PHYTOCONSTITUENTS UTILITY IN ETHIOPIAN KALE AND AFRICAN CABBAGE ORPHAN LEAFY VEGETABLES AND POTENTIAL CULTIVATION OF THE VEGETABLES AS FUNCTIONAL FOODS

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EXTENDED ABSTRACT

Various studies have recently highlighted the importance of African leafy vegetables (ALVs), which refer to plants whose leaves are accepted and utilized as vegetables by communities through tradition. Due to their higher concentrations of nutritious and non-nutritive compounds than widely cultivated and consumed "exotic" vegetable species, ALVs constitute an important part of people's diets. Orphan ALVs, such as *Cleome gynandra* (African cabbage) and *Brassica carinata* (Ethiopian kale), are indigenous, native species that were introduced centuries ago but are still used locally or regionally, having lots of untapped potential to improve nutritional security. Due to their local benefits, occurrence as wild plants, weeds, or volunteer crops, these vegetables are typically characterized as minor, neglected, underutilized, and/or unimproved; and they are almost entirely ignored by farmers, researchers, crop breeders, and even policymakers.

Consumers have recently placed a high value on vegetables as functional foods in their diets, with reports indicating that they are not only nutritionally dense, but also contain high levels of some health-beneficial phytochemicals when compared to commonly consumed staple crops, and that they have a high potential to contribute not only to food security, but also to nutritional security. In this thesis, an extensive study was conducted in order to understand the secondary metabolites present in African cabbage and Ethiopian kale vegetables, their utility in promoting human health, possible strategies to improve the vegetables, and the potential application of these vegetables as functional foods and as a source of natural bioactive compounds in the food and pharmaceutical industries.

Firstly, this study involved a systematic review to determine the composition and health beneficial compounds obtainable from leafy vegetables and the possible strategies for improving such compounds during vegetable growth. Then using seeds supplied from a gene bank in Kenya, the Centre for Biodiversity in Kenya Resources Centre for Indigenous Knowledge, National Museums of Kenya (eight accessions for African cabbage and one accession for Ethiopian kale), the vegetables were cultivated in the greenhouse of KIST. Plant materials were collected at different stages and separated into several organs for bioactive component profiling. Target chemicals such as glucosinolates (GLs) and phenolics were detected in the vegetables using chromatographic techniques combined with mass spectrometry, based on their fragmentation patterns and mass to charge ratio. The identified compounds were quantified with commercial pure standards or relative response factors of the compounds using High performance liquid chromatography (HPLC) techniques. Upon identification and quantification of the compounds in the different vegetable parts and accessions, the adaptation of the vegetables for application in a vertical smart farming system was determined by checking the agronomic characteristics/desired traits. Furthermore, the possibility of improving vegetables by accumulating the bioactive secondary metabolites and their associated biological properties was evaluated using elicitors treatment. Different types and concentrations of elicitors and their applicability to vegetables grown in vertical farming system were chosen.

In the findings from the review, I obtained crucial data on the major composition of leafy vegetables which include GLs and polyphenols, the type of biological activities associated with these compounds such as anticancer, anti-inflammatory, antioxidant activities as well as the different strategies that can be used to improve vegetables at different stages of growth. For the compositional data, I observed variable components and concentrations of the identified compounds in each vegetable, the accessions under study and the vegetable parts used. During the evaluation of the well adapted vegetable to be used in the vertical farming system, Ethiopian kale vegetables were best suited for this type of farming and the cultivation had positive results in improving not only yield but also the quality of the vegetables and the inherent biological activities attributed to them. This vegetable was the choice for further objectives and analysis in this research and the target development stages were chosen depending on their ability to accumulate the bioactive compounds. Ethiopian kale's samples extracts were subjected to various chemical and *in vitro* biological assays including antioxidant and anti-inflammatory potential, chosen depending on the identified compounds and effective activities were attributed to these compounds based on the literature review. The findings on elicitation demonstrate how elicitors could be used as a simple strategy to obtain quality plant functional foods with increased quantities of the health promoting compounds.

This study shows the utility of both the edible and non-edible parts of the orphan leafy vegetables. It reduces the gap of unknown compounds in the vegetables and the identification of new compounds and provides a future reference for further studies. The evaluation of potential smart farming techniques to improve both yield and content of essential phytochemicals in the vegetables creates a good opportunity to venture and apply this emerging farming techniques for the production of high-quality functional foods and vegetables with accumulated drug target compounds for use in the pharmaceutical industry. The identification of superior accessions provides potential research targets to be used breeding programs for improving available vegetable varieties while the strategies used in production of quality vegetables are useful in the food industry for the production and processing of functional foods from the orphan leafy vegetables.

DECLARATION

I Sylvia Wairimu Maina, do hereby declare to the Senate of Sokoine University of Agriculture that this thesis is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

This work is dedicated to my family members who have always believed in me and supported my dreams throughout my education journey. I appreciate the various roles each and every one has played in my life and your contribution towards the success of my studies. You have molded me into the person I am today.

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2. Maina S, Ryu DH, Bakari G, Misinzo G, Nho CW, Kim HY. Variation in Phenolic Compounds and Antioxidant Activity of Various Organs of African Cabbage (*Cleome gynandra* L.) Accessions at Different Growth Stages. ***Antioxidants (Basel). 2021 Dec 6; 10(12):1952. doi: 10.3390/antiox10121952***

3. Maina S, Ryu DH, Bakari G, Misinzo G, Nho CW, Kim HY. Enhancement of glucosinolates, polyphenols and antioxidant potential in Ethiopian kale (*Brassica carinata L.*) sprouts using biotic elicitors. ***Journal of Food Research International (submitted)*****.**

4. Maina S, Ryu DH, Cho JY, Jung DS, Park JE, Nho CW, Bakari G, Misinzo G, Jung JH, Yang SH, Kim HY. Exposure to Salinity and Light Spectra Regulates Glucosinolates, Phenolics, and Antioxidant Capacity of *Brassica carinata* L. Microgreens. ***Antioxidants (Basel). 2021 Jul 26; 10(8):1183. doi: 10.3390/antiox10081183.***

LIST OF ABBREVIATIONS AND SYMBOLS

ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid
AC	Absorbance of the control
AChE	Acetylcholinesterase
$AlCl_3$	Aluminum chloride
ALVs	African leafy vegetables
ANOVA	A one-way analysis of variance
AOA	Antioxidant activity
AS	Absorbance of tested samples
B	Blue LED light
B1R1	Blue plus red LED light
BC	*Brassica carinata*
CAT	Catalase
CCl_4	Carbon tetrachloride
CG	*Cleome gynandra*
CO_2	Carbon dioxide
Cont	Control
DAD	Diode array detector
DAS	Days after sowing
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
DS-GLS	Desulfo-glucosinolates
DW	Dry weight
ESI	Electrospray ionization spray source
FA	Formic acid
FF	Flowers in flowering stage
FL	Fluorescent
GA	Gibberellic acid
GAE	Gallic acid equivalent
GBS	Glucobrassicin
Glu	Glucose
GMG	Glucomoringin
GNBS/NGBS	Neoglucobrassicin
GNL	Gluconapoleiferin
GPx	Glutathione peroxidase
GS/GLs	Glucosinolates
GSHPs	Glucosinolate hydrolysis products
GTR	Glucotropaeolin
H_2O_2	Hydrogen peroxide
HAG1	High aliphatic gucosinolate-1
HCT116	Human colorectal carcinoma cells
HGBS	Hydroxy glucobrassicin
HO-1	Heme oxygenase-1
HPLC	High performance liquid chromatography
HRP	Horseradish peroxidase
ITCs	Isothiocyanates

JA	Jasmonic acid
KIAT	Korea Institute for Advancement of Technology
KIST	Korea Institute of Science and Technology
LC-MS	Liquid chromatography- mass spectrometry
LED	Light emitting diode
LS	Leaves and stem
LSF	Leaves and stem at flowering stage
LSS	Leaves and stem at seed set stage
LSV	Leaves and stem at vegetative stage
$[M-H]^-$	Depproronated ions
MeJA	Methyl Jasmonic acid
Met	L-methionine
MDR	Multi drug resistant
MGBS/MGB	Methoxy glucobrassicin
MS	Mass spectrometry
M/Z	Mass to charge ratio
NaCl	Sodium chloride
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
NT	Non-treated cell group
NrF2	Nuclear transcription factor-erythroid 2 related factor
OPLS-DA	Orthogonal partial least squares discriminant analysis
PAL	Phenylalanine ammonialyase
PASET-RSIF	Partnership for Skills in Applied Science, Engineering and Technology, Regional Scholarship and Innovation Fund
PBS	Phosphate-buffered saline
PCA	Principal Component Analysis
PLS-DA	Partial least square discriminant analysis
PRO	Progoitrin
PVDF	Polyinylidene fluoride
QTOF	Quadrupole time of flight
R	Red LED light
RC_{50}	Dose required to scavenge fifty percent of radicals
RE	Rutin equivalent
RIPA	Radioimmunoprecipitation assay
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RRF	Relative response factor
SACIDS	Southern African Centre for Infectious Diseases Surveillance
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SIN	Sinigrin
SOD	Superoxide dismutase
SS	Siliques in the seed set stage
SUA	Sokoine University of Agriculture
Suc	Sucrose

TFC	Total flavonoid content
TPC	Total phenolic content
UHPLC	Ultra high-performance liquid chromatography

CHAPTER ONE

General Introduction

1.1 Background

African leafy vegetables (ALVs) refer to plant species which are genuinely native to a particular African region and whose leaves are acceptable and used as vegetables by communities through traditions. They include *Amaranthus species* (Amaranths, pigweed), *Cleome gynandra* (African cabbage), *Brassica carinata* (Ethiopian kale), *Vigna unguicalata* (cowpea), *Citrallus lanatus* (bitter lemon) and *Cucurbuta species* (traditional pumpkin) (Shayanowako *et al.,* 2021). These ALVs are an integral constituent of the diets due to their remarkable high concentrations of essential micronutrients including minerals, vitamins and proteins (Sogbohossou *et al.,* 2018). Furthermore, these vegetables play very significant roles in livelihoods and health of local African communities (Mensah *et al.,* 2008). It is thus true that they have the ability to contribute greatly to human health and nutritional security due to their available constituents and due to the fact that they thrive in poor soil conditions and harsh environments with great tolerance to stresses such as diseases, pest, weed and water (Gahukar, 2014).

There are hundreds of different ALV species that have been identified with few of them being utilized as foods. In fact, the majority of these are considered as minor or poor man's crops and they are often overlooked by policy makers, researchers and in development programs as well as farmer who either semi cultivate or collect them from the wild (Maseko *et al.,* 2018). Most of these indigenous species were introduced centuries ago but are still used locally or even regionally, with much untapped potential to increase their uses. Although they are usually adapted to local environmental stresses, little investment is done to improve their quality (Gahukar, 2014). These species, which have been marginalized by special modern agriculture production systems, are commonly described using adjectives such as underutilized, neglected, orphan, minor, local, rare, unimproved, niche, and traditional vegetables (Hunter *et al.,* 2019).

Currently, one of humanity's major issue is ensuring global access to sufficient, nutritious, healthy and affordable food produced in a sustainable manner (Bailey, 2016), making malnutrition, poor health, hunger and starvation a great challenge worldwide. With the introduction of green revolution, most institutions mainly focused on increasing crop productivity but did not consider the nutritional quality and even the health benefits of ALVs of crops as important issues. Therefore, while the global population of hungry people has decreased since, trends in hunger and food insecurity reveal that the population remains poorly nourished and at risk of diet related diseases (Hunter *et al.,* 2019). Lack of knowledge and limited research activities, such as breeding efforts, germplasm characterization, and understanding of species distribution as well as production levels has impeded the promotion and usage of ALVs to solve these problems (Meldrum *et al.,* 2018).

Worldwide, low vegetables intake has been ranked among the top ten risk factors contributing to mortality and the risk of chronic diseases; and these figures tend to be high in under developed and developing countries where the diets of the poor are predominantly cereal based and nutrient poor (Van Jaarsveld *et al.*, 2014; Siegel *et al.,* 2014). In fact, recent studies report that in most developing countries, there has been dramatic increase in such chronic diseases as cancer, diabetes and cardiovascular diseases caused by unbalanced diet (Baldermann *et al.,* 2016). Therefore, enabling the regular access to health benefiting vegetables at an affordable price is both an emerging priority and challenge for policy makers as populations become increasingly urbanized and reliant on purchased foods and conscious of nutritional health.

1.1.1 ***Cleome gynandra***

Cleome gynandra, also known as *Gynandropsis gynandra,* cat's whiskers, spider plant, shona cabbage or African cabbage is a tropical leafy vegetable that belongs to the family *Cleomaceae* in the order Brassicales. It is considered as an orphan ALV whose tender leaves, young shoots and flowers are used as potherb, stew or side dish while the fresh leaves are used as ingredients in mashed foods or ground and incorporated in weaning foods. Its leaves are relatively bitter and therefore usually cooked solely or in combination with other leafy vegetables, milk or butter (Van den Heever and Venter, 2006). In traditional medicine, the herb is used as an anti-inflammatory, antioxidant and an immune booster. The nutritional value of *C. gynandra* is attributed to the high content of carotenoids, vitamins C, E, folic acid and iron while the non-nutritional value is attributed to the presence of phenolic compounds and glucosinolates (GLs) (Neugart *et al.,* 2017; Sogbohossou *et al.*, 2019).

1.1.2 ***Brassica carinata***

Brassica carinata, also known as Abyssinian cabbage, Ethiopian kale, Ethiopian mustard, Ethiopian rape, or African kale, is an oilseed crop that originated in Ethiopia and belongs to the *Brassicaceae* family (Seepaul *et al.,* 2021). This high-yielding but neglected vegetable is rich in folic acid, ascorbic acid, carotenoids, and vitamin E, as well as GLs and phenolic compounds (Hagos *et al.,* 2020) and has been used to treat cancer and analgesic activities by easing pain (Odongo *et al.,* 2017). *B. carinata*, due to its increasing significance and nutritional value, is being promoted in urban agriculture across the African continent as an indigenous alternative to exotic vegetables (Ambrose-Oji, 2009).

1.2 Production and Utilization of African Leafy Vegetables

Although ALVs are recognized as important source of food and medicine for a variety of populations and especially for the survival of rural communities living in marginal areas with water scarcity and where crops struggle to survive, they are still largely ignored (Voster Ineke *et al.,* 2007). Their cultivation is limited and lacks appropriate agronomic guidelines and practices because they are still considered wild species, with some consumers arguing that they are only required in small quantities and that naturally occurring amounts should suffice. As a result, most of these species have never been considered for commercial production on a large scale (Maseko *et al.,* 2018).

Despite their nutritional value, rich bioactive compounds, and possible commercial applications, the use of ALVs has remained low in many areas, particularly among urban populations, compared to exotic vegetables (Ayanwale *et al.*, 2016). During the last decade however, demand for these ALVs has increased, and the expansion in their production, marketing, and consumption can be ascribed to consumer awareness of their health and nutritional benefits (Gido *et al.,* 2017b; Neugart *et al.,* 2017).

1.3 Nutritional and Non-nutritional Value of African Leafy Vegetables

According to research findings, ALVs are rich in micronutrients, vitamins, minerals, antioxidants, anti-inflammatory and anti-cancer compounds, making them possible contributors to dietary reference intakes and important for human health and infection prevention (Abukutsa-Onyango, 2003; Van Jaarsveld *et al.,* 2014). As a result, these vegetables can contribute to more than just filling nutritional deficiencies by providing healthy and economical medicinal compound alternatives (Moyo *et al.,* 2021).

Many indigenous ALVs have higher nutritional and non-nutritional compounds levels than commonly grown “exotic” species (Nesamvuni, Steyn, and Potgieter, 2001). For instance, the vegetables are a good dietary source of polyphenol compounds such as flavonoids and tannins, which have a wide range of physiological properties such as antioxidants, anti-inflammatory, anti-allergenic, anti-microbial, and cardiovascular protective effects (Afolayan and Jimoh 2009; Lippmann *et al.,* 2014; Maseko *et al.,* 2018). Besides, vegetables in the order brassicales are distinguished by a specific group of compounds known as GLs (Verkerk *et al.*, 2009) and their hydrolysis products, which have anti-inflammatory, anticarcinogenic, and antidiabetic properties (Lippmann *et al.,* 2014; Waterman *et al.,* 2015; Herz *et al.,* 2016).

1.4 Factors Affecting Utilization of ALVs

The major constrains and challenges that impede the use and conservation strategies of orphan vegetable crops include the perception around them as food for the poor, lack of innovative processing and value adding techniques, limited distribution and marketing as well as poor nutritional information (Jaffee, 2003). In addition, other contributing factors include low productivity, limited variety development, lack of consumer awareness, loss of knowledge, and lack of value addition as well as farmers' debt burden that has compelled them to adopt cash crops cultivation oriented toward making money (Gahukar, 2014).

The lack of development on the global agricultural research agenda is most likely owing to the fact that each orphan crop's relevance is usually local and culturally specific, and funds are rarely allocated to enable their research and improvement. On the other hand, loss of ALV knowledge related to limited research and extension has led to the labels of "backward knowledge" and "poverty food" in various regions, causing people to change their eating habits and willingness to learn about such orphan vegetables (Voster Ineke *et al.,* 2007). For most of the vegetables, basic understanding about reproductive biology, physiology, resistance and tolerance levels to biotic and abiotic challenges, the degree of natural diversity, and the genetic basis behind features of interest are still lacking.

Orphan vegetables' unavailability is due to low production because they are still classified wild species hence cultivation and production are still on a small scale. Since the vegetables are obtained through collection rather than production, they are vulnerable to overexploitation. Drought and heavy rainfalls have also resulted in loss of seeds and vegetables depleting seed banks (Voster Ineke *et al.,* 2007). The cytotoxicity associated with non-nutritional factors and bitter taste of vegetables has also contributed to their low consumer acceptance and low consumption (Gido *et al.,* 2017a; Moyo *et al.,* 2021). Ethnicity, gender and cultural preferences have also shown different preferences in the consumption of vegetables affecting their production and use (Ambrose-Oji, 2009).

1.5 Alternative Farming Systems for Production of Vegetables

Emerging problems in food security, climate change, greenhouse gas emissions, globalization, and the needs of a growing population have put pressure on natural resources and the ecosystem in recent years. The negative consequences of these have been observed in vegetable production for both rural and urban households, particularly in most developing African countries where vegetables provide reliable and inexpensive supplies of nutrients for human health and disease prevention (Shackleton *et al.,* 2009).

As a result, populations have remained vulnerable to food and nutritional insecurity and subsistence farming practices have faced a number of challenges, including soil damage and insufficient yields as a result of farmers' reliance on rainfed agriculture, as well as the overuse of pesticides that remain as residues in crops and the environment (Kpéra *et al.,* 2017). In this regard, the development of sustainable and ecologically friendly farming practices, such as smart farming systems for the production of high-quality vegetables, is very important (Walter *et al.,* 2017).

Smart farming systems are highly mechanized and automated using advanced technologies such as mobile applications, sensors and mainly utilize soil free based techniques to optimize high quality vegetable production (Muangprathub *et al.,* 2019). Advantages of such systems include: -

1) Efficient nutrient delivery and uptake by the plants; this maximizes growth enabling plants to flourish and produce high yields
2) Production of high-quality plants (clean, healthy green, leafy, nutritious, and tasty)
3) Reduced costs (handling, labour, and water consumption)
4) Eco-friendly and enhanced resilience; this includes reduced vulnerability to climate related risks (drought, pests, diseases) and improved capacity to adapt and grow in stresses such as shortened seasons
5) Less application of pesticides and herbicides

   Vertical farming systems for instance, have been identified as an eco-friendly, energy-efficient, and promising alternative to the conventional farming for agriculture's bright future. Crops are cultivated indoors in soil-free, stacked vertical structures where the environment is regulated and chemicals are used rarely, with the goal of increasing efficiency (Benke and Tomkins, 2017).

The Figure 1.1 below displays an example of a vertical farm system with light emitting diode (LED) illumination in a regulated environment (temperature, humidity, CO_2 supply).

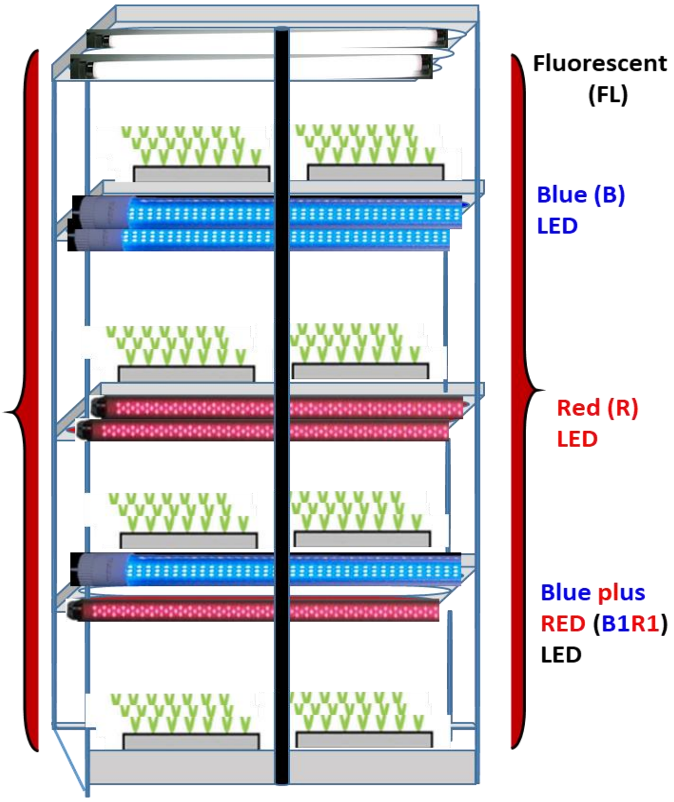

Figure 1.1: **A layout of a vertical farm utilizing light emitting diode (LED) lighting and automatically controlled hydroponic system**

1.6 Vegetables as a Source of Plant-based Functional Food

Functional foods are foods that, when consumed regularly, may give additional functional benefits to consumers' health and well-being. They include brassica vegetables that have long been associated with the prevention of noncommunicable diseases due to their ability to accumulate vital functional components like antioxidants and GLs that are beneficial to human health in treatment and prevention of diseases (Ferreira *et al.,* 2018; Ashaolu and Adeyeye, 2022; Revelou *et al.,* 2022).

Sprouts and microgreens, which are edible seedlings of vegetables harvested at a young stage, have received a lot of attention in recent years and are often regarded as super functional foods due to their beneficial health benefits. They appear to be the most promising when compared to adult vegetables, where studies show that they accumulate the majority of secondary metabolites such as polyphenols, carotenoids, GLs, and anthocyanins, all of which have been linked to anti-inflammatory, antioxidant, and anticancer properties (Baenas, Moreno, and García-Viguera, 2012; Le, Chiu, and Weber, 2017; Hsieh, 2020).

1.7 Problem Statement

One of the major problems facing humanity currently is to secure universal access to sufficient, nutritious, healthy and affordable food that is produced in a sustainable manner. Poor diets are the principal cause of multiple health burdens with studies indicating that three out of four deaths are caused by non-communicable diet related diseases particularly in emerging economies and in low-to-middle income countries (Forouzanfar *et al.*, 2015; WHO, 2022).

Worldwide, low intake of vegetables has been associated to be among the top ten risk factors contributing to mortality and the risk of chronic diseases especially in least developed and developing countries. In Sub Saharan Africa, which accounts for 9% of the global population, high food and nutritional insecurity is mainly due to lack of food diversification. Most ALVs species which are considered as useful plant species using recent research information and due to the significant potential they hold in diets are marginalized and ignored by researchers, breeders and policy makers; these species are either cultivated on small scale or occur in the wild; making their production and availability limited (Muhanji *et al.,* 2011; Aworh, 2015).

The availability of naturally occurring bioactive compounds, which continue to be the primary source of materials for drug development and discovery, is similarly constrained in most ALVs due to the lack of improved species that constrain both the supply and the levels of such phytocompounds in the vegetables.

Furthermore, despite their high nutritional value and perceived health benefits, vegetables are poorly researched on, still downgraded and negatively regarded, with attitudes toward local and traditional foods equating them with poor people's food, leading to consumer rejection (Baldermann *et al.,* 2016).

1.8 Justification of the Study

The alarming increase in diet related diseases, emphasizes the need for new food policies, as well as nutritional and health education programs, to better address issues like unbalanced diets, malnutrition, and the emergence of chronic diseases. Finding strategies to bring rich, neglected plants out of their niche in order to provide solutions that are sustainable, robust, and practical to the diet-related challenges that humans face is critical.

Neglected edible plants including vegetables, represent a natural wealth for many countries although most of these countries fail to use them adequately for this purpose. These plants are usually rich in nutrients and health promoting compounds that can help prevent malnutrition and chronic diseases. As a result, they play an important role in diversifying food systems for healthier diets and they are an excellent tool for improving human health and nutrition (Baldermann *et al.,* 2016). Several studies show that although neglected vegetables are under-researched, they often have superior nutrition content compared to most crops dominating our food systems and are thus recognized not just by local importance, but also by the potential to improve diets on a greater regional level (Bharucha and Pretty, 2010).

To realize the full potential of indigenous vegetables for human health and nutrition, we must identify the ALVs, learn how they are used in different societies and then analyse them to identify the bioactive compounds that contribute to the health benefits. This will increase our understanding and evidence basis, as additional food composition data on the world's neglected and underutilized vegetables is essential.

In addition to improve the utilization of the ALVs bioactive compounds, the vegetables cultivation techniques need to be advanced by adapting technologies that satisfy the consumer demands. Also, for maximum advantages to humans, it is crucial to improve the quality of such vegetables, such as through elicitation to promote the accumulation of bioactive compounds. These enhanced vegetables have potential uses as functional foods in the food industry and as a source of bioactive compounds in the pharmaceutical industry.

1.9 Study Objectives

1.9.1 General objective

To investigate the significance of phytoconstituents in orphan African green vegetables *Brassica carinata* (Ethiopian kale) and *Cleome gynandra* (African cabbage) in human health, and analyze potential production of improved functional foods from these vegetables.

1.9.2 Specific objectives

i. To identify the profile of bioactive compounds in Ethiopian kale and African cabbage orphan leafy vegetables
ii. To assess the potential of cultivating these orphan leafy vegetables in a smart farming system
iii. To investigate the biological properties of extracts prepared from the orphan leafy vegetables
iv. To evaluate appropriate elicitors for improving the orphan leafy vegetables functional foods.

1.10 Research Questions

1. Which secondary metabolites are present in Ethiopian kale and African cabbage orphan leafy vegetables?
2. Are the vegetables under consideration suitable for growing in a vertical smart farming system?
3. Which biological activities can be associated with extracts prepared from the orphan leafy vegetables?
4. Is elicitation an effective technique for improving the functional foods of the orphan leafy vegetables?

1.11 General Materials and Methods

This thesis details a study that was conducted to understand the secondary metabolites present in African cabbage and Ethiopian kale vegetables, their utility in promoting human health, possible strategies to improve the vegetables, and the potential application of these vegetables as functional foods and as a source of natural bioactive compounds in the food and pharmaceutical industries. The study involved a detailed literature review to determine the composition and health beneficial compounds obtainable from leafy vegetables and the possible strategies for improving such compounds during vegetable growth. Then using seeds supplied from a genebank, a preliminary study was performed to determine the potential cultivation of the vegetables in a vertical smart farm system as the vegetables were also being cultivated in the greenhouse of KIST. Plant materials were collected at different stages and separated into several organs for bioactive component profiling. Target compounds including GLs and polyphenols were detected in the vegetables using chromatographic techniques combined with mass spectrometry, based on their fragmentation patterns and mass to charge ratio. The identified compounds were quantified with commercial pure standards or relative response factors of the compounds using HPLC techniques. Upon identification and quantification of the compounds in the different vegetable parts and accessions, and on checking the adaptation of the vegetables for application in a vertical smart farming system based on the agronomic characteristics it was observed that only Ethiopian kale showed positive features of adaptation. The vegetable was used in further research objectives and to assess the possibility of improving them by accumulating the bioactive secondary metabolites and their associated biological properties. Different types and concentrations of elicitors and their applicability to vegetables grown in vertical farming system were chosen and treated to juvenile stages of the vegetable. In addition, its extracts were subjected to various chemical and *in vitro* biological assays including antioxidant and anti-inflammatory potential, chosen depending on the identified compounds and effective activities were attributed to these compounds. Various analytical techniques were applied in this study to process, analyze and interpret data including using Microsoft™ Excel application, agricolae package in R software and IBM Statistical Package for Social Science (SPSS) software version 26.

1.12 Study Limitations

The small numbers of orphan ALV species, including a large collection of most of their accessions, were one of the study's apparent limitations. The ability to generalize the findings to the majority of vegetable species would have grown as a result. This would have been beneficial in identifying how differently the vegetables were adapted to the vertical smart system. The fact that the vegetables in the smart farm system were grown under predetermined conditions is another limiting factor. To reduce the likelihood of confounding variables, it would be required to optimize the environment for our ALVs given that the existing vegetables were already suited to such environmental conditions.

1.13 Organization of the Thesis

The following important sections make up this thesis:

An extended abstract that describes the study's goal, research material and general methodologies employed in the investigation, as well as major research findings and a general conclusion.

Chapter one: The first chapter that provides the study's background information, problem statement, research justification, and research objectives.

Chapter two: In this chapter, the findings from the study objectives are summarized in peer-reviewed research articles that have been published (papers one, two, and four) or are in a publishable format (paper three). Paper one is a summary of the findings from the literature review. Paper two is the summary of the findings from the research objective targeting *Cleome gynandra* at different stages of growth. Paper three is the summary of the findings from the research objective targeting sprouts of *Brassica carinata* treated to different elicitors and the evaluation of the antioxidant activity of the sprouts' extracts and this is included and formatted as per the target journal requirement. Paper four is the summary of the findings from the research objective targeting *Brassica carinata* microgreens under different elicitors.

Chapter three: The chapter contains the research's general discussion, conclusion, and recommendations, as well as the areas for further research that were identified after the research was completed.

CHAPTER TWO

Paper One

Human, Animal and Plant Health Benefits of Glucosinolates and Strategies for Enhanced Bioactivity: A Systematic Review

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Paper Two

Variation in Phenolic Compounds and Antioxidant Activity of Various Organs of African Cabbage (***Cleome gynandra*** L.) Accessions at Different Growth Stages

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Paper Three

Enhancement of glucosinolates, polyphenols and antioxidant potential in Ethiopian kale (*Brassica carinata* L.) sprouts using biotic elicitors

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Abstract

Brassica carinata (BC) is an important vegetable crop in Africa with a multitude of therapeautic potentials attributed to the available phytochemicals. However, inadequate studies on strategies to increase accumulation of these phytochemicals have hampered production of quality vegetables. Thus herein, the content of individual and total glucosinolates (GLs), polyphenols, and the antioxidant capacity was investigated in 8-days-old BC sprouts treated with sucroce, glucose and L-methionine elicitors. While sinigrin, the major compound in the sprouts increased in response to all the treatments, the pattern of accumulation of the aliphatic and indole GLs as well as the polyphenols varied depending on the elicitor type and concentration, with low elicitor concentrations generally having a positive effect on their contents. Specifically, L-methionine (3 mM) was the most effective elicitor, resulting in a 1.21-fold increase in polyphenols and much lower efficient dosages responsible for scavenging radicals which were 0.61 and 0.66 times lower in the ABTS and DPPH assays, respectively, compared to the control sprouts. These findings suggest that elicitation is a cost-effective strategy for improving the metabolites and health benefits of functional foods such as BC sprouts.

Keywords: Elicitation, functional foods, Ethiopian kale, bioactive compounds, radical scavenging

Abbreviations

BC, *Brassica carinata*; GLs, glucosinolates; met, L-methionine; Glu, glucose; Suc, sucrose; Cont, control, ABTS, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); DPPH, 2,2-diphenyl-1-picrylhydrazyl; PAL, Phenylalanine ammonialyase; HPLC, High performance liquid chromatography; TPC, total phenolic content; GAE, gallic acid equivalent; DW, dry weight; AC, absorbance of control; AS, absorbance of tested extract sample; RC_{50}, dose required to scavenge fifty percent of radicals; dsGLs, desulfo glucosinolates; LC-MS, liquid chromatography- mass spectrometry; QTOF, quadrupole time of flight; ESI, electrospray ionization spray source; DAD, diode array detector; ANOVA, A one-way analysis of variance; PCA, principal component analysis; SD, standard deviation; PRO, progoitrin; SIN, sinigrin; GNL, gluconapoiferin; HGBS, hydroxyglucobrassicin; GTL, glucotropaeolin; GBS, glucobrassicin; MGBS, methoxyglucobrassicin; NGBS, neoglucobrassicin; RRF, relative response factor; HAG1, high aliphatic gucosinolate-1.

Introduction

Brassica carinata (BC), an orphan traditional African leafy vegetable also known as Ethiopian kale, has received considerable attention due to its nutritional and economic importance, leading to the recent development of specific lines for human consumption and industrial use (Odongo *et al.,* 2017; Schreiner *et al.*, 2009; Xin *et al.,* 2014). This vegetable is widely distributed and consumed in most parts of East and Southern Africa, frequently accompanying starch staples (Odongo *et al.,* 2017). The young leaves and fleshy stems of BC contain high nutritious and non-nutritious compounds such as glucosinolate (GLs) and polyphenols with health-promoting benefits (Maina *et al.,* 2021). Owing to the quenching ability of these compounds against reactive species (reactive oxygen species, ROS; and reactive nitrogen species, RNS), they are an excellent focus in the food industry; due to their great contribution in the prevention of chronic diseases including cancers, inflammations, and cardiovascular diseases (Lozano-Baena *et al*., 2015; Maina *et al.,* 2020, 2021; Odongo *et al.,* 2017).

Over the years, interest in leafy vegetables has grown, with particular emphasis as a source of vitamins, proteins, minerals, and phytochemicals such as GLs and phenolic compounds (Manchali, Murthy, and Patil, 2012). GLs are a group of sulfur and nitrogen-containing metabolites that are grouped into aliphatic, aromatic, and indolic groups based on their precursor amino acid and their different side-chain structure (Prieto, López, and Simal-Gandara, 2019). Particularly in vegetables, sprouts are considered as the better source of these compounds than mature plants because the levels of compounds decrease during the plant growth due to plant tissue expansion, which results in a dilution effect (Baenas, García-Viguera, and Moreno 2014a; Baenas *et al.,* 2012). Numerous research shows that consumers reap the most health benefits from young tender vegetables (Baenas *et al*., 2012; Kyriacou *et al.,* 2016; Xiao *et al.,* 2019) therefore making young vegetables stage such as sprouts, a good target to improve these phytochemicals. Sprouts are considered natural functional foods with numerous advantages because they can be easily grown at home regardless of the season and consumed raw, avoiding loss of bioactive compounds during cooking or processing (Natella *et al*., 2016).

Elicitors induce various physiological and molecular factors that induce chemical defense as a stress response in plants resulting in secondary metabolite accumulation (Gorelick and Bernstein, 2014). Due to their ability to activate different signaling pathways in the plant defense system, elicitors stimulate stresses that have a large impact on plant development, causing them to accumulate specific or a mixture of defensive metabolites (Angelova, Georgiev, and Roos, 2006). Therefore, has been regarded as a successful strategy for improving secondary metabolites and their associated biological properties in vegetables. It is typically classified into two groups (abiotic and biotic elicitors) and sugar and amino acid treatment are involved in biotic elicitors (Gorelick and Bernstein, 2014).

Sugars play important roles in plant growth as sources of carbon and energy and they modulate several developmental processes including seedling development, germination as well as have a variety of effects on the metabolism of plant secondary metabolites at various stages of development (Miao *et al.,* 2013, 2016; Smeekens *et al.,* 2010*)*. Glucose and sucrose, in particular, are recognized as effective signaling molecules that influence various metabolic and developmental processes such as photosynthesis, stress responses, and germination (Baenas *et al.,* 2014a; Miao *et al.,* 2013). These sugars, together with amino acids such as methionine have been reported as elicitors that positively regulate the accumulation of antioxidant polyphenols and individual as well as total GLs in various brassica crops (Baenas *et al.,* 2016; Guo, Yuan, and Wang 2011; Liu *et al.,* 2019; Pérez-Balibrea, Moreno, and García-Viguera, 2011).

In addition to the GLs, the plant polyphenols are important antioxidants of leave vegetables, and these are synthesized via the phenylpropanoid pathway, which is initiated by the enzyme phenylalanine ammonialyase (PAL). In Chinese kale, Pak choi and broccoli sprouts, the activity of PAL was observed to be induced by various stress treatments including glucose and sucrose causing changes in phenolic compound content (Guo *et al.,* 2011; Wei, Miao, and Wang, 2011). Therefore, it is expected that the biotic elicitation including glucose, sucrose, and L-methionine can stimulate the biosynthesis of antioxidants of BC as well as enhance the pharmacological properties of BC.

Currently, very scarce information is available on the accumulation of health-beneficial phytochemicals and their biosynthesis in Ethiopian kale vegetables, which has hampered studies on strategies for the production of quality functional foods. This study, therefore, aimed at evaluating in the Ethiopian kale sprouts, the effect of different concentrations and types of elicitors on the total phenolics content, individual and total GLs content, and antioxidant capacity.

Materials and Methods

Plant material and reagents

The seeds of Ethiopian kale were provided by the Kenya Resource Centre for Indigenous Knowledge, National Museums (Nairobi, Kenya). HPLC grade analytical solvents including water and acetonitrile, and elicitors sucrose, glucose elicitors were purchased from Sigma-Aldrich (St. Louis, MO, USA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), Folin Ciocalteu’s phenol reagent, gallic acid (CAS No. 149-91-7) and rutin (CAS No. 205-814-1) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

L-Methionine was from Junsei Chemical Co. (Tokyo, Japan) while formic acid (98/100%) was purchased from Fisher Scientific Co. (New Jersey, USA) and glucotropaeolin (GTR CAS. No.499-26-3) was obtained from Extrasynthese (Genay, France).

Sprouts Growth Conditions

Seeds were cultivated in the Smart-farm of the Korea Institute of Science and Technology (Gangneung, South Korea). The seeds were first rinsed in distilled water and then sterilized by immersion into 0.5% sodium hypochlorite for 2h. The sterilized seeds were soaked in distilled water for 12 h and then spread on the meshed plate of the sprouting trays (30 cm × 20 cm × 5 cm) containing water. The trays, each containing three replicates (approximately 150 seeds), were kept in a dark condition for 2 days, at 25 ^{o}C to allow stem elongation of the germinating sprouts. These trays were then transferred to the growth chamber with controlled conditions of photoperiod 16 h and 8 h; temperature, 25^{o} C and 20^{o} C, a relative humidity, 60% and 70% for 5 days and 5 nights. The plants were exposed to 200 ± 11 μmol/m^{2}s of light intensity from fluorescent lamps (TL-D 18W/865; Philips Electronics, Seoul, South Korea) at 20 cm until day 8 when the sprouts were harvested.

Elicitors Treatment

Elicitors selected for this experiment included L-methionine, glucose, and sucrose. Different concentrations of L-methionine (3 mM, 6 mM and 9 mM), glucose (2%, 4% and 6%) and sucrose (70 mM, 140 mM and 210 mM) were prepared. Glucose and sucrose were dissolved in distilled water while the L-methionine was dissolved in distilled water containing 0.04% ethanol (*v/v*). The elicitors (10 mL per replicate) were applied through exogenous spraying onto the plants' cotyledons for 5 days (from day 3 to day 7) and distilled water served as the control. Three replicates per treatment, (each containing 120 plants of the 8 days old sprouts) were rapidly harvested for further analysis. Upon harvesting, the sprouts were weighed and flash frozen in liquid nitrogen and stored at −80 °C before freeze-drying for 4 days for chemical analysis and biological activities of interest. The experiment was performed in a complete randomized block design.

Sample Preparation

For the determination of phenolic contents and biological assays, 0.1g of freeze-dried samples were extracted two times with 70% ethanol (2 mL) at 40 °C for 15 min. The extracts were filtered and concentrated to dryness before re-dissolving in dimethyl sulfoxide solvent.

Determination of total phenolic contents (TPC)

The total polyphenols of the BC sprouts treated to various elicitors were determined using Folin-Ciocalteu's reagent method as previously described using gallic acid as a standard (Chang *et al.,* 2019). Briefly, into a 96 well plate with 10 μL of the sprouts' extracts, 100 μL of sodium carbonate (Na_2CO_3, 2%) reagent was added and allowed to react for 3 min. Then, 10 μL of the Folin reagent was added, mixed thoroughly, and allowed to stand for 30 min in the dark before measuring the optical density of the plates at 750 nm using a multi detection microplate reader (Synergy HT; BioTek Instruments, Winooski, VT, USA). For the standard curve, serially diluted gallic acid standards were added instead of sample and reacted with the reagents as mentioned above and the content of the total phenolics was determined using an equation ($y = 1.7387x - 0.4772$, $r^2 = 0.999$). Data on the polyphenols were expressed as milligrams (mg) gallic acid equivalent (GAE) per 100 g of the dry weight (DW).

Measurement of Antioxidant Activity

DPPH radical scavenging activity

To determine the antioxidant activity, DPPH free radical scavenging assay was performed as described by (Thaipong et al. 2006) with slight modifications. Briefly, to determine the electron-donating ability to the DPPH radical, 10 μL of graded concentrations of each sample extract in a 96 well plate was reacted with 190 μL of freshly prepared DPPH reagent (0.2 mM). The mixture was mixed well and placed in the dark for 30 min after which the absorbance was read using a multi detection microplate reader spectrophotometer set at a wavelength of 517nm. The percentage DPPH radical scavenging activity was determined using the following equation: % inhibition = (AC-(AS/AC)) * 100 (AC is the absorbance of the control and AS is the absorbance of the tested extract sample) while the RC_{50} (dose required to scavenge fifty percent of radicals) was determined using the inhibitory concentrations of several concentrations of the sprouts' extracts determined in the experiment.

ABTS antioxidant assay

$ABTS^{\cdot+}$ free radical cation decolorization assay was performed using a protocol described by (Thaipong *et al.,* 2006) with slight modifications. Briefly, the ABTS reagent was prepared by dissolving the tablet in water and reacting the mixture with 2.45 mM potassium persulfate solution, then incubating the reagent overnight at 4 °C in the dark to generate free radicals. The ABTS solvent was diluted with ethanol to achieve a concentration of 7 mM. During the assay, 10 µL of different concentrations of each sample extract in a 96 well plate was reacted with 190 µL (Doheny-Adams *et al.,* 2017)of $ABTS^{\bullet+}$ reagent, while for the blank, the sample was reacted with ethanol in place of the $ABTS^{\bullet+}$ reagent. The mixtures were reacted for 5 minutes and the absorbance was read at 734 nm using a multi detection microplate reader (Synergy HT; BioTek Instruments, Winooski, VT, USA). The radical scavenging activity of the extracts and the RC_{50} were calculated as mentioned above.

Extraction, Identification, and Quantification of Glucosinolates

The extraction of GLs and conversion into their desulfo forms was performed following previously described protocols with minor modifications (Doheny-Adams *et al.,* 2017). Powdered freeze-dried samples (100 mg) were homogenized with 70% methanol, and the mixture was extracted twice with heating at 90 °C for 30 min, then centrifuged at 2063 ×*g*, for 15 min at 4 °C. 1.2 mL of the supernatant was transferred into tubes containing 1.0 mM glucotropaeolin (GTR) and 0.15 mL mixture of 1M lead acetate and barium acetate (1:1, *v/v*), then centrifuged for 5 min. The supernatants (1 mL) were loaded onto diethyl-aminoethyl Sephadex A-25 anion exchanger columns preactivated with 0.1 M sodium acetate. Prior to loading the samples, the columns were washed with distilled water and upon loading samples were desulphated by reacting them with 0.2 mL of 0.1% purified arylsulphatase (*Helix pomatia* Type H-1; Sigma-Aldrich, St. Louis, MO, USA) for 16 h at room temperature. Desulfo-GLs (dsGLs) were eluted with 1.0 mL of distilled water and filtered through a 0.2 µm polyvinylidene difluoride filter for analysis.

LC-MS analysis

GLs in their dsGLs forms were identified following their MS^2 $[M\text{-}H]^-$ fragmentations through LC-MS analysis conducted using an Agilent HPLC 1200 system (Agilent Technologies, Waldbronn, Germany). For the analysis, a reverse phase C18 column (YMC-pack ODS-AQ, 12 nm, 150x 4.6 mm, 5 µm particle size) set at 45 ^{O}C was used. Samples (20 µL) were injected into the system, and separation was achieved using a mobile phase composed of water containing 0.2% formic acid (solvent A) and acetonitrile containing 0.2% formic acid (solvent B) with a flow rate of 0.7 mL/min. The gradient started with 100% solvent A up to 1 min, reaching 92% at 7 min, 90% between 7–10 min, 65% between 10-17 min and this percentage was maintained up to 18 min, and then increased to 100% at 20 min.

The LC system was coupled to a quadrupole time of flight (QTOF) mass spectrometer (Agilent Corp., Santa Clara, CA, USA) equipped with a binary pump, autosampler, and an electrospray ionization spray (ESI) source. The DAD detector was set at 229 nm for acquiring the chromatograms. Analysis was performed on a full scan mode and the mass range was set at *m/z* 100 -1000 in the positive mode. The conditions of the ESI source were as follows: drying gas (N_2), flow rate 12.0 L/min, drying gas temperature 350 ^{o}C, ion spray voltage. All the operations, acquisition, and analysis were controlled by Chemstation software (Agilent technologies, USA).

The samples were further analyzed and quantified in an HPLC-DAD system, HPLC 1260 series using a mobile phase composed of water containing 0.2% formic acid (solvent A) and acetonitrile containing 0.2% formic acid (solvent B), in a similar separation gradient as in the detection process and following the UV spectra and order of elution acquired in the identification process, GLs were quantified using GTR as internal standard at 229 nm.

Statistical Analysis

All the experimental data were performed in three independent replicates and represented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used for data analysis to determine the significant differences between the means of the experimental groups of the treatments in the agricolae package in R (De Mendiburu 2014). ANOVA was followed by post-hoc analysis with Tukey's multiple comparison test to determine the specific groups responsible for the significant differences. Principal component analysis (PCA) was performed in R to determine the relationship in all the variables of the different treatments. In the analysis, statistically significant results were considered at *P* < 0.05.

Results

Ethiopian kale sprouts biomass

The effect of different concentrations of three elicitors on 8 days old sprouts' fresh weight is shown in Figure 1. Methionine treatment resulted in a significant increase in sprout fresh weights when compared to the control; however, monosaccharide treatment resulted in a slight decrease in fresh weight, and the effects were greatest when the sugars were applied in high concentrations. The highest concentration of glucose resulted in the most drastic fresh weight reduction in Ethiopian kale sprouts.

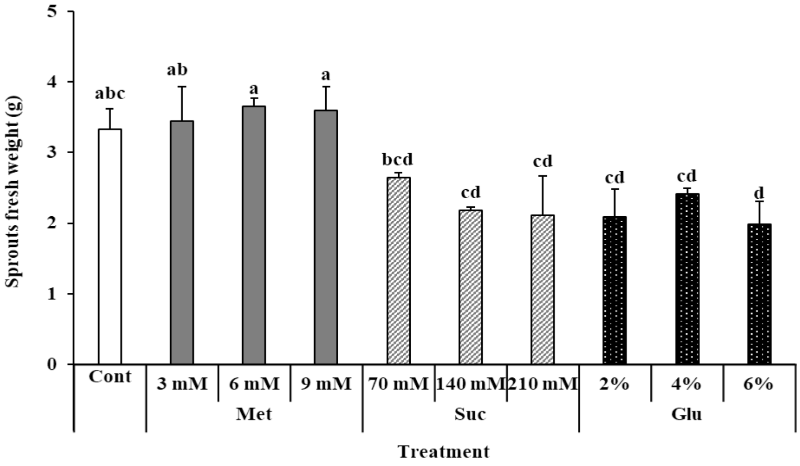

Figure 3.1: Effect of elicitors treatment on the fresh weights (g) of 8 days old sprouts. 120 uniformly grown sprouts were randomly selected to make one replica and values indicate the means of three replicates per treatment (*n*=3, mean ± standard deviation). ANOVA test was performed and bars not sharing the same letters are significantly different at *P*<0.05. Cont = control (distilled water treatment); Met = L-Methionine; Suc = Sucrose; Glu = Glucose

Content of polyphenols of Ethiopian Kale Sprouts under Different Treatments

The influence of different elicitors on the polyphenols in the Ethiopian kale sprouts is shown in Figure 2. TPC was positively and significantly influenced by all the evaluated concentrations of methionine and the lowest concentrations of both glucose and sucrose, but not higher concentrations of the monosaccharides.

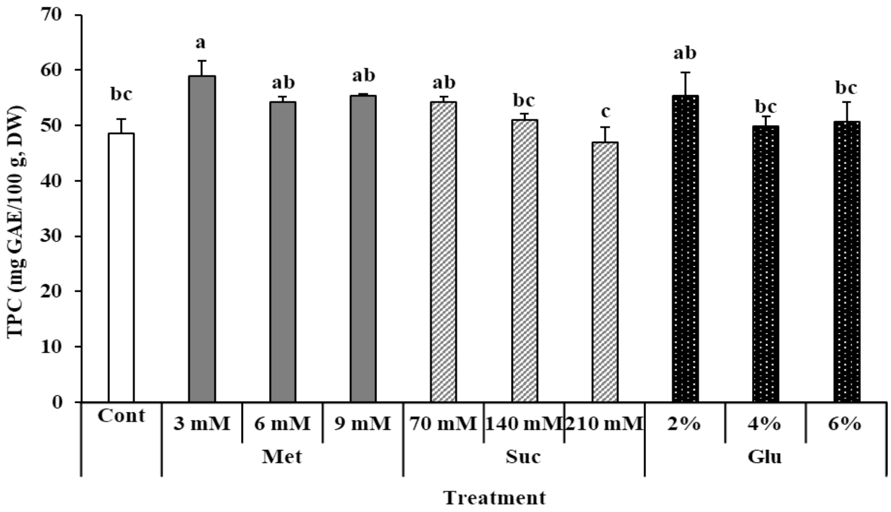

Figure 3.2: Effect of elicitors treatment on the total phenolic contents of the *8 days old sprouts.*

The values indicate the means of three independent replicates (*n*=3, mean ± standard deviation). Bars not sharing the same letters are significantly different at* P*< 0.05. Cont = control; Met = L-Methionine; Suc = Sucrose; Glu = Glucose; mg GAE/100 g DW= milligram Gallic acid equivalent in 100 grams of the extract dry weight.

Antioxidant potential

As shown in Figure 3.3 and Table 3.1, L-methionine treated sprouts had the strongest radical scavenging abilities that were significantly higher than sprouts treated with sucrose and glucose as well as the control sprouts. Sprout extracts from lower concentrations of the monosaccharaides also showed stronger inhibitory abilities which were also significantly higher than the control group sprout extracts. Sprouts treated with 3 mM L-methionine specifically, had the highest antioxidant capacity when compared to other treatments with the percentage inhibition ranging from 11.66 to 79.08 % in the DPPH assay and from 17.73 to 89.03 % in the ABTS assay (Figure 3.3).

The extracts displayed a concentration-dependent scavenging effect (25 - 500 µg/mL) at $P < 0.05$ for the various extracts tested and the ABTS assay displayed a much higher scavenging activity (up to two folds for specific treatment at specific concentration) compared to the DPPH assay.

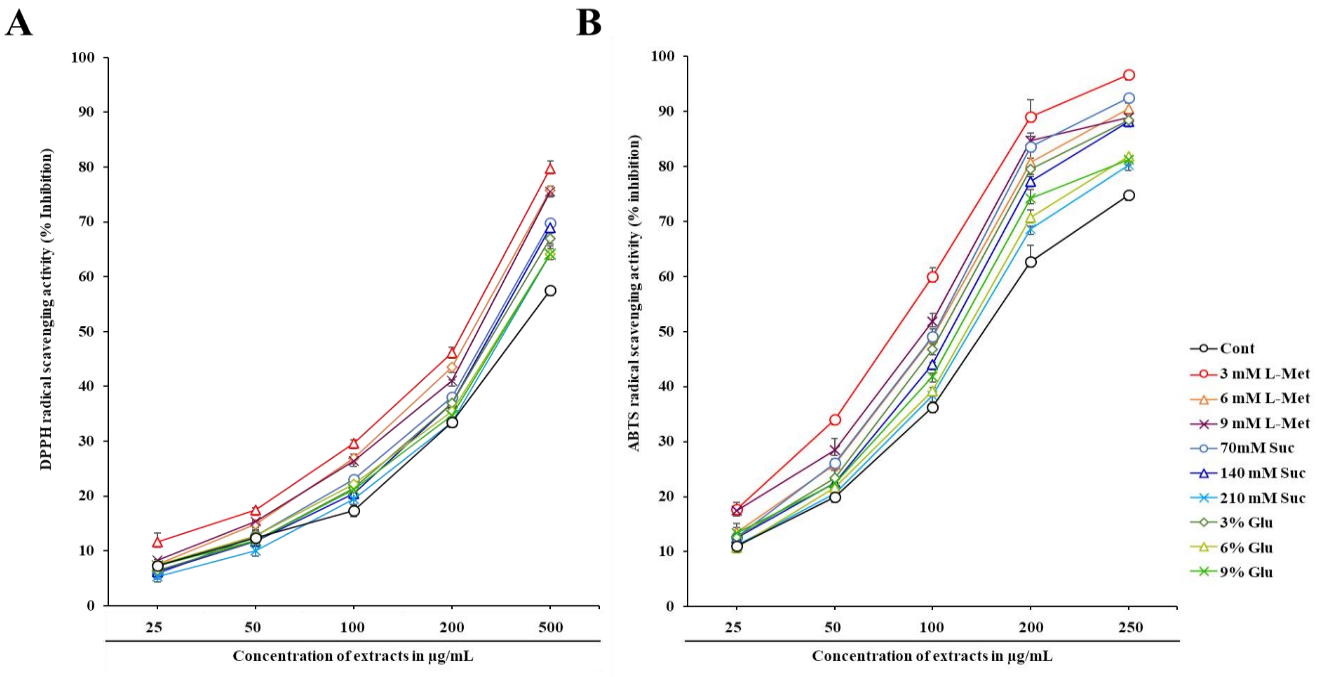

Figure 3.3: The percentage radical scavenging activity of extracts of Ethiopian kale sprouts under different treatments. Experiments were conducted with three replicates and results are means ± standard deviation, and are expressed as the percentage inhibition of radicals by extracts at various concentrations. Cont = Control; Met = L-Methionine; Suc = Sucrose, Glu = Glucose, DPPH = 2,2-diphenyl-1-picrylhydrazyl, ABTS = 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid).

For the various sprouts extracts, the dose required to scavenge fifty percent of the radicals (RC_{50}), ranged between 268.76 ± 8.41 and 409.86 ± 7.10 µg/mL with DPPH assay; and between 93.47 ± 5.76 and 154.24 ± 12.44 µg/mL with the ABTS assay. The L-methionine treated sprouts had the most potent activity followed by the sprouts treated with lower concentrations of the sugars. For extracts from different treatments, the doses required to scavenge ABTS radicals were at least three times lower than those required to scavenge DPPH radicals.

Table 3.1: The doses of sprout extracts required to scavenge fifty percent of the radicals (RC_{50}). Each data represents the mean of three independent replicates per treatment (mean ± standard deviation)

Treatment		DPPH (RC_{50}) µg/mL DW	ABTS (RC_{50}) µg/mL DW
Control		409.86 ± 7.10^{d}	154.24 ± 12.44^{f}
L-Met	**3 mM**	$\mathbf{268.76 \pm 8.41^{a}}$	$\mathbf{93.47 \pm 5.76^{a}}$
	6 mM	292.69 ± 6.84^{a}	114.29 ± 3.31^{bc}
	9 mM	297.35 ± 2.62^{a}	105.08 ± 6.12^{ab}
Suc	**70 mM**	330.20 ± 5.08^{b}	111.88 ± 6.28^{bc}
	140 mM	338.35 ± 8.96^{bc}	123.28 ± 2.39^{cde}
	210 mM	369.41 ± 2.91^{c}	140.75 ± 2.18^{ef}
Glu	**2%**	346.75 ± 19.83^{bc}	118.55 ± 7.38^{bcd}
	4%	362.26 ± 17.74^{c}	136.31 ± 3.75^{de}
	6%	365.19 ± 13.88^{c}	128.55 ± 5.42^{cde}

Values in the same column not sharing the same letter are significantly different at *P* ≤ 0.05, GAE = Gallic acid equivalent, DW = dry weight; DPPH = 2,2-diphenyl-1 picrylhydrazyl, ABTS = 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); RC_{50} = dose required to scavenge fifty percent of radicals.

Identification and Content of GLs in Ethiopian Kale Sprouts

Based on UV spectra and retention time, GLs were identified in Ethiopian kale sprouts as desulfo-glucosinolates. The GLs included the aliphatic progoitrin (PRO), sinigrin (SIN), and gluconapoleiferin (GNL), as well as indole Hydroxyglucobrassicin (HGBS), Glucobrassicin (GBS), Methoxyglucobrassicin (MGB), and Neoglucobrassicin (NGBS) as shown in Figure 3.4 and Supplementary Figure 3.1.

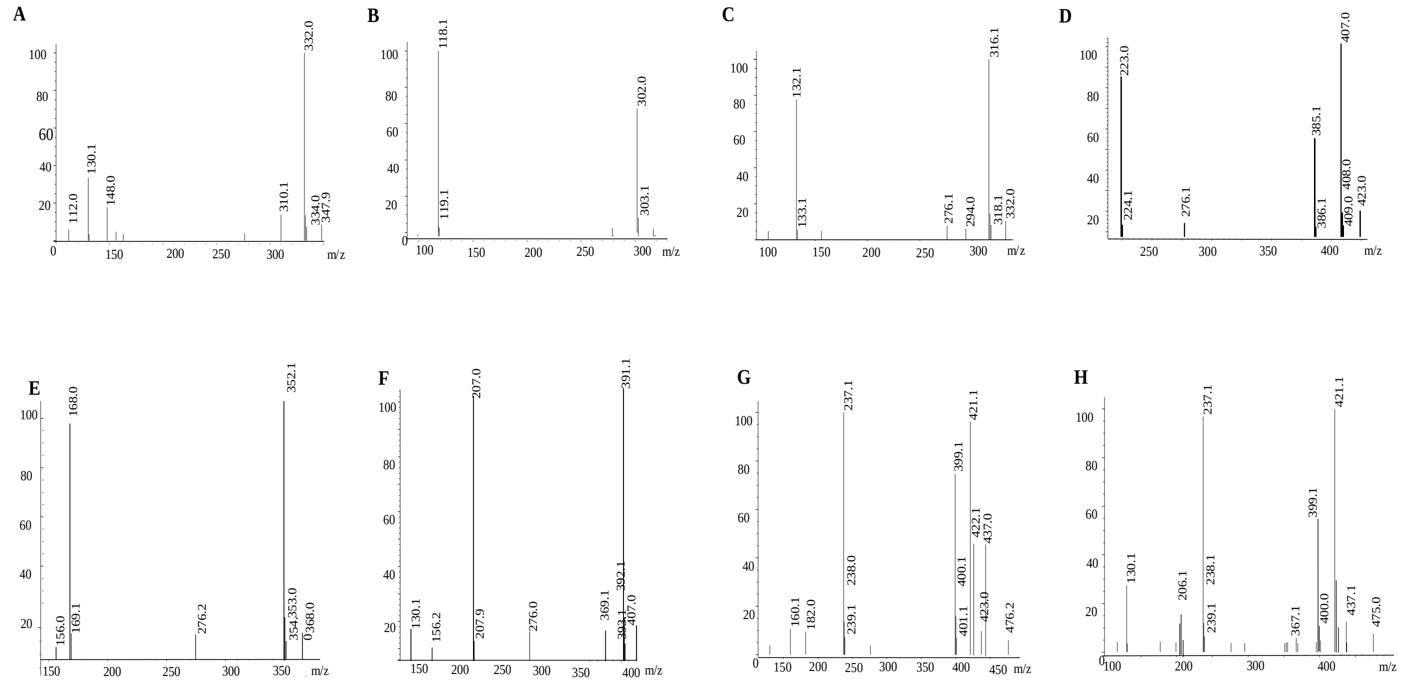

Figure 3.4: LC-MS/MS of GLs identified in the Ethiopian kale sprouts extracts.
The fragment ions were annotated with the molecular structures of desulfo glucosinolates and identified as: -
(A) progoitrin (PRO), (B) sinigrin (SIN),(C) gluconapoleiferin (GNL), (D) hydroxyglucobrassicin (HGBS),
(E) glucotropaeolin (GTL), (F) glucobrassicin (GBS), (G) methoxyglucobrassicin (MGBS), (H) neoglucobrassicin (NGBS)

Effect of elicitors on the content of identified individual and total GLs

The contents of GLs in the 8 days old sprouts after treatment with the different elicitors were quantitatively determined (Table 3.2). SIN was the most abundant GL in the Ethiopian kale sprouts contributing approximately 50% of the total GLs followed by NGBS.

All the elicitors promoted the accumulation of the GLs however, there were differences detected in the quantified total and individual GLs due to the specific nature and the concentrations of the elicitors used. All the concentrations of the methionine elicitor markedly increased the SIN content in a comparable manner, and the lower concentrations of the glucose and sucrose were better elicitors of SIN compared to the higher concentrations. Like SIN, NGBS, the major indole GLs identified in the sprouts, increased on treatment with a lower concentration of all the three elicitors although methionine best increased this compound. Specifically, the highest increase (73.8%) for NGBS was observed in 6 mM methionine compared to a 67.1% increase in 3 mM methionine.

The undesirable PRO GLs increased in a range between 4.8 % and 49.3% with the highest increase being in 9 mM methionine. In the case of GBS, the precursor GL for the indole GLs, a different trend in its increment was observed after treatment with the elicitors. Contrary to what was observed on other individual GLs, methionine was not the best elicitor of the GBS.

Regarding the total GLs, all elicitors increase the total GLs content significantly compared to the control with the methionine elicitors showing the best results followed by the monosaccharaides’ lowest concentrations.

Table 3.2: Effect of elicitors on the content of individual and total GLs (µmoles g-1 DW) in the 8 days old Ethiopian kale sprouts

								L-Methionine											
No	**Rt**	**Compound name**	**Formula**	**Cont**				**3 mM**				**6 mM**				**9 mM**			
Group 1 (Aliphatic GLs)																			
1	7.52	Progoitrin	$C_{11}H_{18}N_{10}S_2$	19.26	±	1.08	de	24.39	±	1.35	bc	25.94	±	0.78	ab	28.76	±	2.00	a
2	8.27	Sinigrin	$C_{10}H_{17}NO_9S_2$	206.86	±	5.70	d	352.71	±	8.01	a	354.01	±	9.81	a	334.29	±	15.91	a
3	11.33	Gluconapoleiferin	$C_{12}H_{21}NO_{10}S_2$	3.941	±	1.21	d	6.44	±	0.42	abc	6.92	±	0.18	ab	6.99	±	0.43	a
Total aliphatic GLs content				230.03	±	5.8.3	e	383.54	±	9.36	ab	386.87	±	9.25	a	370.04	±	14.41	ab
Group 2 (Indolic GLs)																			
4	12.28	Hydroxyglucobrassicin	$C_{16}H_{20}N_2O_{10}S_2$	0.28	±	0.09	e	7.49	±	0.82	a	6.61	±	0.87	ab	4.93	±	0.7	bcd
5	15.32	Glucotropaeolin (IS)	$C_{14}H_{19}NO_9S_2$	18.05	±	0.80		18.42	±	0.16	NS	18.67	±	0.45	NS	18.18	±	0.84	NS
6	16.59	Glucobrassicin	$C_{16}H_{20}N_2O_9S_2$	15.12	±	4.50	e	29.78	±	0.32	d	31.02	±	3.92	d	32.14	±	2.59	d
7	17.82	Methoxyglucobrassicin	$C_{17}H_{22}N_2O_{10}S_2$	75.28	±	5.14	c	132.20	±	5.28	ab	132.30	±	13.00	ab	153.44	±	9.05	a
8	19.42	Neoglucobrassicin	$C_{17}H_{22}N_2O_{10}S_2$	111.51	±	222.90	bcde	186.35	±	6.3.2	a	193.86	±	12.33	a	126.83	±	6.69	bc
Total indolic GLs content				202.20	±	32.25	f	346.80	±	9.95	ab	363.78	±	25.03	a	317.34	±	13.60	abc
Total GLs content				432.24	±	36.74	g	730.36	±	9.77	ab	750.65	±	19.17	a	687.38	±	5.26	abc

No	Compound name	Sucrose												Glucose											
		70 mM				**140 mM**				**210 mM**				**2%**				**4%**				**6%**			
Group 1 (Aliphatic GLs)																									
1	Progoitrin	22.12	±	0.73	bcde	20.18	±	1.15	cde	19.09	±	1.65	e	23.43	±	0.26	bcd	21.95	±	2.95	bcde	20.89	±	0.76	cde
2	Sinigrin	342.49	±	10.59	a	304.25	±	9.52	abc	255.46	±	24.93	cd	310.91	±	17.60	ab	269.08	±	30.20	bc	251.97	±	28.33	cd
3	Gluconapoleiferin	6.66	±	0.29	ab	6.27	±	0.23	abc	5.05	±	0.86	bcd	5.62	±	0.63	abcd	5.21	±	0.51	abcd	4.72	±	0.96	cd
Total aliphatic GLs content		371.27	±	10.01	ab	330.70	±	10.69	bcd	280.00	±	27.22	de	339.96	±	17.64	abc	296.24	±	33.59	cd	277.58	±	29.83	de
Group 2 (Indolic GLs)																									
4	Hydroxyglucobrassicin	5.76	±	0.10	abcd	3.66	±	0.42	d	3.97	±	1.93	cd	4.32	±	0.17	bcd	6.15	±	0.59	abc	4.24	±	0.24	cd
5	Glucotropaeolin (IS)	18.81	±	1.10	NS	18.04	±	0.28	NS	18.15	±	0.59	NS	18.04	±	0.62	NS	19.12	±	0.60	NS	18.19	±	0.63	NS
6	Glucobrassicin	35.16	±	3.94	cd	48.37	±	2.79	ab	48.72	±	6.04	ab	44.57	±	4.21	abc	54.85	±	2.88	a	39.33	±	5.96	bcd
7	Methoxyglucobrassicin	110.83	±	3.91	b	126.98	±	3.49	ab	76.24	±	12.62	c	119.88	±	18.03	ab	108.93	±	13.35	bc	107.11	±	19.77	bc
8	Neoglucobrassicin	137.00	±	18.30	b	99.42	±	3.16	cde	77.31	±	15.12	e	120.42	±	0.82	bcd	95.53	±	8.38	cde	87.03	±	15.79	de
Total indolic GLs content		288.75	±	25.10	bcd	278.43	±	1.71	bcde	206.25	±	35.63	ef	289.19	±	21.15	bcd	265.45	±	20.80	cdef	237.72	±	40.56	def
Total GLs content		660.02	±	26.50	abcd	609.12	±	9.42	cde	485.84	±	62.10	fg	629.15	±	38.67	bcd	561.69	±	45.17	def	515.30	±	70.38	efg

Data is presented as mean ± standard deviation; different letters following the mean values in each row indicate statistically significant differences among the treatments at $P \leq 0.05$.

DW= dry weight, dsGLs= desulphoglucosinolates, RRF= relative response factor

Principal component analysis (PCA)

To evaluate the relationship between the sprouts under elicitor treatments, the sprouts' various compound variables, as well as the antioxidant activity of the Ethiopian kale sprouts extracts, the data sets were analyzed by PCA. Figure 5 shows the scores for the two principal components (PC1 and PC2), indicating the differences in variable distribution with the elicitor treatments and clear discrimination between the elicitor treated and untreated sprouts. PC1 and PC2 accounted for 76.2 % and 8% respectively of the variances and these were sufficient to explain the total variance (84.2 %). Results indicate that the biological replicates for the control as well as for the different elicitors treated groups were always clustered together, demonstrating high reproducibility of the elicitor treatments and their significant effects on the changes in the variables. The positive region of PC1 and the negative region of PC2 were occupied by water-treated sprouts (control) while high concentrations of sucrose lay in the positive regions of PC1 and PC2. This analysis revealed a positive correlation between sprouts compound variables which were linked to the methionine elicitors and low concentrations of sucrose. The antioxidant activities were more influenced by the low concentrations of the methionine elicitors while the MGB and GNL compounds were associated with the lowest concentrations of sucrose.

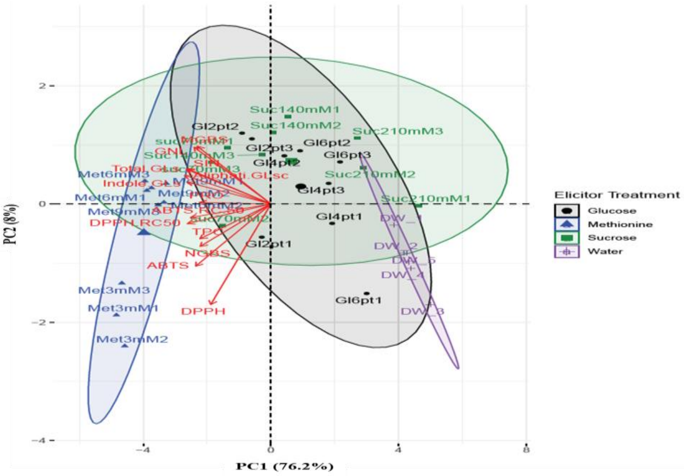

Figure 3.5: PCA biplot of the analyzed variables from sprouts elicited with various concentrations of glucose, methionine and sucrose at different concentrations.

methionine 3 mM (Met3mM), methionine 6 mM (Met6mM), methionine 9 mM (Met9mM), glucose 2% (Gl2pt), glucose 4% (Gl4pt), glucose 6% (Gl6pt), sucrose 70 mM (Suc70mM), sucrose 140 mM (Suc140mM), sucrose 210 mM (Suc210mM), distilled water (DW), progoitrin (PRO), sinigrin (SIN) and gluconapoleiferin (GNL), hydroxyglucobrassicin (HGBS), glucobrassicin (GBS), methoxyglucobrassicin (MGB) and neoglucobrassicin (NGBS), total polyphenols contents (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), the dose required to scavenge fifty percent of the radicals (RC_{50}).

Discussion

The exposure of elicitors to growing plants affects their growth, yield, and accumulation of secondary metabolites including GLs and antioxidants (Baenas, García-Viguera, and Moreno 2014b; Jahangir *et al.,* 2009). In this study, methionine had a positive effect on sprout biomass while sugars slightly reduced the sprouts biomass, with the high concentration of glucose having the greatest reduction. Previously, exposure to methionine was linked to an increase in the fresh weight of broccoli, turnip, China rose radish, and red radish sprouts (Baenas *et al.,* 2014a). Also, upon methionine elicitation, the overexpression of the high aliphatic gucosinolate-1 (HAG1) (also known as MYB28) gene, a positive regulator of methionine-derived aliphatic GLs, was found to be positively correlated with phenotypic changes in *Arabidopsis thaliana* (Gigolashvili *et al.,* 2007). Such previous findings agree with our observations on the effect of L-methionine Ethiopian kale sprouts. In the case of sugars, similar observations in broccoli sprouts found that glucose, fructose, and mannitol reduced the sprouts' fresh weight, whereas sucrose had no effect (Guo *et al.,* 2011).

Elicitors are reported to activate a wide range of signal transduction pathways, resulting in the accumulation of different levels of polyphenols and GLs (Baenas *et al.,* 2014b; Guo *et al.,* 2011; Sánchez-Pujante *et al.,* 2018; Wei *et al.,* 2011). The effectiveness of elicitation on secondary metabolites is due to the combined effect of several factors, including the genetic characteristics of the sprouts, the nature of the elicitor, the dose of the elicitor used, and the method of application of the elicitor (Wei *et al.,* 2011). It is, therefore, true that a small difference in elicitation causes different effects on metabolites in sprouts. In this study, we observed that the elicitation of the Ethiopian kale sprouts with the different concentrations of L-methionine, glucose, and sucrose significantly influenced the accumulation of phenolic compounds and the specific, as well as total GLs differently. Overall, the sprouts elicited with L-methionine and with the lower concentrations of the monosaccharides showed enhanced levels of these compounds more, in comparison to the control.

Previously elicitation with methionine was reported not to significantly influence the phenolic contents of broccoli sprouts but increased GLs content in older sprouts (Pérez-Balibrea *et al.,* 2011). However, sugars, were reported to significantly influence and enhance total polyphenols and GLs in Chinese kale, pak choi, rutabaga cabbage, China rose radish, and red radish (Baenas *et al.,* 2014a; Guo, Yuan, and Wang, 2013; Natella *et al.,* 2016; Wei *et al.,* 2011) and in broccoli, they enhanced anthocyanin antioxidants alongside the GLs (Guo *et al.,* 2011). The dramatic increase in total phenolic on elicitation was attributed to the compounds' de novo synthesis and transformations (Baenas *et al.,* 2016, 2012; Guo *et al*., 2011; Liu *et al.,* 2019; Natella *et al.,* 2016; Pérez-Balibrea *et al.,* 2011; Wei *et al*., 2011). Elicitors stimulated the compounds biosynthetic pathway by increasing the activity or expression of the genes for the key regulatory enzymes such as PAL, which is involved in the conversion of the aromatic amino acid L phenylalanine to ammonia and transcinnamic acid that is converted to various polyphenols, as evidenced in broccoli sprouts treated with sucrose (Cartea *et al.,* 2010; Guo *et al.,* 2011; Jeong *et al.,* 2018).

The induction of the GLs biosynthetic process by inducers is reflected in the content of GLs in the plant (Wei *et al.,* 2011). Sugars and amino acids were reported to influence individual and total GLs content, with low L-methionine concentrations enhancing both aliphatic and indole GLs groups to varying degrees depending on the plant species (Baenas *et al.,* 2014a; Lozano-Baena *et al.,* 2015; Pérez-Balibrea *et al.,* 2011; Scheuner *et al.,* 2005). An increase in GLs content is attributed to be a result of one of two processes: induction of the biosynthetic pathway by the inducer or induction of the hydrolysis process by the enzyme myrosinase (Mithen *et al.,* 2000). In the current study, exogenous treatment of L-methionine increased the aliphatic GLs more than the indole GLs, which corresponded with our hypothesis however, the 6 mM displayed a better effect than the lowest 3 mM concentration. L-methionine has been successfully applied in the elicitation of the GLs where this precursor amino acid for the aliphatic GLS undergoes transamination to α-keto acid before its side chain is elongated to produce the GLs core structures .(Gigolashvili *et al.,* 2007) Sugar treatment too had a positive effect on aliphatic and indole GLs as well and the effects were greatest in sprouts elicited with the lowest concentrations. And in contrast to previous studies that found a decrease in the undesirable GLs progoitrin upon elicitation (Baenas *et al.,* 2014a) we found a slight increase in progoitrin upon sprout elicitation in the current study.

Sugars have been reported as a source of biotic challenge, where the signal molecule induces transcriptional regulatory mechanisms for the integration of carbohydrate availability and hormone action in plants; additionally, they have been shown to synergistically induce the expression of aliphatic GLs controlling genes such as *Bo-ELong*, HAG1/MYB28 and MYB29 genes, with MYB28 expression predominating over MYB29 expression (Liu *et al.,* 2019; Miao *et al.,* 2016; Sánchez-Pujante *et al.,* 2018). Sugars have also been shown in studies to alter myrosinase activity, increasing the content of GLs (Liu *et al.,* 2019) and in the case of indole GLS, sugar treatment was found to be a positive influencer and inducer of the accumulation of these compounds, increasing the expression of key transcription regulator genes such as MYB34, MYB51, and MYB 122 (Wei *et al*., 2011), as well as inducing the assimilation of sulphur to GLs (Miao *et al.,* 2016).

GLs along with polyphenols, are among the most important health-beneficial compounds in Brassica crops, owing to remarkable anticarcinogenic, anti-inflammatory, antioxidant, and antimutagenic activities from their hydrolysis products (Maina *et al.,* 2020). Numerous studies on sprouts have reported the influence of elicitors on antioxidant activities, which is attributed to changes in antioxidant compounds such as phenolic and GLs (Liu *et al.,* 2019; Moreno-Escamilla *et al.,* 2017). Vegetables with high antioxidant activity were associated with high levels of phenolic compounds (Rashmi and Negi, 2020), and it was suggested that the antioxidant activities of these vegetables be evaluated using a variety of methods to avoid underestimation of their potential (Baenas *et al.,* 2016). The DPPH and ABTS reagents are relevant reagents whose activities are used to determine the free radical scavenging activity of plant extracts; they are indicator compounds for testing the antioxidants' hydrogen donating capability (Hoyos-Arbeláez, Vázquez, and Contreras-Calderón, 2017). In this study, elicitor-treated sprout extracts exhibited significantly higher free radical scavenging than non-treated sprouts. These findings suggest that sprout extracts had hydrogen donating ability and antioxidant activity that could be significantly improved by treating them with elicitors. Furthermore, the ABTS assay better reflected the antioxidant contents of the sprouts than the DPPH assay, as has been reported to be possibly due to differences in the extracts' complexity, polarity, and chemical properties, which could result in varying bioactivity depending on the method used, as well as differences in the molecular structures of the two radicals as previously reported (Floegel *et al.,* 2011). According to reports, the ABTS assay is based on the generation of a cation $ABTS^{\cdot +}$, which is applicable to both hydrophilic and lipophilic antioxidants, as opposed to the DPPH assay, which generates a radical that is dissolved in organic media and is thus applicable to hydrophobic antioxidant systems (Floegel *et al.,* 2011). We observed that the antioxidant capacities as evaluated by both assays were more strongly correlated to the total phenolic and GLs contents as previously reported in several cruciferous vegetables (Chang *et al.,* 2019; Sun *et al.,* 2012; Xiao *et al.,* 2019).

Conclusion

This study demonstrates how elicitors could be used as simple strategies to obtain plant functional foods with increased quantities of health-promoting compounds. According to the findings of this study, elicitation has a significant impact on the quality of treated vegetables and may thus be a cost-effective tool for increasing the health usefulness of Ethiopian kale sprouts. Sugars play an important role in the sprouting process of these vegetables, not only as carbon and energy source, but also as powerful signaling molecules while methionine serves not only as a precursor for compound biosynthesis but also as an inducer of biosynthetic pathways. The low concentrations of these elicitors selectively induce secondary metabolites such as phenolic and GLs, as well as their biological activity significantly. More research is however needed to understand the changes in the metabolism of primary and secondary metabolites in Ethiopian kale sprouts in order to develop such effective strategies for enhancing the sprouts for nutritional and health benefits.

Supporting information

The following supplementary data is available online at elsevier publication website. **Figure S1**: The chromatograms of desulfoglucosinolates in Ethiopian kale sprouts, **(1)** refers DAD detection at 229nm, **(2)** MS (+) detection in full scan mode with positive ionization. Peaks (A) progoitrin, (B) Sinigrin, (C) gluconapoleiferin, (D) hydroxyglucobrassicin, (E) glucotropaeolin, (F) glucobrassicin, (G) methoxyglucobrassicin and (H) neoglucobrassicin.

Authors contributions

Development of concept, methodology, statistical analysis and manuscript writing S.W.M.; validation, review, and editing D.H.R.; investigation; supervision, reviewing and editing, G.B. and G.M; funding acquisition, resources and supervision H-Y.K. This version has been read and approved by all the authors of this manuscript.

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Paper Four

Exposure to Salinity and Light Spectra Regulates Glucosinolates, Phenolics, and Antioxidant Capacity of Brassica carinata L. Microgreens

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CHAPTER THREE

General Discussion, Conclusions and Recommendations

3.1 General Discussion

Studies on neglected ALVs are critical for guiding the approach to their consumption, importance in health, production, and possible improvement strategies. This includes exploring the potential and appropriate time for harvesting the most useful parts of the plant for consumption and use as a source of natural bioactive compounds as an important step towards drug discovery, as well as the important role of vegetables in diversifying food systems for healthier diets; and also, generation of production guidelines for these crops that will enable match supply and demand. However, there is a scarcity of information on: (i) the phytoconstituents present in such ALVs and their usage for health benefits, (ii) the techniques for producing high-quality, low-cost ALVs, and (iii) the prospect of using ALVs as functional foods to prevent non-communicable diseases (Forouzanfar et al. 2015). This is because: (i) most ALVs are marginalized and still considered poor man's food; (ii) their cultivation is done on a small scale, or they grow in the wild as weeds (Aworh, 2015); and (iii) there is limited research information available and no consumer awareness on the health benefits of the ALVs because they are ignored by researchers and policymakers. However, vegetable consumers have recently begun to prioritize vegetables in their diets as a result of reports and understanding that they are not only nutritionally dense, but also contain significant levels of health-promoting chemicals (Neugart *et al.,* 2017).

The relevance of recognizing vegetable phytochemicals and their health benefits in lowering diet-related disorders and understanding the biological properties these compounds can have in cell and animal models is crucial. The first article in this study looked on the benefits of synthetic and vegetable-sourced GLs compounds, as well as ways for improving these compounds for high-quality vegetable production. GLs and phenolic compounds have been identified as the most abundant compounds in green leafy vegetables in previous studies, and they play important roles in the prevention of chronic diseases (Clarke, 2010; Luna-Guevara *et al.,* 2018; Possenti *et al.,* 2017; Rashmi and Negi, 2020). We observed that many studies have found that GLs and phenolic compounds have numerous health benefits, particularly for humans, including antioxidant, anti-inflammatory, and anti-cancer properties. Phytohormones, sugars, salt, and changes in the quality and type of light all were mentioned in several studies as ways to improve these compounds during the vegetable cultivation process. This prompted us to target identifying and evaluating the benefits of these two major groups of compounds in the African cabbage and Ethiopian kale vegetables in further study.

The second research article in this study summarizes the profile and variation in quantity of selected polyphenol compounds in various parts of eight African cabbage vegetable accessions during growth, and links the quantity of the compounds to antioxidant activity. Plants have peak levels of secondary metabolites like polyphenols (Feduraev *et al.,* 2019; Fernando, Abeysinghe, and Dharmadasa, 2013), and the developmental stage, genotype, and plant organ all influence these metabolites' levels and biological properties (Chepel, Lisun, and Skrypnik, 2020; Ghimire *et al.,* 2021; Neugart *et al.,* 2017). As a result, this research fills in the gaps in terms of unquantified and unidentified compounds in various organs of African cabbage vegetable accessions, as well as the possible use of the vegetable's edible and non-edible portions as a source of polyphenol compounds and their antioxidant activity. Furthermore, it reveals possible accessions that can be used in breeding efforts to improve this ALV varieties and generate polyphenol-rich foods.

In the third research article, the study suggested that, in addition to boosting the utilization of ALVs bioactive substances, vegetable cultivation techniques must be upgraded by adapting technologies that meet customer needs. According to our preliminary findings, Ethiopian kale vegetables were well adapted for indoor growing in a smart vertical farm system. Vertical farming systems have been highlighted as a possible alternative to conventional farming systems for increasing crop yield and improving quality (Agrilyst, 2017; Benke and Tomkins, 2017). Our study focused on improving the production of Ethiopian kale sprouts by eliciting GLs and polyphenol compounds, as well as their properties, based on such information and numerous studies that show how consumers reap the most health benefits from young tender vegetables, including sprouts and microgreens (Baenas *et al.,* 2012; Kyriacou *et al.,* 2016; Xiao *et al.,* 2019).

Sprouts are regarded natural functional foods with a number of benefits, including the ability to be grown at home regardless of the season and consumed raw, preventing the loss of bioactive compounds during cooking or processing (Natella *et al.,* 2016). Elicitors, on the other hand, stimulate a wide range of signal transduction pathways, inducing secondary metabolite accumulation (Baenas *et al.,* 2014a). We observed that different types and concentrations of elicitors have diverse effects on secondary metabolite production and biological properties, with low elicitor concentrations being particularly effective. This research showed how elicitors can be used as a simple technique for obtaining high-quality plant functional foods rich in health-promoting compounds.

The goal of our last research paper was to apply elicitors to an advanced growing stage of Ethiopian kale vegetables, microgreens, to determine the elicitor effects on production of the main bioactive compounds, and to evaluate the biological activities of their extracts in a much more advanced assay utilizing cell lines. Overall, this research helped to: (i) establish the microgreens' optimal growth conditions and variations in bioactive compound production as well as the biological properties when exposed to various light emitting diode wavelengths and cultivated under salt stress conditions in an indoor vertical growing system; (ii) determine the quantitative measure of the extract's potency and assign it to the standards, and ensure the extract's quality by assessing its toxicity to cells.

3.2 Conclusions

Neglected ALVs are an excellent source of bioactive phytochemicals with excellent biological properties on human health and they are potential targets to be used in reduction of non-communicable diseases. The lack of research on identifying their phytochemical constituents, variation of the phytoconstituents in different accessions as well as various plant parts, their benefits to human health and use of modern cultivation strategies to improve the vegetables have limited knowledge around them and their utilization. It is possible that the key to future natural bioactive compounds sources may very well lie in the untapped potential of such neglected vegetables. Therefore, it is critical that we investigate locally available neglected and underutilized vegetables, assess their health benefits to humans, and identify modern farming systems that allow for high production of natural bioactive compounds. As more information about the major phytochemicals and their physiological effects becomes available, efforts should be made to regulate their biosynthesis using particular growth methods in order to take advantage of their protective properties. Understanding the production of key bioactive substances in different plant accessions as well as different parts of the plants is extremely beneficial to help substantially with breeding for essential traits, making developed vegetable varieties available, providing targets for pharmaceutical and food industry as well as improving quality especially of functional foods obtained from the vegetables. Additionally, there is need to address factors on their effect on digestion and bioavailability together with factors that limit the vegetables availability, commercialization and consumption. Addressing these limitations can be used to promote the benefits of these vegetables to humans.

3.3 Recommendations

Based on the findings from this study and the needs for reduction of diet related diseases, recommendations can be addressed as follows: -

i To meet the population's health needs, there need to be an integrated coordinated approach on the local, regional and international level that demands the involvement of various stakeholders. The global nutrition and agricultural communities must find innovative ways to shift the focus in food systems towards diets that are more diversified, sustainable and beneficial to humans. This could be done by increasing vegetable production, value addition, maintaining their good quality by improving the post-harvest handling techniques, linking farmers to their markets and promoting their consumption. Additionally, undertaking studies that contribute to a broader scientific understanding of important constraints and drivers in ALV promotion should be prioritized.

ii Successful models for incorporating natural bioactive compounds sources into the local, regional and international markets should be launched. They include emphasizing the cultivation of ALVs, which are classified as minor crops, well-suited to climate change due to their ability to withstand harsh environments. These vegetables can be used as alternatives in the event of drought, flooding, or crop failure, and they can be grown with little or no chemical input, reducing pollution and the risk of residual toxicity to human health.

iii The domestication of wild plant species and improvements in neglected ALVs such as African cabbage and Ethiopian kale quality parameters (including content of bioactive chemicals) are also promising avenues for increasing access to nutritional security and lowering diet-related no communicable diseases. These can be accomplished by promoting the adoption and implementation of modern farming ideas with high yielding crops and using strategies such as elicitation which have an impact not only on productivity but also on quality.

iv In order to tap into the nutritional power of these ALVs, particularly their contribution to health and economy, it is important to raise awareness of their contribution, particularly among farmers and consumers. Doing so will encourage producers to explore different sources of these ALVs at affordable prices, such as growing their own vegetables.

v All along supply chain, it is crucial to promote the consumption of vegetables by educating consumers and highlighting the consumption's scientific value through workshops and seminars. This can be enhanced by offering options for maintaining ALV quality and increasing the use of vegetables through value addition to raise consumer acceptance. Regarding raising consumer knowledge of its health benefits, impersonal public relations and marketing strategies—such as advertising and media articles in local languages—that convey critical information about the ALV's contribution to dietary diversity are crucial. To encourage the use of ALVs in communities, behavioural changes such kid exposure to and teaching on ALVs in primary schools, as well as including these products, are recommended.

vi It is considered that if the rise in ALV promotion and consumption is not matched by an increase in ALV propagation or cultivation, this could lead to an unstable increase in wild harvesting and the extinction of these species. We recommend promotion of conservation and collection of genetic resources of the neglected ALVs variety germplasms on the verge of extinction and their preservation in genebanks for use by public and private sectors as well as researchers and breeders to develop products that are more acceptable to the local people for consumption.

Areas of Further Research

Due to financial and time constraints, the study did not include areas that would have provided a more comprehensive assessment of the two neglected ALVs, their production, improvement, and utilization. As a result, it is proposed that the following areas be explored further: -

1. To expand research on the health beneficial properties including toxicity, antioxidant, anti-inflammatory and anticancer properties, different ALVs solvent extracts and their effect on digestion using simulated and advanced bioavailability model be assessed.

2. Identify enzymes and critical regulatory factors by elucidating the biosynthetic pathways of bioactive compounds in order to better understand and produce these high-value natural bioactive compounds on a large scale, which will help both human health and plant resource conservation.

3. Considering our target ALVs lack a well-studied genetic background and genome sequence, we can use high-throughput sequencing technology to generate transcriptome data in a short period and at a low cost. A comparison of transcriptome, chemical components, and gene expression patterns could be utilized to find candidate genes in the biosynthesis pathways of bioactive compounds.

4. Employ modern modelling tools to quickly assess bioactive chemical production scenarios among a variety of accessions collected from various locales.

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Appendices

Appendix 1: Supplementary data: paper two

Appendix 2: Supplementary data: Paper Three

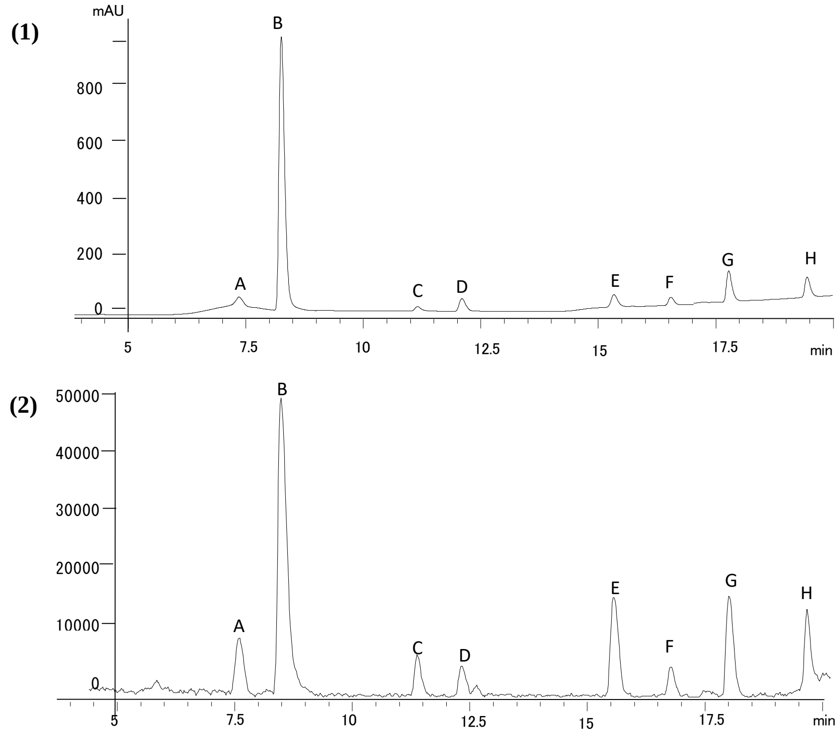

Figure S1: The chromatograms of desulfoglucosinolates in Ethiopian kale sprouts, **(1)** refers DAD detection at 229nm, **(2)** MS (+) detection in full scan mode with positive ionization.

Peaks (A) progoitrin, (B) Sinigrin, (C) gluconapoleiferin, (D) hydroxyglucobrassicin, (E) glucotropaeolin, (F) glucobrassicin, (G) methoxyglucobrassicin and (H) neoglucobrassicin.

Appendix 3: Supplementary data: Paper Four

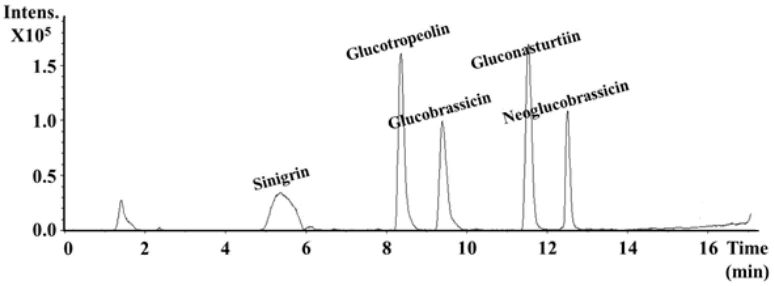

Figure S1. UPLC-DAD chromatogram of available standards detected at 229 nm.

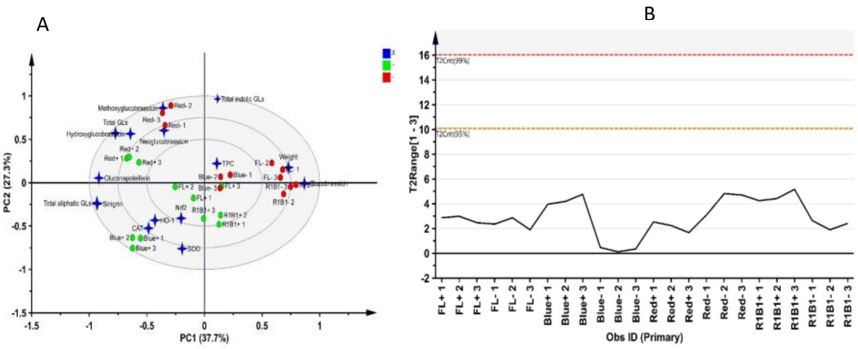

Figure S2: PCA bi-plot of *B.carinata* microgreens (A) showing separation of groups on the space. Green hexagons represent the group of microgreen samples under different lights that were treated to salt stress. Red hexagons: represent the group of microgreen samples that were not treated with salt. Blue stars: X-variable. (B) Hotelling's T2 range plots of the PCA belong to thenormal area.