic acid (Vitamin C)	86.5	78.1	57.1	79.7	mg/g DM
Total phenolics	11.9	2.1	2.4	3.9	mg GAE/g DM
Total flavonoids	28.6	3.3	11.7	51.7	mg QE/g DM
Total protein	104.7	53.7	61.1	81.5	mg/g DM
Antioxidant activity [DPPH (diphenyl-1-picrylhydrazyl)]	105.3	94.9	90.5	95.4	mg AAE/g DM

\* For mineral composition, plant extracts were analyzed using inductively coupled plasma mass spectrometry (ICP-MS), whereas the analyses of phytochemicals were carried out following methods of Boonkasem et al. [15] (ascorbic acid concentration), Wang et al. [16] (total phenolics), Gu et al. [17] (total flavonoids), Bradford [18] (total protein) and Rocchetti et al. [19] (antioxidant activity-DPPH) using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

#### 2.4. Determination of vegetative growth, certain physiological, morphological and yield parameters

##### 2.4.1. Plant height and number of leaves

Plant growth parameters, including plant height and number of fully expanded leaves were recorded from the first treatment application until stage 4 (tuber bulking) of potato growth and development at 7-day intervals. Plant height (cm) was measured from the base of the stem to the tip of the terminal bud using a tape measure. The total number of leaves per plant were counted manually. These parameters were measured in all 25 plants used in this experiment.

##### 2.4.2. Leaf area

From the first treatment application until the tuber bulking stage, leaf area of the entire potato plant was estimated directly from leaf length

and width measurements. These measurements were taken from 20 leaves per treatment ( $\approx 4$  leaves per plant). Leaf area was then calculated using the formula described by Bhatt and Chanda [20].

$$LA = 11.98 + 0.06L \times W,$$

where LA = Leaf area ( $\text{cm}^2$ ), L = leaf length (cm) and W = leaf width (cm).

#### 2.4.3. Leaf chlorophyll index

Leaf chlorophyll index was determined using a portable, non-destructive and lightweight instrument (CCM-200plus - Opti-Sciences Inc., Hudson, NH, USA). At tuber bulking stage (2 week after treatment application), the chlorophyll content index was measured from three fully developed functional leaves on each potato plant. A total of four plants, randomly selected from each treatment, were measured.

#### 2.4.4. Fresh and dry above-ground biomass

Both fresh and dry above-ground biomass (g/plant) were determined using a fine balance in all 25 plants used in this experiment. Fresh mass was recorded immediately after harvesting (mid-August, early spring), whereas dry mass was measured after four days of oven-drying at  $80^\circ\text{C}$ .

#### 2.4.5. Yield and fresh tuber mass

At the mature tuber stage, all tubers were harvested from all replicates. Total tuber yield (tuber number/plant) and tuber mass (g) were recorded immediately after harvesting (mid-August, early spring).

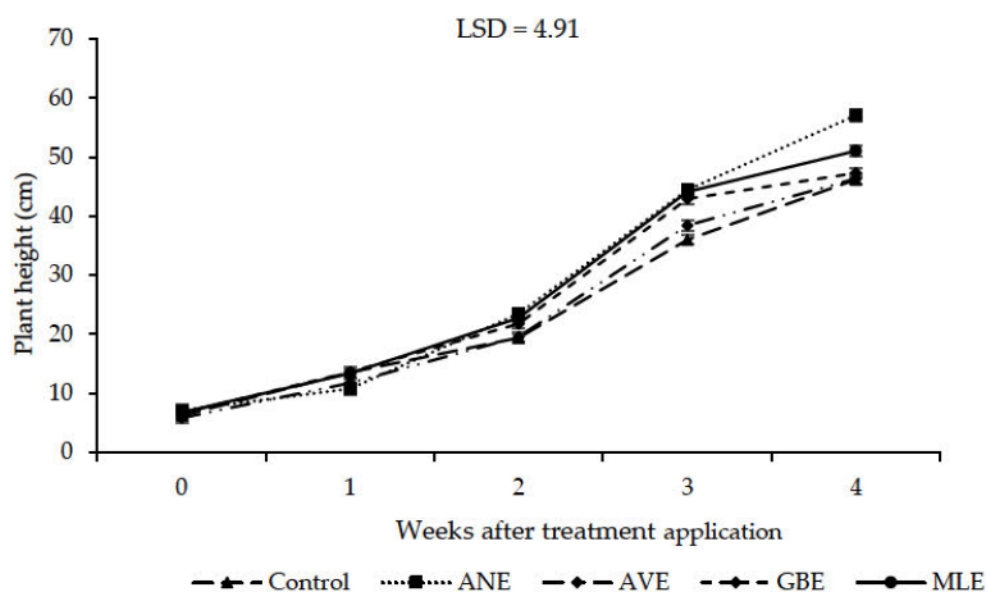
### 2.5. Statistical analysis

Results obtained were subjected to one-way analysis of variance (ANOVA) using GenStat statistical software (GenStat®, 18th edition, VSN International, UK) and plotted using Microsoft Excel®. Means separation were carried out using Duncan's multiple range test with a difference at  $p \leq 0.05$  considered significant; LSD (Least Significant Difference) values were compared at a 5 % significance level.

## 3. Results

### 3.1. Plant height

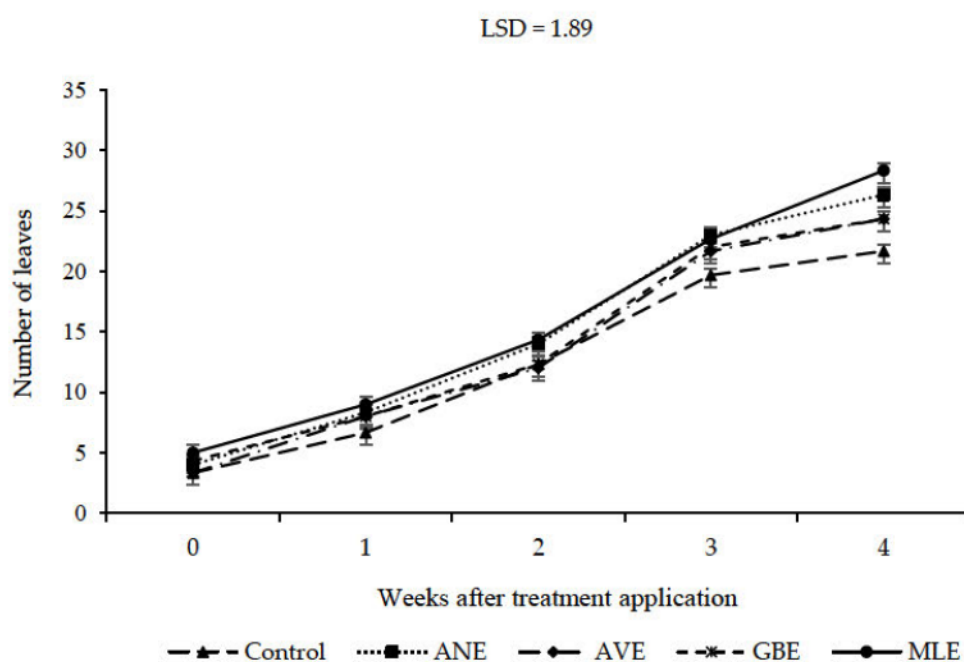
Variations in morphological data revealed a significant effect of plant extract application on the growth of potatoes in terms of plant height. The results indicate that pre-harvest application of ANE at the vegetative stage significantly influenced ( $p \leq 0.05$ ) potato plant height, unlike other treatments, four weeks after treatment application (Fig. 1). This treatment, hence, resulted in the tallest plants, followed by MLE-treated plants on the 4<sup>th</sup> week after treatment application (Fig. 1). There were, however, no significant differences between values recorded in AVE, GBE and the control (Fig. 1).



**Figure 1.** Effect of foliar-applied plant extracts (g/L) on the height of potato plants. Control (no application), ANE (*Ascophyllum nodosum* extract), MLE (moringa leaf extract), GBE (garlic bulb extract) and AVE (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 4.91.

### 3.2. Number of leaves

Four weeks after treatment application, number of leaves in all treatments was significantly higher than in the control ( $p \leq 0.05$ ). The MLE treatment recorded the highest number of leaves, followed by ANE (Fig. 2); the number of leaves recorded in the AVE and GBE treatments were less than that of ANE and MLE.



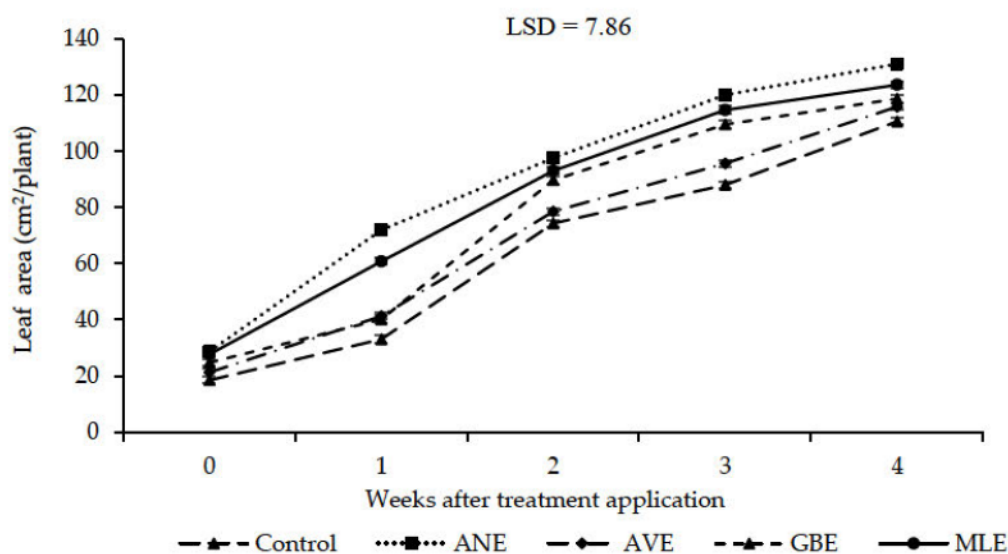
**Figure 2.** Effect of weekly applied plant extracts (g/L) on the number of potato leaves. Control (no application), ANE (*Ascophyllum nodosum* extract), MLE



(moringa leaf extract), GBE (garlic bulb extract) and AVE (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 1.89.

### 3.3. Leaf area

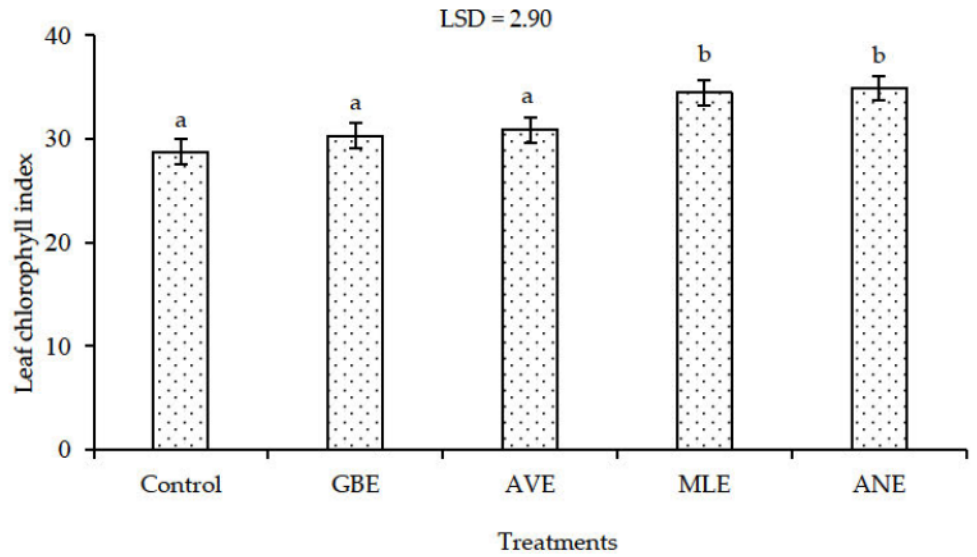
The treatment of potato plants with various plant extracts positively influenced leaf area development. Four weeks after treatment application, the largest leaf area was obtained by potato plants treated with ANE, followed by plants treated with MLE, GBE and AVE. The smallest leaf area was, however, recorded for the control. Obtained results were significantly different ( $p \leq 0.05$ ) from each other (Fig. 3).



**Figure 3.** Effect of foliar-applied plant extracts (g/L) on the leaf area of potatoes. Control (no application), ANE (*Ascophyllum nodosum* extract), MLE (moringa leaf extract), GBE (garlic bulb extract) and AVE (aloe vera leaf extract). LSD at ( $p \leq 0.05$ ) = 7.86.

### 3.4. Leaf chlorophyll index

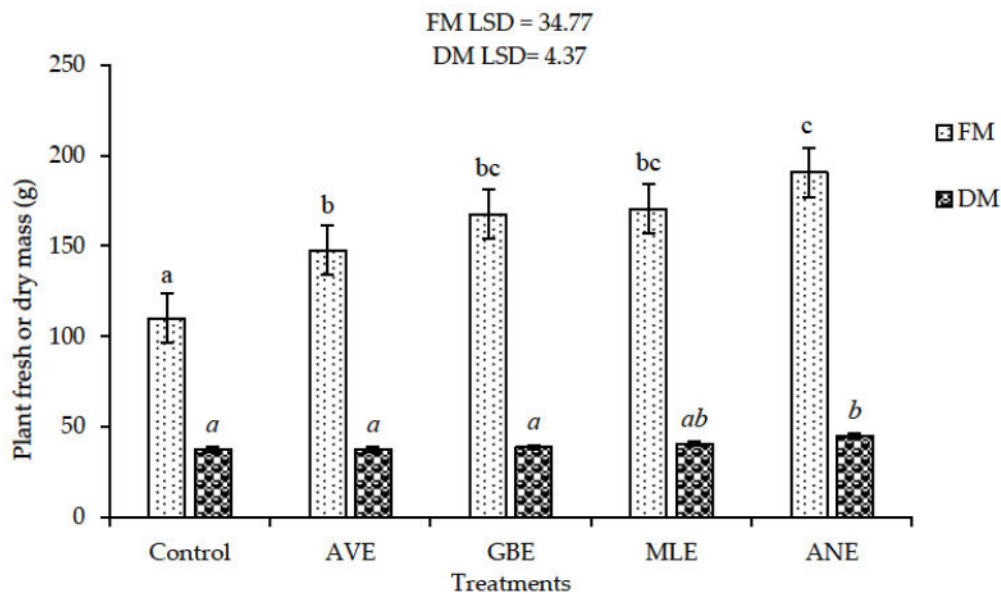
The treatment of potato plants with various plant-derived extracts significantly enhanced the leaf chlorophyll index ( $p \leq 0.05$ ). The highest leaf chlorophyll index was obtained in potato plants treated with ANE and MLE; however, the two latter treatments did not differ significantly from each other. The control recorded the lowest leaf chlorophyll index, with a value that does not differ significantly from GBE and AVE (Fig. 4).



**Figure 4.** Effect of foliar-applied plant extracts (g/L) on leaf chlorophyll index of potatoes. Control (no application), ANE (*Ascophyllum nodosum* extract), MLE (moringa leaf extract), GBE (garlic bulb extract) and AVE (aloe vera leaf extract). Different lowercase letters above each column indicate significant differences between treatments ( $p \leq 0.05$ ) according to Duncan's multiple range test.

### 3.5. Fresh and dry above-ground biomass

Analysis of variance of the fresh and dry mass of above-ground potato plant biomass indicates a significant difference ( $p \leq 0.05$ ) between treatments (Fig. 5). The potato plant treatments showed a pronounced effect on the accumulation of above-ground fresh and dry mass (Fig. 5). Specifically, ANE significantly accumulated the highest fresh and dry mass compared with other treatments, although this treatment does not differ significantly from GBE and MLE. The control treatment, however, accumulated the lowest fresh and dry above-ground biomass.

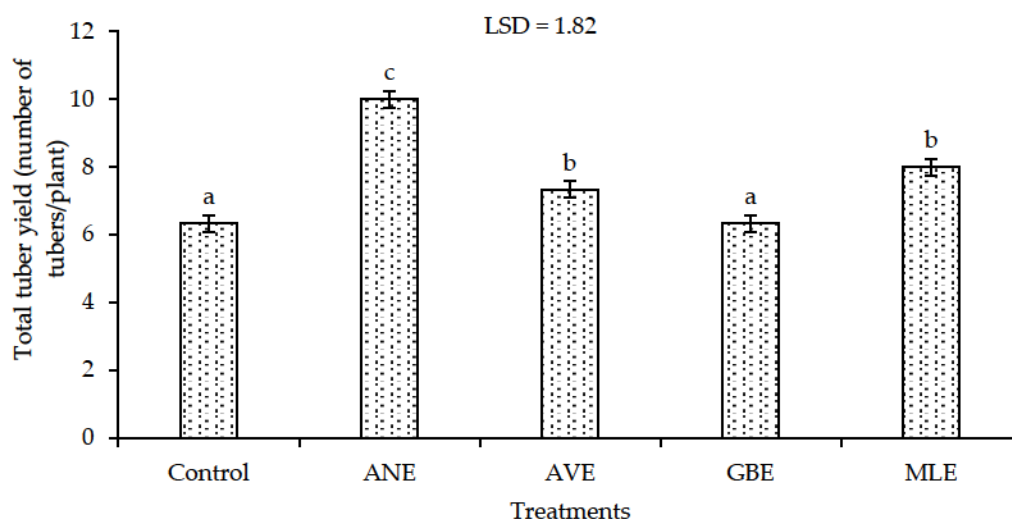


**Figure 5.** Effect of foliar-applied plant extracts (g/L) on fresh and dry mass of potato above-ground biomass. Control (no application), ANE (*Ascophyllum*

*nodosum* extract), MLE (moringa leaf extract), GBE (garlic bulb extract) and AVE (aloe vera leaf extract), FM (fresh mass), DM (dry mass). Different lowercase letters above each column indicate significant differences amongst treatments ( $p \leq 0.05$ ) according to Duncan's multiple range test.

### 3.6. Total tuber yield (number of tubers/plant)

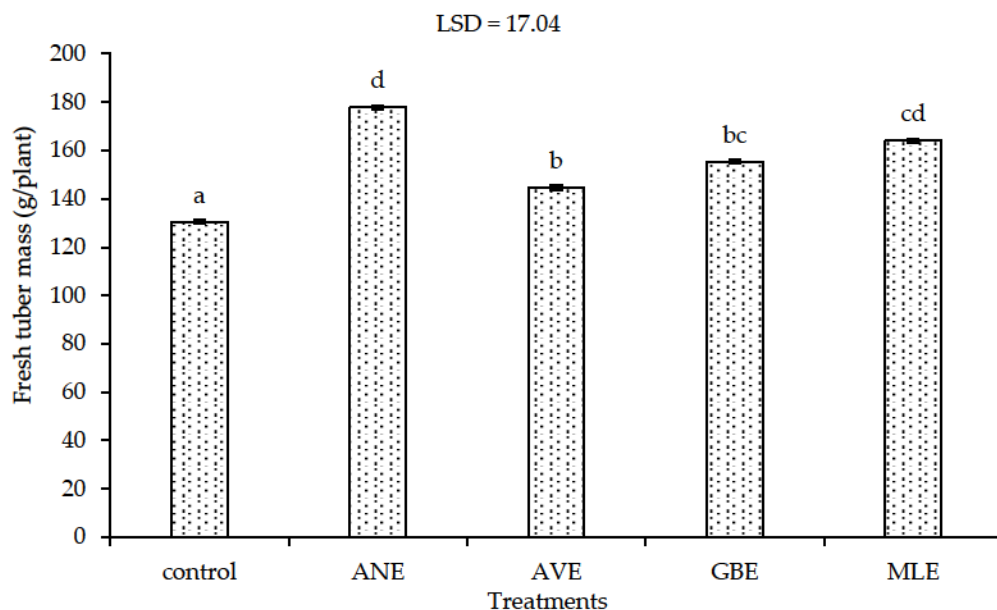
The obtained results indicate that pre-harvest application of all plant extracts significantly influenced ( $p \leq 0.05$ ) total tuber yield, except for the GBE treatment (Fig. 6). While ANE treatment resulted in the most significant tuber number increase compared with other treatments.



**Figure 6.** Effect of foliar-applied plant extracts (g/L) on total tuber yield. Control (no application), ANE (*Ascophyllum nodosum* extract), MLE (moringa leaf extract), GBE (garlic bulb extract) and AVE (aloe vera leaf extract). Different lowercase letters above each column indicate significant differences amongst treatments ( $p \leq 0.05$ ) according to Duncan's multiple range test.

### 3.7. Fresh tuber mass

The treatment of potato plants with various plant extracts positively affected fresh tuber mass. The greatest fresh tuber mass was obtained by potato plants treated with ANE, followed by MLE, GBE and AVE treatments. The control recorded the least fresh tuber mass. Overall, the obtained results indicate significant differences ( $p \leq 0.05$ ) in fresh tuber mass between treatments (Fig. 7).



**Figure 7.** Effect of foliar-applied plant extracts (g/L) on fresh tuber mass. Control (no application), ANE (*Ascophyllum nodosum* extract), MLE (moringa leaf extract), GBE (garlic bulb extract) and AVE (aloe vera leaf extracts). Different lowercase letters above each column indicate significant differences amongst treatments ( $p \leq 0.05$ ) according to Duncan's multiple range test.

#### 4. Discussion

The use of plant extracts as biofertilizers, biostimulants and soil ameliorants has gained increasing attention owing to the adverse effects of synthetic pesticide and inorganic fertilizer application on human health and the environment [21]. Plant extracts have the ability to increase plant growth and yield attributes by positively interfering with plant physiological processes [7]. The present study, therefore, intended to evaluate the growth and yield promotion effects of various plant extracts. The foliar application of the plant extracts made from *Ascophyllum nodosum* (ANE), aloe vera leaves (AVE), garlic bulbs (GBE) and moringa leaves (MLE) had a positive impact on growth and yield parameters of potato.

Increased vegetative growth, leaf chlorophyll concentrations and yield parameters could be attributed to the biofertilization effect of natural plant extracts, especially ANE and MLE [22]. This phenomenon has been previously reported on a wide variety of crops, such as soybean (*Glycine max* L.), sweet pepper (*Capsicum annuum* L.), wheat (*Triticum aestivum* L.) and tomato [11,23–25]. Both ANE and MLE are excellent sources of minerals, including macro- and micro-nutrients (*i.e.*, N, P, K, Ca, Mg, Zn and Na) [26,27]. Mineral elements, particularly N and P, have been known for their vegetative growth promotion in plants, hence, exogenous application of plant extracts increased the availability of N and P to the plant, thereby boosting vegetative growth and above-ground biomass [28]. In addition, enhanced leaf chlorophyll concentration could be due the presence of the minerals Mg and N, which are vital components of the chlorophyll molecule [7,26]. Magnesium is the central atom in the porphyrin ring of the chlorophyll molecule, while N, is also a



structural component of the chlorophyll molecule; this results in a green leaf colour representing a high leaf chlorophyll concentration; both elements are necessary to allow the capture of light energy during photosynthesis [29,30]. Minerals, especially K and Zn, are mainly responsible for yield and quality promotion in crops. Cakmak et al. [29] revealed that adequate K nutrition is vital in crops to enhance photosynthate translocation (via the phloem from source to sink). Potassium also plays a critical role in the partitioning of carbohydrates by improving photosynthate translocation and the growth rate of sink and/or source organs [29]. Furthermore, Zn is an essential component in the biosynthesis of tryptophan, which is involved in the synthesis of indole acetic acid (IAA) [31]; this plant hormone is responsible for cell growth and cell elongation; thus, increasing tuber growth substantially.

Besides biofertilization, enhanced vegetative growth, leaf chlorophyll concentration, plant above-ground biomass and yield attributes following plant extract applications could also be ascribed to their bio-stimulatory effect [32]. Plant extracts, especially ANE and MLE, are natural sources of several bioactive compounds, including phytohormones, ascorbic acid, polysaccharides, betaines and phenolics [26,33]. Exogenous application of such extracts could potentially provide plants with these essential phytochemicals, with many of these compounds working in a synergistic manner [32]. Both, ANE and MLE contain phytohormones, such as auxins [indole-3-acetic acid (IAA)], gibberellins (GAs) and cytokinin (zeatin) [34,35]. The presence of such growth-promoting plant hormones in ANE and MLE could possibly induce cell expansion and cell division, thereby improving vegetative growth, plant biomass and yield attributes, as observed by Rayorath et al. [26] in *Arabidopsis thaliana* (L.). Rioux et al. [36] reported that, besides containing plant hormones, *Ascophyllum nodosum* extract can exhibit a wide range of growth-stimulatory effects because of the polysaccharides present in the extract. Such compounds include laminarin [ $\beta$ -glucan – ( $\beta$ -D-glucose polysaccharide)] and fucoidans (fucose-rich sulphated polysaccharides, consisting primarily of 1,2-linked  $\alpha$ -L-fucose-4-sulfate units with very small amounts of D-xylose, D-galactose, D-mannose, and uronic acid), both exhibiting radical scavenging antioxidant activity [37].

Rayorath et al. [26] also reported significant amounts of betaine (trimethylglycine, a non-protein, methyl-derivative of glycine) present in ANE, which plays a significant role in counteracting metabolic dysfunctions brought on by stress, thereby improving plant growth and survival. In addition to betaines, ANE and MLE contain several antioxidant compounds, including ascorbic acid, tocopherols, flavonoids and polyphenols; their presence triggers antioxidant biosynthesis, thereby reducing stress caused by reactive oxygen species (ROS) [38]. These ROS can cause cell and membrane degradation, which may fast-forward chlorophyll degradation and senescence; hence, these antioxidant compounds found in ANE and MLE could promote growth and developmental processes and maintain leaf greenness by reducing ROS levels in potato plants [39]. Increased leaf chlorophyll concentrations due to the foliar application of ANE and MLE could possibly be due to enhanced gene transcripts involved in photosynthesis, cell metabolism and stress response. Application of ANE and MLE suppresses cysteine protease activity [40], which ultimately results in the inhibition of

chlorophyll degradation, thus delaying senescence in plants, as observed by Kałużewicz et al. [41] in broccoli (*Brassica oleracea* var. *italica*).

The results obtained in the present study confirm findings by Haider et al. [9], who demonstrated a significant improvement in growth attributes of potato plants due to various ANE treatments. Rajendran et al. [21] also demonstrated that growth parameters, such as plant height, number of leaves and branches and leaf area of sweet pepper plants were significantly enhanced by foliar ANE and MLE application; these findings correspond well with the present study. In addition, ANE and MLE applications to tomato plants grown under water-deficit conditions significantly improved plant height, number of leaves and branches, as well as leaf area [12]. Ahmad et al. [42] also noted similar effects on leaf chlorophyll in bulbous cut flowers (*Freesia hybrida*), reporting a significant improvement in leaf chlorophyll concentration following MLE application. Similar results were also observed by Elzaawely et al., [43] in snap bean (*Phaseolus vulgaris* L.) and Ali et al., [44] in okra (*Abelmoschus esculentus* L.). Various authors noted a considerable improvement in yield attributes of many crop species, such as lima beans (*Phaseolus lunatus* L.) [45], tomato and sweet pepper [24], following ANE application, similar to what is observed in this study. These results also coincide with those of Ali et al. [46], who noted that foliar ANE application improved total fruit yield and fruit mass of tomato. Similarly, a significant increase in total tuber yield, following foliar ANE application, was observed by Haider et al. [9]; our results confirm the findings of these authors. In addition, Taskos et al. [47] demonstrated a profound effect of ANE application on total grape yield; moreover, obtained results concur with Rajendran et al. [21], who noted a positive response in total sweet pepper yield and fruit mass, following ANE foliar application. Therefore, the obtained results demonstrate the usefulness of ANE in potato production.

## 5. Conclusions

The present study encourages the use of various plant extracts in the crop farming community. The pre-harvest foliar application of these plant extracts considerably enhanced vegetative growth, physiological and yield attributes of potato. Since modern agriculture necessitates financially feasible and easily accessible organic inputs, use of plant extracts, as biofertilizers, biostimulants and bio-elicitors could effectively be used as an ideal multi-active organic input to improve crop growth and yield potential of agricultural crops. This research has shown that foliar applications of plant extracts, especially of ANE and MLE, have the potential to improve crop productivity and yield. Results presented in this study are, hence, of high significance to commercial as well as small-scale potato growers, as use of organic plant extracts is an environmentally friendly and sustainable approach towards increasing crop productivity. Plant extracts have shown beneficial effects on other crops, but further validations of these effects on other crops is still necessary in an attempt to popularize and commercialize such applications.

**Author Contributions:** Conceptualization, Siphokuhle Mbuyisa. and Isa Bertling.; methodology, Siphokuhle Mbuyisa.; software, Siphokuhle Mbuyisa.;

validation, Siphokuhle Mbuyisa., Isa Bertling. and Bonga Ngcobo.; formal analysis, Siphokuhle Mbuyisa.; investigation, Siphokuhle Mbuyisa.; resources, Bonga Ngcobo.; data curation, Bonga Ngcobo.; writing-original draft preparation, Siphokuhle Mbuyisa.; writing-review and editing, Isa Bertling and Bonga Ngcobo.; visualization, Bonga Ngcobo.; supervision, Isa Bertling.; project administration, Isa Bertling; funding acquisition, Isa Bertling. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by National Research Foundation (NRF), grant number PMDS22061523208.

**Data Availability Statement:** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Acknowledgments:** The technical support from Mr. Nkosi, is gratefully acknowledged. Siphokuhle also thanks NRF for funding. Family and friends are also well-appreciated for their physical and psychological support they provided to make this research successful.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.



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