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How Substances in Agricultural Waste Material Can Be Used as Active Ingredients in Skin Care Products

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Master's Thesis
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Abstract

Aging of the skin is an inevitable process that occurs throughout a lifetime and exposure to environmental pollutants is a factor accelerating this process. Phytochemicals present in plants possess biological activities known to protect or repair the skin cells and when used in skin care products they can slow down the skin aging process. In this project agricultural waste materials are investigated to see if they contain compounds with biological activity on human skin and can be used as active ingredients in skin care products. Secondary metabolites were extracted from up to six fractions from seven different crops. 76 compounds were found and identified using liquid chromatography-mass spectrometry and 68 human targets were found using in-silico evaluation. The extracts were also investigated for antioxidant activity, inhibition of glycation processes and inhibition of the enzymes tyrosinase and kallikrein-related peptidase 5. Each of the seven plants showed some promising results and 10 extracts had high activity for one of the four primary screening assays, one extract had high activity for two. There is a possibility for some of these extracts being used as active ingredients in skin care products. However, more research is needed to fully know which extracts and for what purpose. The method can be used to investigate the activity of other plant extracts to broaden the research and increase the chances of finding a new active ingredient for skin care.

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Chapter 1: Introduction

Right now, the world is facing a challenge it has never faced before: reducing our negative impact on the environment. The success of this challenge is depending on support from every country, every industry and every individual. For as long as we can remember, the human population has been using the resources of earth to create a life where development and achievement have been the main goals. In this process of becoming who we are today and creating the society we now live in we have discovered the results of our actions. The United Nations has decided on global goals of sustainable development to deal with this challenge and in 2030, the aim is that these 17 goals are adopted by every member who thereby contributes to a safe and green world. The industry of cosmetic products is not an exception and more companies strive to use sustainable resources in their products and packages. One big driving force of this change is the global awareness among consumers and their request for using sustainable skin care products. Along with awareness and people wanting to contribute to a healthier world, the trend of using natural products for personal care is greater than ever. People are nowadays more aware of what they put on their skin and they want the products to be as natural as possible.

Oriflame is a Swedish cosmetic company founded in 1967 and is now existing on the market in over 60 countries. Their products are inspired from the Swedish nature and are therefore based on plant extracts. They have, from the beginning, focused on being natural and not tested any products on animals. Year 2009 Oriflame opened a skin research institute in Stockholm where research scientists search for new ingredients and create new methods for skin health research in order to fashion unique skin care products. Oriflame's skin research institute (SRI) is today organised by over ten scientists who discover, develop and test new useful active skin care ingredients. Among other things, they explore the plant kingdoms and the marine searching for potent ingredients that might have different protecting or healing qualities, such as anti-aging profits. Oriflame works with natural ingredients and strive to use sustainable resources for their skin care products.

This project was a part of one of Oriflame's newly started collaboration projects together with The Plant Protein Factory (PPF). PPF is currently working on becoming a new supplier of raw material such as plant protein and other nutritional material from the plant parts that are left behind and not used today. Their vision is to minimise the waste and find useful applications for these materials in, among others, the food and cosmetic industry. In this collaboration, PPF provided Oriflame with fractionated samples of waste material from different crops cultivated in south of Sweden. These fractions were then examined for their potential use in coming skin care products to take advantage of the biological activity of compounds produced by the crops. Cellulose, proteins and sugars are some of the components present in plants, phytochemicals or phytonutrients that are also present in plants are more specific and unique for each species. It is the phytochemicals (e.g. polyphenols) that are interesting when in search of biologically active ingredients for skin care products since they are metabolites produced by the plants as protection to ensure survival in their environment.

1.1 Aims and Objectives

The main aim of this project is to investigate if extracts from various sample fractions produced from agricultural waste material can be used as active ingredients in skin care products. The specific objectives for this project are:

- to analyse and identify the content of the extracts using Liquid Chromatography-Mass Spectrophotometry (LC/MS),
- to examine some biological activities of the extracts known to protect or repair skin cells using different primary activity screening methods,
- to examine the protective properties against pollution using a cell based in-vitro assay, and
- to find potential targets using an in-silico method.

1.2 Outline

The rest of this report is divided into five chapters covering theoretical background, method, results, discussion and conclusion. The theoretical background in Chapter 2 describes the environmental mechanism of skin aging and how plant secondary metabolites can be used to protect the skin against pollution. Chapter 3 covers the method of the laboratory procedures used for the project. In Chapters 4 and 5 the results of the biological activities of the extracts are evaluated and discussed. Finally, the report is concluded in Chapter 6.

Chapter 2: Theoretical Background

This chapter describes relevant topics to provide knowledge to understand the project: facts about the skin and the mechanism behind skin aging, environmental pollution and its contribution to skin aging and various diseases, the protective properties of plant secondary metabolites, some sustainability aspects and information about the crops examined. It also describes the assays used in this project to extract these compounds and examine their properties.

2.1 Skin Structure

The skin is the largest organ of the body and serves as a barrier to protect us from intruding materials and organisms as well as keep us contained. The skin structure is mainly two layers called epidermis and dermis containing cells such as keratinocytes, melanocytes and fibroblasts that can be viewed in figure 1. Epidermis is the outer layer of the skin while dermis is the inner part of the skin and both consist of multiple layers of cells and tissues. Hypodermis is deeper, beneath dermis and connects the skin to the rest of the body, e.g. muscles and bones but is not considered a part of the skin. (Lawton 2019)

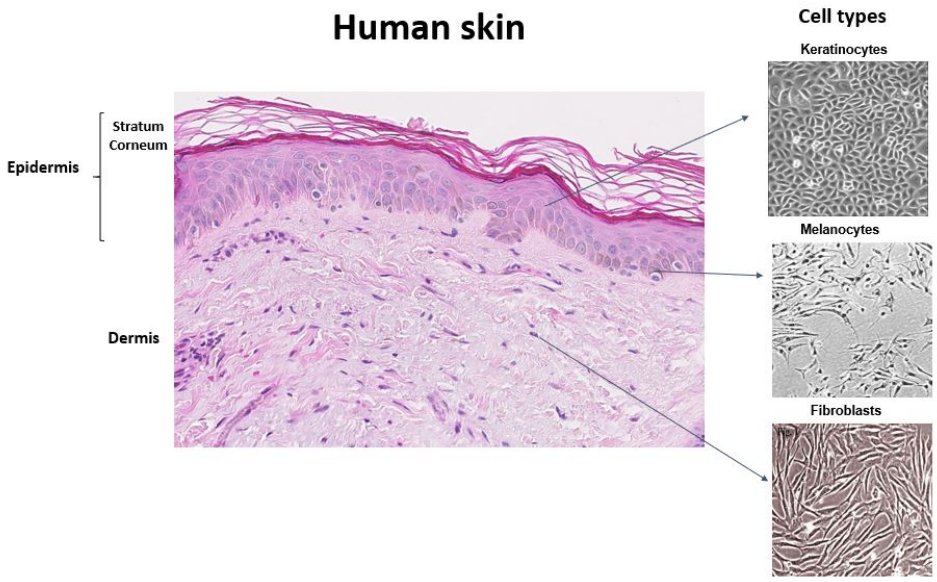


Figure 1. Dermatological picture of the different layers of the human skin (epidermis and dermis) including the three cell types; Keratinocytes, Melanocytes and Fibroblasts. (Taken from the skin lab at Oriflames head office in Stockholm)

The cells of epidermis are keratinocytes that produces keratin, a protein that provides the skin with its hard texture. The most superficial layer of keratinocytes are dead cells accumulated with keratin called corneocytes. This layer is called stratum corneum and is a complex structure of fatty acids, cholesterol and corneocytes. It is a lipid barrier directly exposed to the outer environment protecting the body from penetration of foreign particles and microbes as well as water loss. This layer is regularly replaced by new dead cells and every month we have a completely new layer of corneocytes. The inner keratinocytes lay where epidermis connects to dermis and they bind together through collagen fibers. This part of epidermis also contains melanocytes and Merkle cells that play important roles. Merkel cells cooperate with the nerve

cells of the brain which makes us feel the sensation of touch. Melanocytes are cells producing melanin which is a pigment and the reason for our skin colours. (Lawton 2019) Only 1% of cells in the epidermis are melanocytes and one melanocyte is therefore surrounded by about 36 keratinocytes. Melanin is produced and distributed by melanosomes to keratinocytes where they are accumulated. The skin cells are protected by melanin since it blocks ultraviolet radiation (UVR) from damaging the cells. About 50% of the UVR is absorbed by melanin and therefore not penetrating through epidermis. Darker skin has higher absorbance and thereby protection, a demonstration of melanin production and distribution can be viewed in figure 2. (Brenner, Hearing 2008)

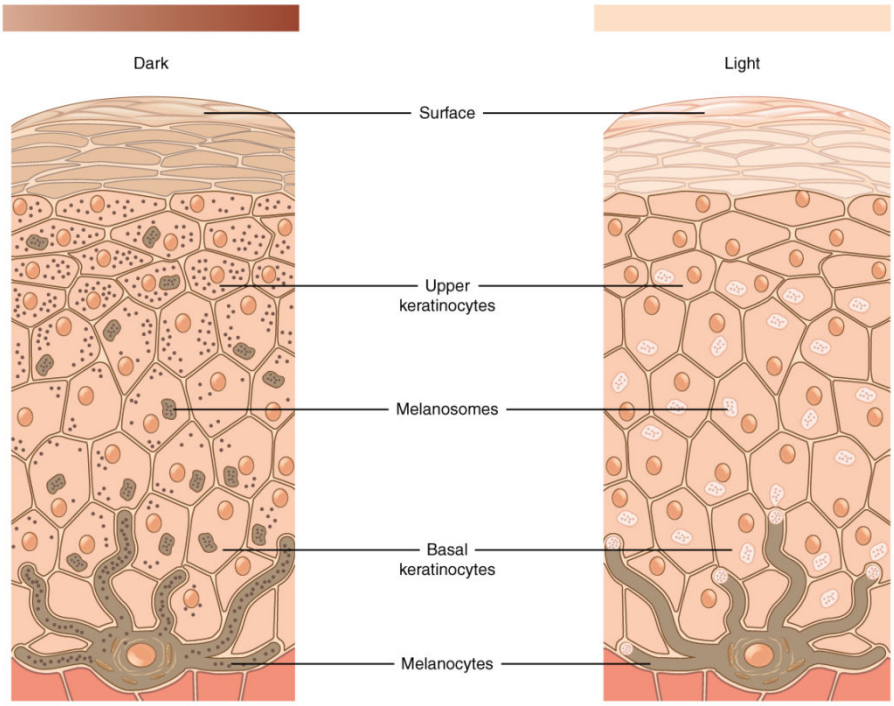


Figure 2. Melanin production and distribution from melanocytes to keratinocytes in dark and light skin. (Betts, J.G et. al 2013)

Unlike epidermis, dermis contains blood vessels and nerves. The fibroblasts in the dermis layers produce collagen fibers to keep the structure and strength of the skin as well as to bind water for hydration. Fibroblasts also produce elastin fibers that give the skin its elasticity which is an important property of the skin. (Lawton 2019)

2.2 Skin Aging

The human skin naturally undergoes changes throughout the years. When skin gets damaged, we say it ages, it gets filled with wrinkles, pigment spots and it loses lots of its elasticity. The aging of the skin occurs mainly in two different ways. Firstly, photoaging which relates to the exposure of UV radiation and other environmental pollutants and results in coarsely wrinkled skin and can even lead to chronical diseases. Lastly, we have the natural aging of the skin which is dependent on ethnicity and genetics and is considered to give a smoother wrinkle appearance. (Rhie, Shin et al. 2001) What they both have in common is the process of oxidative stress, where the accumulation of reactive species in the skin cells is the greatest harm.

2.2.1 Reactive Species and Oxidative Stress

As mentioned before, reasons for skin aging is related to the presence of reactive species (RS) that are formed in the body from metabolic reactions as well as from interference from environmental pollutants. The term RS includes radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS). (Rhie, Shin et al. 2001) When they are produced in a faster pace or a larger amount than what the body is in need of the balance between oxidation and antioxidation is disrupted. This leads to over oxidation of components in the cells as well as outside of the cells, and proteins, lipids, barriers and DNA are only a few examples of components that get damaged through this interference. One oxidation process can easily lead to another by chain reactions. If, for example, phospholipids in the membranes of the skin cells are oxidised, lipid peroxide is formed causing inflammation and then upregulation of specific enzymes degrading collagen leading to looser skin. (Dayan 2008)

2.2.2 Intrinsic Skin Aging

The natural aging process is called intrinsic skin aging and is determined by genetics. The results of this process are multiple, among other, fine wrinkles, hollowed eye sockets and cheeks due to loss of underlying fat and that the skin gets more transparent and thinner. (Ahsanuddin, Lam et al. 2016) For intrinsic aging, ROS is generated in the cells both enzymatically and non-enzymatically. In mitochondria, ROS particles are produced as a by-product as the cell respiration occurs. During oxidative phosphorylation in the mitochondria, electrons from the electron transport chain (ETC) are unintentionally released to oxide molecules O_2 resulting in the generation of superoxides O_2^-. (Rinnerthaler, Bischof et al. 2015) ROS can also be produced in a non-mitochondrial way, for example, through a Fenton reaction which is a catalysed degradation process of H_2O_2 by free bivalent iron ions. (Ahsanuddin, Lam et al. 2016)

The skin gets older as we grow older for primarily the reasons above. Over the years, ROS production increases and thereby the exposure of them to the cells, at the same time there is a decrease in antioxidant activity and the cell's ability to repair the oxidative damage caused by ROS. (Ahsanuddin, Lam et al. 2016) Every year, the skin loses 1% of its elasticity due to lowered production of collagen and elastin (Obagi 2005), the change in collagen-elastin structure is demonstrated in figure 3, where a comparison between the structure of the collagen and elastin in young skin and aging skin is shown.

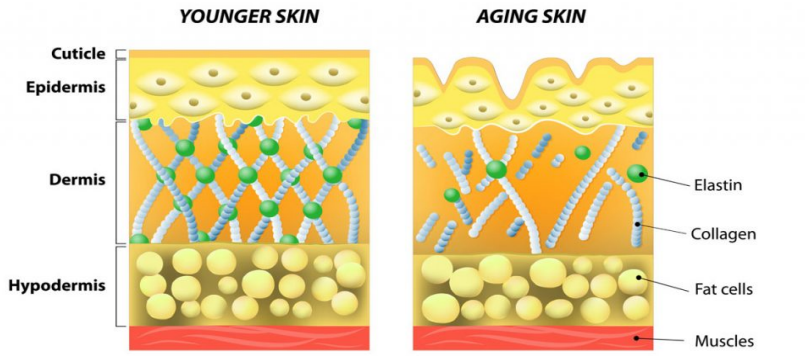


Figure 3. The figure demonstrates the complex structure of collagen and elastin in the skin. The left picture demonstrates the structure and elasticity of young skin while the right picture shows the broken structure of an older skin that has lost its former elasticity. (Kwall, R 2016)

2.2.3 Extrinsic Skin Aging or Photoaging

Photoaging is the part of skin aging that is dependent on exposure to several pollutants in our surrounding. UV radiation is the largest source of this type of aging and studies show that the skin areas that are mostly exposed to the sun: face, neck, hands, forearms and upper chest are significantly more photodamaged than the rest of the skin. Photoaging appears in a different way than intrinsic aging, not at all as smooth and finely wrinkled but rather a dry, sallow and coarsely wrinkled finish with changes in pigmentation. This aging process, in contrast to intrinsic, is more severe since exposure to UV radiation and environmental pollutants can lead to chronical diseases such as allergic reactions, acne and skin cancer. (Helfrich, Sachs et al. 2008)

What happens is that the epidermal skin gets thicker, solar elastosis, which is an elastin containing material, is accumulated and the collagen production and structure are disrupted. Transforming growth factor (TGF) and activator protein (AP-1) are two collagen regulators that promotes collagen formation and collagen breakdown respectively. The breakdown is induced as a result of the up-regulation of matrix metalloproteinase (MMPs: enzymes that degrades collagen) in presence of AP-1. A study showed that within 15 minutes after irradiation of UVA/UVB light on the skin, hydrogen peroxide was generated and the levels of AP-1 noticeably increased as well as the MMPs and the collagen levels were then decreased because of degradation. The changed levels remained for 24 hours. This indicates that the aging procedure starts immediately after exposure of pollutants. (Helfrich, Sachs et al. 2008)

2.3 Environmental Pollution

Environmental pollutants are agents that, in a negative way, modify the natural composition and characteristics of the atmosphere. They originate from different sources that could be human made (e.g. fossil burning and car emissions) or natural (e.g. vulcanic activities and forest fires). Air pollution, which is one kind of environmental pollutant, and the most interesting one for skin health concerns, is present in various forms and phases. (Puri, Nandar et al. 2017) Exposure to environmental pollutants can contribute to oxidative stress in the cells of the body. Pollutants are, or can generate, free radicals which disturb the oxidant and antioxidant balance of the cells. Overproduction, or lack of antioxidant defence lead to oxidative stress. This is because many compounds are involved in production of ROS and RNS and an overproduction of these due to pollution can lead to various chronic diseases. (Poljšak, Fink 2014)

Example of environmental pollutants are:

- Oxides
- Volatile Organic Compounds
- Ultraviolet radiation
- Ozone
- Polycyclic aromatic hydrocarbons

Other broader pollutants are for example:

- Particulate matter such as particles from traffic and cigarette smoke

2.3.1 Oxides

Different pollutive oxides present in the air are nitrogen oxides (NOx), sulphur dioxide (SO_2) and carbon monoxide (CO) that all have damaging effects on the cells. Nitrogen dioxide (NO_2) can cause oxidation of amino acids in proteins and CO, which is a well-known pollutant, acts damaging on the metabolism of the cells. (Drakaki, Dessinioti et al. 2014)

2.3.2 Volatile Organic Compounds

Organic solvents present in paints, tobacco smoke and fuels lead to emissions of volatile organic compounds (VOCs) that can induce inflammation and allergic reactions such as eczema. They react with sunlight and NOx to form ozone molecules that have negative effects on the skin, see section 2.3.3. (Drakaki, Dessinioti et al. 2014)

2.3.3 Ultraviolet Radiation and Ozone

Ultraviolet radiation can be divided into three different types according to wavelength: UVA, UVB and UVC. 95% of all UVR that reaches the surface of the earth is UVA which has wavelengths between 320-400 nm. The rest are UVB (280-320 nm) and UVC (180-280 nm). UVA reaches the skin and penetrates to epidermis and dermis and is a leading cause for photoaging. Together with UVB, that reaches only to epidermis, they can lead to immunosuppression and skin cancers. (Drakaki, Dessinioti et al. 2014) UVR decreases the activity of antioxidants in the skin as well as generates ROS that damage the cells. (Poljšak, Fink 2014)

The stratosphere and troposphere naturally contain ozone. If the skin is exposed to high levels of ozone the levels of antioxidants such as vitamin E and ascorbic acid (vitamin C) can be decreased, epidermis can be inflamed, and cells damaged due to oxidative stress. Moreover, extracellular matrix components such as collagen and elastin are degraded which results in extrinsic aging of the skin. When exposed to ozone in presence of other pollutants (e.g. UVR) the effects can be intensified. (Drakaki, Dessinioti et al. 2014)

2.3.4 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are organic pollutants that originate from wood burning, car fumes and cigarette smoke and are easily spread in the air. PAHs can produce epoxides and diols that act carcinogenic by binding to DNA molecules and they can transform into redox-cycling chemicals that generate ROS particles and induce oxidative stress. Other diseases can form from halogenated PAHs, e.g. chloracne and arthritis. (Drakaki, Dessinioti et al. 2014)

2.3.5 Particulate Matter

Particulate matter (PM) is a name of all the different complex particles suspended in the air. They are classified by their sizes and range from ultrafine (<0.1 μm), fine (<2.5 μm) to coarse particles (<10 μm) that can penetrate the skin. (Jin, Li et al. 2018) They appear from different sources, but the ones originating from traffic events are considered highly dangerous since they are prone to react with the human body causing, among other, oxidative stress. The effects of

such exposure are pigment changes as well as wrinkles, but PMs can also cause more severe diseases such as cancer and cardiovascular diseases. (Drakaki, Dessinioti et al. 2014) Cigarette smoke is another source of particulate matter and hold thousands of harmful substances like ROS and RNS, free radicals, carcinogens and oxygen radical forming substances. They are responsible for causing oxidative stress in the skin cells, trans-epidermal water loss, degeneration of collagen and elastin and the levels of certain antioxidants such as vitamin C and glutathione are decreased. Moreover, smoking cigarettes will lead to deep wrinkled skin and skin discoloration. Studies have shown that smoking is also connected with development of acne and psoriasis. (Drakaki, Dessinioti et al. 2014)

2.4 Diseases Caused by Oxidative Stress

As a result of increased amount of air pollution and thereby our exposure to it, we tend to develop various diseases. Many diseases are linked to the skin and some examples are acne, psoriasis, arthritis, chloracne, atopic dermatitis, eczema and skin cancer but we can also develop other serous conditions such as cataracts, cardiovascular diseases and other types of cancer. (Poljšak, Fink 2014, Puri, Nandar et al. 2017)

2.5 Protective Compounds Present in Plants

The body has ways to protect the skin from aging processes like oxidative stress, different substances and mechanisms work to prevent or repair such processes. These compounds work by, for instance, having inhibitory effects on the enzymes kallikrein-related peptidase 5 or tyrosinase. Kallikrein-related peptidase 5 (KLK5) is a serine protease present in epidermis where it is part of the renewal process of skin cells and the skin barrier. The enzyme helps release the old and dead corneocytes as they get closer to the skin surface by degrading one key player in the junction matrix, the protein corneodesmosomes. When the KLK5 enzyme is overactivated this process is accelerated, damaging the function of the barrier leading to skin diseases such as rosacea. By suppressing the activity of the enzyme, such diseases can be prevented or treated. (Matsubara, Matsumoto et al. 2017) Tyrosinase is another enzyme which is involved in the synthesis of melanin in the skin. Its activity is highly connected to the skin pigmentation and it is shown to be much more active in darker skin than in lighter skin. Regulating its activity will therefore affect the pigmentation of the skin. (Iwata, Corn et al. 1990)

There are also non-enzymatic processes affecting the aging of the skin, glycation and oxidation are two examples. The glycation process is a multiple step reaction between proteins, nucleic acids and lipids with reducing sugars that lead to the formation and accumulation of advanced glycation end products (AGEs). These end products are key players in skin aging as well as in other damaging processes in the body. They contribute to oxidative stress in the cells as well as damage important factors like proteins, all affecting the aging of the skin in a negative way. AGEs have multiple damaging properties such as decreasing skin elasticity by cross-linking with the extracellular matrix (ECM), affecting cellular functions by modifying proteins as well as binding to specific receptors for AGEs (RAGE) and thereby induce inflammation. (Kim, Park et al. 2017) Studies have shown that the accumulation of AGEs is increased or accelerated in presence of UV-irradiation and cigarette smoke but also in people that have diabetes. (Gkogkolou, Böhm 2012) Knowing how to hinder this process is not as simple as for an enzyme, but phytochemical compounds have shown inhibitory effects of the formation of AGEs, compounds possessing high antioxidant activity is one of them. (Kim, Park et al. 2017)

Plant secondary metabolites have shown to possess inhibitory qualities as such and therefore, looking through the plant kingdom for active compounds to be used as protectors and repairers in skin care products is highly interesting. Just like all other organisms, plants produce chemicals to survive and function, phytochemicals are specific chemicals produced by plants and therefore only occurring naturally in plants. There are five types of phytochemicals: carbohydrates, lipids, phenolics, terpenoids and alkaloids where the three latter are bioactive, meaning they have biological activity in humans. (Huang, Xiao et al. 2016) Phytochemicals are present in various plant parts in high concentrations (e.g. flowers, leaves, stems and roots) and some are said to have protective properties against cell damage from free radicals. (Altemimi, Lakhssassi et al. 2017) Polyphenols are phytochemicals that have multiple phenolic rings and possess various amounts of biophysiochemical properties (e.g. anti-inflammatory and antimicrobial activities and gene expression influence). They are plant secondary metabolites and can be divided into different subgroups, the two general classes are: flavonoids and phenolic acids but there are also tannins, lignans and more (figure 4). (Chu, Gross et al. 2000)

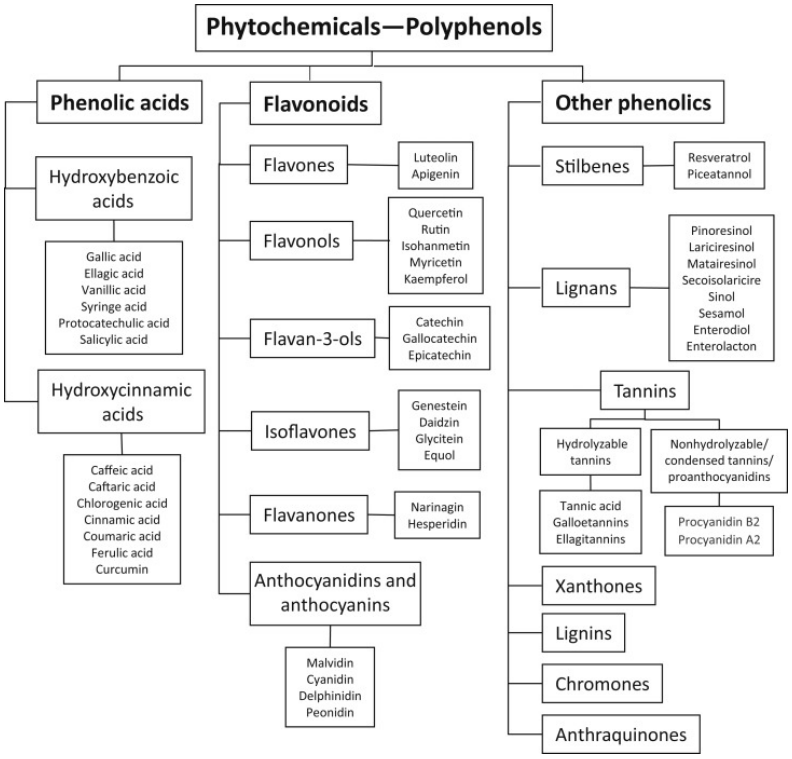


Figure 4. A schematic overview of polyphenolic subgroups and some polyphenolic compounds. (Martinez, K.B et. al 2017)

Flavonoids, which are polyphenols, are antioxidants present in the leaves, flowers, seeds, berries, bark and roots of green plants. They are mostly present as flavonoid glucosides, and play great roles in plant growth, disease resistance, protection from free radicals, UV radiation and bacterial and viral attacks. Some flavonoid subclasses are flavones, flavonols, isoflavones, flavanones and anthocyanidins and anthocyanins. The structure of flavonoids does not differ much between molecules and they all contain two phenolic rings, A and B, and a heterocyclic ring, C, which contain an oxygen atom. What does differ between the subgroups of flavonoids is the structure of the C ring, more specifically, there are different levels of oxidation of the ring. Flavonols are the most common flavonoid in plants, examples of flavonols are: Quercetin, Kaempferol, Myricetin, Isohamnetin, Rhamnetin and Rutin. (L Miranda, F Stevens et al. 2012)

Phenolic acids is the other general subgroup of polyphenols and are present in various parts of plants, such as seeds, fruits and leaves. They are known to possess very high levels of antioxidant activity and by eating these compounds they can reduce the risk of oxidative stress in the cells. The level of antioxidant activity is dependent on the compounds but generally, by the radical scavenging mechanism, they provide antioxidation by donating H-atoms. Besides being antioxidants, phenolic acids act antimicrobial and anti-inflammatory in the human body. Examples of phenolic acids are caffeic acid, ferulic acid, benzoic acid and syringic acid. (Kumar, Goel 2019)

2.6 The Examined Crops

This report investigates the possibilities of using wasted fractions from plant materials as a source of active ingredients in skin care. The plant materials originate from Sugar beet, Lucerne, Kale, Phacelia, Crimson clover, Oil radish and Red beet leaves. Fractions, such as white, green and brown juices, fibers, proteins and whole leaves are produced as biproducts when retrieving the wanted parts of the plant. These could therefore be interesting sources of natural active ingredients for health care products. The process of retrieving these fractions is shown in figure 5 and is done by The Plant Protein Factory using wasted agricultural material.

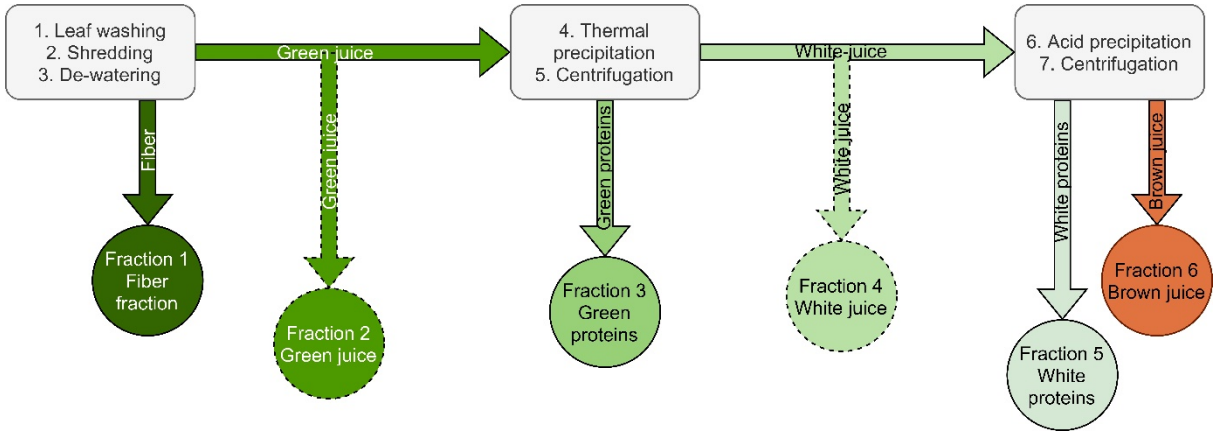
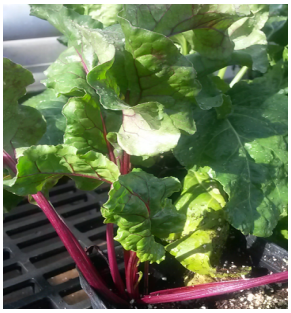


Figure 5. The process where the different fractions are derived. The fractions are produced by the Plant Protein Factory in Alnarp where they harvest the plants and collect the waste. The dashed fractions are optional fractions that are only produced if needed.

Information regarding the composition of phytochemicals within these plants can be found in scientific studies. Mostly the studies focus on the active compounds present in the roots and flowers and might or might not be present in the leaves which is the focus of this project. Thus, the results could be used as a prediction or a guide to know what to look for in the fractions. In table 1, the plants examined in this project are briefly described.

Table 1. Description and prediction of compounds of the plant materals examined in the project.

Plant name	*Picture of the plant*	*Description and prediction of compounds*
Kale *(Brassica oleracea var. Sabellica)*	 Photo by Rasbak (2005)	Kale is a leafy vegetable that has high concentrations of flavonoids, low molecular weight polyphenols, that have multiple protective characteristics to human health. The flavonoids in plants are often conjugated with glucose and are therefore present as glycosides. (Schmidt, Zietz et al. 2010) A study of Kale extract using LC/MS showed high levels of flavonol glucosides, such as quercetin, kaempferol and isorhamnetin. (Neugart, Krumbein et al. 2016)
Sugar beet *(Beta Vulgaris)*	 Photo by Jacopo Werther (2010)	Sugar beet is mainly a source of sucrose and substrate for ethanol production, but apart from that it appears to be beneficial for human health. Sugar beets consist of phenolic compounds and saponins whom both have significant relevance as active ingredients in nutritional and cosmetic products. (Mikołajczyk-Bator, Błaszczyk et al. 2016) The family of the sugar beet and the sugar beet itself and their leaves, are known to contain various phenolic acids and flavonoids, such as quercetins and vitexins. (Vissers, Kiskini et al. 2017)
Lucerne (*Medicago sativa)*	 Photo by Robert Flogaus-Faust (2018)	Lucerne is an herbal plant with blue coloured flowers and deep roots. (Dolling, Revell 2018) The levels of phenolic compounds in Lucerne are dependent on what part of the plant that is investigated. The leaves have a higher concentration of phenolic acids compared to the flower and stalk whereas the flower contained the highest level of flavonoids. Antioxidants identified in Lucerne are 4-hydroxybenzoic acid, Rutin, Quercetin, Formononetin, Taxifolin, Coumestrol (phytoestrogen) and formononetin-7-O-glucoside-6″-malonate (Bajkacz, Baranowska et al. 2018)
Phacelia *(Phacelia tanacetifolia)*	 Photo by Faxstaff (2006)	Phacelia is an herbal plant that is commonly used as green manure to improve the soil structure and give nutrition between periods of cultivation. A study of the antioxidant activity in the seeds and sprouts of Phacelia showed, using HPLC method, that the total content of free phenolic acids and flavonoids in Phacelia sprouts was 5.20 mg/100g dry mass while the total content of bound phenolic acids and flavonoids was 61.82 mg/100g dry mass. The antioxidant activity was analysed for DPPH radical and measured to be 7.35 mg trolox/g dry mass which is not considered to be high. (Pająk, Socha et al. 2019)

Crimson clover *(Trifolium incarnatum)*	 Wikimedia Commons (2010)	Crimson clover in an herbal plant with red flowers used to temperate and moist fields. According to a study where multiple *Trifolium* species were investigated, there were multiple phytochemicals found (e.g. flavonoids such as quercetin and kaempferol). (Sabudak, Guler 2009) Another study of the antioxidant activity of red clover (*T. pratense*), which comes from the same family and even genus as Crimson clover but are different species, showed that the total amount of polyphenols was 153 mg/g. (Kroyer 2004) The results of this study does not guarantee similarities to the content of Crimson clover but due to similarities between the species it can be used to predict the amount of polyphenols present.
Oil radish (*Raphanus sativus)*	 Photo by KENPEI (2007)	Radishes have a history of being used in medicinal contexts treating digestion issues and liver failures. They contain multiple beneficial compounds such as phenolic acids and anthocyanins and they claim to have antioxidant activity. (Hanlon, Barnes 2011)
Red beet *(Beta vulgaris)*	 Photo by Horticulturalist RJ (2016)	The leaves of Red beet are a source of numerous compounds with antioxidant capacities. Studies show that they, among others, contain polyphenols, flavonoids, vitamins, betalains and folic acid. The extract of red beet leaves could therefore be a great source of ROS scavengers and therefore have protective properties on the skin cells. (Lee, Son et al. 2009)

Chapter 3: Method

This chapter describes the methodology of the project which includes a detailed description of the laboratory procedures. All the steps of the examination process are described: the process used for extraction of plant secondary metabolites, how compounds were identified, the primary assays used to examine the biological activity of the extracts as well as the in vitro assay used to examine protective properties against pollution.

3.1 Laboratory Procedures

This project was based primarily in the lab. The total process included the following 6 steps, but for this project step 5 was not performed. Steps 1-3 (and a little bit of step 4), were performed in the lab and step 6 was preformed digitally:

1. Extraction
2. Identification
3. Activity screening
4. In-vitro assays
5. Ex-vivo assays
6. In-silico prediction of biological activity and targets

The first step was extraction of secondary metabolites from the plant samples using methanol extraction. In the second step these extracts were analysed using Liquid Chromatography-Mass Spectrophotometry (LC/MS) to identify the compounds present. After this, in the third step, activity screening methods were used to find the extracts showing activity. Those extracts would then have been further studied in step 4-5, by cell-based in-vitro assays and lastly ex-vivo assays. The cell-based assays look for anti-pollution, UV protection or anti-inflammatory properties of the extracts. The ex-vivo assays uses qPCR, flow cytometry and histology to further examine these properties after the substances has been applied to donated skin. The last step was not performed as mentioned earlier. Instead the digital platform Reaxys was used to predict the biological activity of the extracts on the skin, this is step 6. Descriptions and procedures of the methods used in this project were available as standard procedures in the Oriflame network written by members of the SRI team.

To summarize, the laboratory part of the project consists of four main experiment types: extraction of secondary metabolites, identification of compounds, primary activity screening and in-vitro assay. Figure 6 demonstrates a flow chart over the experiments mentioned as well as the previous harvesting and processing steps that is not part of this project.

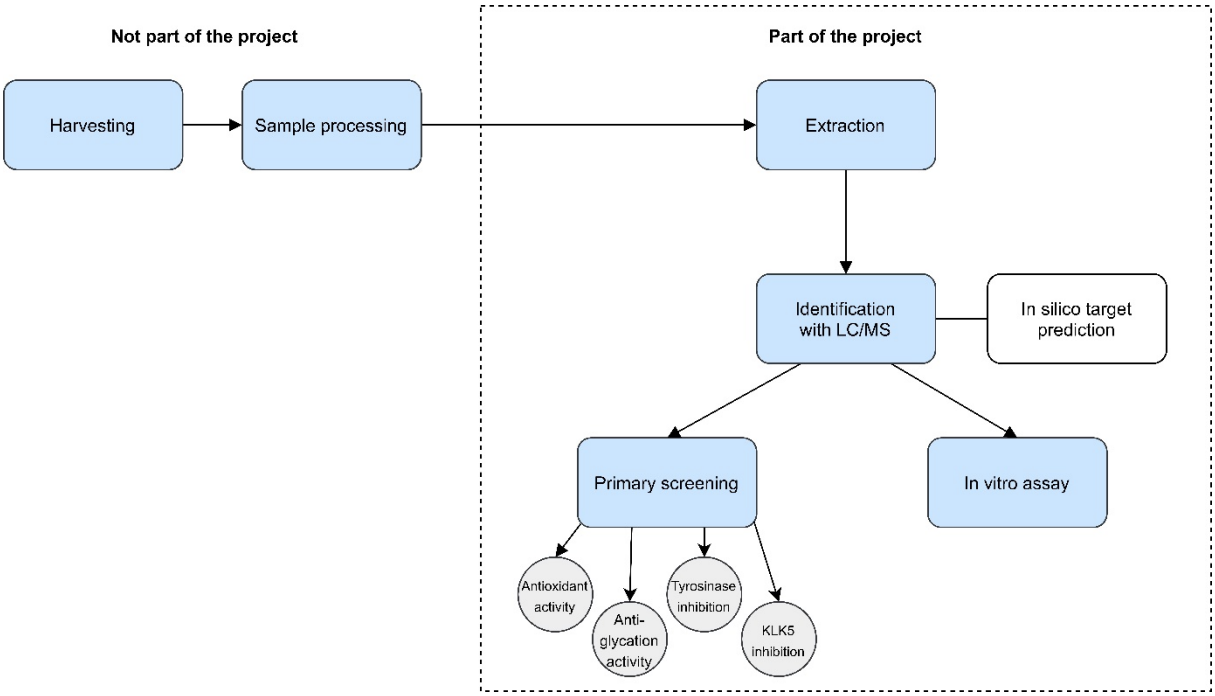


Figure 6. Overview of the analytical steps from harvesting and processing (which is done by the PPF before sent to Oriflame) to extraction, identification, primary screening assays, in-vitro assay and in-silico evaluation which are all done as a part of the project.

3.1.1 Freeze Drying

In this project, the samples investigated are freeze dried multiple times to dry and remove all water to make it possible to dissolve the samples in other liquids, like methanol, which is essential for extraction. When freeze drying the samples, the samples first freeze and then the ice is subliming which means it converts into vapor directly and is removed (Mellor, Bell 2003). The freeze dryers used were a Labconco 4.5 Plus FreeZone and a VirTis Benchtop K and the freeze drying was performed as following. The freeze dryer was turned on and approaching a collector temperature of -50 ºC. In the meantime, the tubes containing the samples or extracts were covered with two layers of parafilm and then, using a small needle, 15-20 small holes were made so that the water could leave the tubes. When the system had reached the desired temperature, the tubes were placed in the system and the vacuum was turned on, decreasing the pressure to 0.45 mbar. The system was running for three days until all water was removed, then the vacuum and the freezer were turned off, and then the whole system. A valve was opened to slowly let air enter the system increasing the pressure back to normal and the tubes, now containing a powder, were taken out and stored in a -20 ºC freezer.

3.1.2 Extraction

For extraction of the secondary plant metabolites from the plant cells, methanol extraction was used. Methanol is known to extract most of the content and is a fitting extraction solvent for a primary investigation of the samples. Figure 7 shows an overview of the extraction method used.

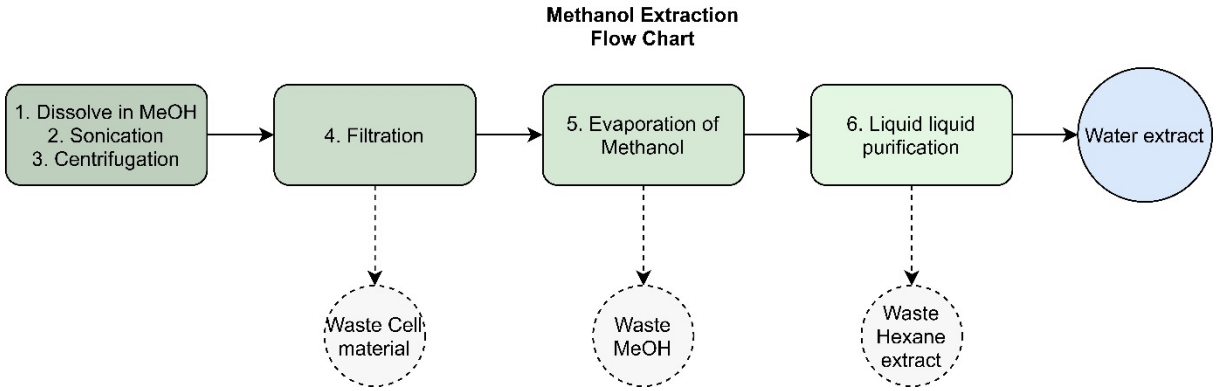


Figure 7. Overview of the steps in the methanol extraction process, showing six laboratory steps as well as three waste lines and the desired water extract.

A total of 38 plant fractions were received from PPF, they are listed in table A1 in Appendix 1. The extraction of the secondary metabolites from these plants fractions was executed as following. The fractions were first freeze dried according to section 3.2.1. About 1-2 g of plant cells of each fraction was weighed and placed in separate 40 mL falcon tubes. 20 mL methanol was then added per gram cells before sonication in ultra-sound bath Fisher scientific, Branson 5510 for 120 min. After sonication, the tubes were centrifuged for 5 minutes at 1000 rcf to spin down all large particles. The extracts were filtered using grade 4 upon grade 1 filter papers, a vacuum flask and a vacuum pump. The filtered extracts were then transferred into round-bottomed flasks before evaporation. Evaporation of the methanol was done using a rotavapor where the flasks containing the extracts were placed in 37 ºC water and the pressure was gradually decreased until it reached ~100 mbar. The rotavapor used was from Büchi Switzerland and included a vacuum pump, vacuum controller, heating bath and a rotavapor. This was done until the extracts were almost dry. After evaporation of methanol, 20 mL distilled water was added to each extract and they were put ultrasound bath for a few minutes to try to get as much extract as possible out into the water. The water extract was transferred to a separatory funnel. 6.5 mL Hexane was added to the round-bottomed flask to clean out the extract residues and then transferred to the same funnel. The solutions were carefully mixed and separated. The hexane extraction was repeated three times until purified. The hexane extracts, containing lipids, cellulose, chlorophyll and other cell components, were discarded, and the water extracts containing phytochemicals were put in new 40 mL purple falcon tubes. The extracts were finally freeze dried again under the same conditions as before. The final extract powders were stored in a -20 ºC freezer.

3.1.3 Identification

The compound contents of the extracts were identified using the equipment and method of Liquid Chromatography-Mass Spectrometry (LC/MS) or more specifically: ultra-high-performance liquid chromatography with quadrupole time-of-flight mass spectrometry (UHPLC-Q-ToF/MS). UHPLC is the first part and is a chromatography technique that separates the compounds in the extracts when passing via a mobile phase through a column with a stationary phase. Each compound will pass through the column in a specific time and give a unique chromatogram. What differs the UHPLC from the HPLC is that the pressure is significantly higher making it possible to use columns with smaller particle sizes resulting in faster analyses, higher resolution and higher separation efficiency of the compounds. (Nováková, Svoboda et al. 2017) After passing through the LC, the extracts reach the Q-

ToF/MS (quadrupole time-of-flight mass spectrometer) where the accurate mass-to-charge ratio (m/z) of each compound is determined which makes the technique reliable and greatly advantageous for compound identification. Q-ToF/MS uses an electrospray ionizing the molecules to then measure the time of flight. If the particles are negatively charged the electric field is adjusted to make the molecules travel to the right direction. (Allen, McWhinney 2019)

The following preparations were done for identification. First, the freeze-dried extracts were weighed separately into 1.5 mL Eppendorf tubes. They were then dissolved 10 mg/mL in H_2O/ACN 95:5. Some extracts were not soluble in H_2O/ACN 95:5 and were instead dissolved in H_2O/ACN 50:50 or in solution with even higher %ACN. Some that were not soluble at all in H_2O/ACN were dissolved in DMSO. If the extracts weren't soluble at 10 mg/mL they were diluted until dissolved. Table A2 in Appendix 1 shows the final concentration of each extract and what solution was used. They were vortexed and sonicated to ensure that the extracts were dissolved. Finally, the extracts were filtered through 1 µM filters into 2 mL vials. The solutions prepared was used for identification via LC/MS.

The identification was performed using the LC/MS system Agilent Technologies 1290 Infinity, 6520 Accurate-Mass Q-TOF LC/MS and gave chromatograms for each extract (section 4.1) as well as m/z value for each peak (compound). By comparing the m/z values from the LC/MS with values from pure compounds found in literature and through Reaxys, they were identified. The Reaxys system contains an enormous amount of information about different compounds: where they can be found (e.g. specific plants), what properties they have as well as known targets. Excel files containing information of what compounds might be present in the plants were downloaded containing compound name, molecular formula and molar weight. The molar weights from this file were then converted to m/z values using a website called Warwick. These were then compared to the m/z values from the files retrieved from the LC/MS system and thereby the peaks (compounds) were identified by names.

From the LC/MS analysis, the ponderate molecular weight for each extract was also retrieved and could then be used in the primary screening assays and the in vitro assay to calculate the mass (g) needed of each extract to receive a specific concentration (mol/L ponderate). The ponderate MW is a MW value that is an average value for the whole extract and is derived from the mentioned LC/MS system. It makes it possible to dissolve the extracts at the same concentration leading to the possibility of comparing them in the activity screening assays. In the rest of this report the molar ponderate will be referred to as M p (e.g. 1 mM p).

3.1.4 Primary Screening

After identification of the compounds the biological activities were examined through four different primary screening assays.

The first method looks for radical scavenging activity by 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) which will, in presence of antioxidant activity, react and the compound inhibition can be determined. DPPH is a free radical and thus it has an unpaired valence electron placed on one of the nitrogen atoms. Due to its purple colour, it absorbs light at a wavelength of 516 nm and after reaction it turns yellow resulting in no such absorbance which makes measuring the inhibition possible. (Chandra Shekhar, Anju 2014) In figure 8 the inhibitory reaction of DPPH is shown, turning DPPH into DPPHH which no longer is a radical.

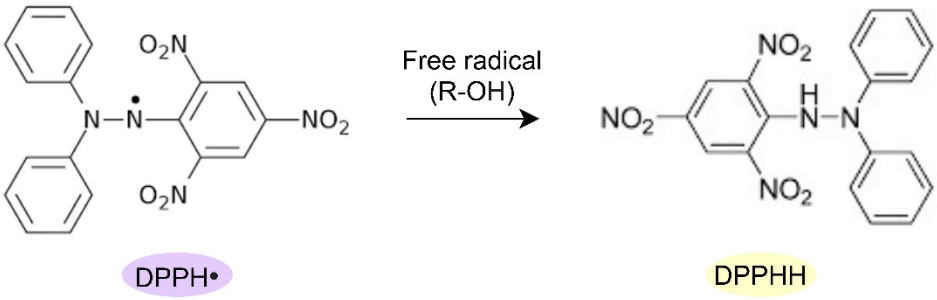


Figure 8. The figure shows the antioxidation reaction where DPPH transforms to DPPHH.

The second method used for primary screening is an anti-glycation assay where the extracts are tested for inhibitory characteristics of the glycation mechanism. The principle of this assay is that ribose will react with bovine serum albumin (BSA) to form AGEs and since it is fluorescence based, the spectrophotometer measures the fluorescence spectra of the formed AGEs. The absorbance will be lower if the extract acts inhibitory of this process. (Séro, Sanguinet et al. 2013)

The third method is a Tyrosinase inhibition assay that is an enzymatic assay screening for possible inhibitory effects on the enzyme tyrosinase. If a compound or an extract is inhibiting this enzyme and lowering the melanin production, they can be used in depigmentation approaches. (Momtaz, Lall et al. 2008)

The fourth and last method is a human tissue kallikrein-related peptidase 5 inhibition assay where the extracts are examined for inhibiting activity towards the enzyme KLK5. This method measures the enzymes ability to cleave the substrate Boc-VPR-AMC by measuring the absorbance of the fluorogenic group AMC. High absorbance is equivalent to the extract having low inhibition activity and low absorbance is equivalent to the extract having high inhibition activity.

The general procedure of the assays can be described using four steps (figure 9). They can be viewed more specifically in table 2.

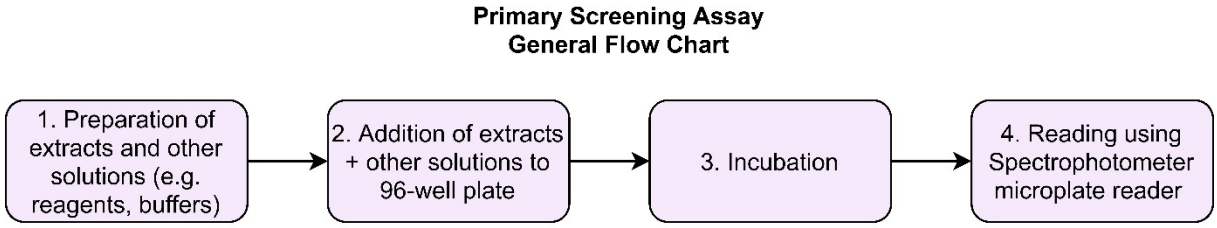


Figure 9. Overview of a general working process of the different primary screening assays. What differs between the assays are reagent preparation, solvent and concentration, volume added to the plates, incubation time and temperature as well as reading parameters.

Each screening method has unique reagents, substrates and solvents, incubation time and temperature as well as reading parameters. The concentrations of the extracts and the solvents used are also specific for each method. The material and solutions used as well as the preparation of the reagents can be viewed in table A3 and A4 in Appendix 1. The spectrophotometer used was a Spectrophotometer Microplate Reader - Cytation 3 – BioTek.

*Table 2. Four steps of primary screening for four different assays: radical scavenging activity, glycation inhibition, tyrosinase inhibition and KLK5 inhibition. These assays should be repeated three times on three different days. Preparation details for extracts, references and other solutions can be found in Appendix 1. *Room temperature*

Step	***Radical scavenging activity***	***Glycation Inhibition***	***Tyrosinase Inhibition***	*KLK5 Inhibition*
1. Preparation	-DPPH solution -Vitamin C stock solution 10000 µM -Vitamin C 100 µM -Extracts 2 mM p in methanol (1 mM p in plate)	-Sodium phosphate buffer 50 mM, pH 7.4 -BSA 10 mg/mL -D-Ribose 0.5 M -Rutin stock solution 100 mM -Rutin solution 10 mM -Extracts 10 mM p in 10% DMSO (1 mM p in plate) -Or 10 mM p in Buffer	-Potassium phosphate buffer 50 mM -L-tyrosine 2 mM -Tyrosinase 333 units/mg solid -Kojic Acid 150 mM -Extracts 100 mM p in DMSO (1 mM p in plate)	-Sodium phosphate buffer 0.1 M pH 8 -Enzyme KLK5 1 ng/µL -Substrate Boc-VRP-AMC 100 µM -Control SBTI 100 µM -Extracts 100 mM p in DMSO (1 mM p in plate)
2. Sampling into 96-well plate	-100 µL of Blank or Standard or extract was added to each well of the plate. -100 µL of DPPH are added to each well of the plate.	-50 µl BSA 10 mg/ml to all wells -40 µl D-ribose 0.5 M or for blank wells: 40 µl buffer -10 µl extract or blank solution	-70 µL reference or Blank or 2.1 µL (100 mM) extract + 67.1 µL Buffer -30 µL tyrosinase or Blank *5 min incubation* -110 µL substrate (L-tyrosine 2 mM) *30 min incubation*	-1 µL blank (DMSO)/ control or extract -50 µL enzyme/buffer for the blank *5 min incubation + pre-reading* -50 µL substrate *15 min incubation + reading*
3. Incubation	30 min at RT*	24 h at 37 ºC	5 min + 30 min at RT	5 min + 15 min at RT
4. Reading parameters	Wavelength: 516 nm	Wavelength: λexc = 370 nm λem = 440 nm	Wavelength: 492 nm	Wavelength: λexc = 380 nm λem = 460 nm

3.1.4.1 Calculations

The radical scavenging by DPPH assay measures the absorbance at λ=516 nm and by using those values the %radical inhibition (or free radical scavenging activity) could be calculated.

$$\%Radical\ Inhibition = \frac{Absorbance\ Blank - Absorbance\ Extract}{Absorbance\ Blank} * 100 \quad (1)$$

The %Inhibition of glycation was calculated using the AGE fluorescence intensity (FI) from the emission and excitation values at λexc=370 nm; λem=440 nm, as following,

$$\%AGEs = \frac{FI\ extract - FI\ blank\ extract}{FI\ control - FIblank\ control} * 100 \quad (2)$$

$$\%Inhibition = 100 - \%AGEs \quad (3)$$

The %Inhibition of the tyrosinase activity was calculated using the absorbance at λ=492 nm and the following equations:

$$\%Inhibition = \frac{Absorbance\ of\ max\ activity - Absorbance\ of\ extract}{Absorbance\ of\ max\ activity} * 100 \quad (4)$$

Where the average absorbance of the blanks was removed from the absorbance of max activity and extract in advance.

The %Inhibition of KLK5 was calculated using the AMC fluorescence intensity (FI) from the emission and excitation values at λexc=380 nm; λem=460 nm pre- and post-reaction, as following,

$$\%Inhibition = \frac{FI\ Max\ Activity - FI\ extract}{FI\ Max\ Activity} * 100 \quad (5)$$

Before calculating, the FI from the pre-reading was subtracted from the final FI for each sample. Then an average FI from the background was subtracted from each sample, see table A40 in Appendix 2 for full calculation.

3.1.4.2 IC50 Evaluation

For all four assays, if an extract shows high activity, they can be further analysed at different concentrations to calculate the inhibitory concentration for which the extract gives 50% inhibition of the enzyme, IC50 value. After knowing the IC50 value it is easier to determine whether the extract is a good inhibitor or not as well as comparing and ranking the performances in the assay. The four assays have two limits each to easier see if the activity is good, medium or not good, table 3 shows the limits used.

Table 3. Guidelines for screening results to evaluate the level of activity for the extracts in each of the four primary screening assays.

Assay	*%Inhibition (below this value, the compound is not considered to be a good inhibitor)*	*%Inhibition (between these values, the compound is a medium inhibitor)*	*%Inhibition (above this value, the compound is a good inhibitor and an IC50 can be determine as a second step)*
DPPH inhibition	30%	30-60%	60%
Glycation inhibition	10%	10-40%	40%
Tyrosinase inhibition	20%	20-60%	60%
KLK5 inhibition	20%	20-60%	60%

An estimation of the IC50 value can, as mentioned, be determined if an extract is considered a good inhibitor. Then the assay would be performed as in table 2 but instead of using the extract concentration described, around six different concentration is used for the extract. That is done to be able to create a curve where the IC50 value can be calculated from.

To calculate the IC50 value the software GraphPad was used. The logarithmic value of the concentration log[C] was plotted on the x-axis against the %inhibition on the y-axis. A non-linear regression was then created to find the log[C] where the inhibition was 50%.

3.1.5 In-Vitro Assay

To examine the extracts protective properties against pollution, a pollution induced CYP1A1 gene expression assay was performed. When CYP1A1 is expressed in human skin it can result in cornification of follicles that later can lead to chloracne outbreak. (Fabbrocini, Kaya et al. 2015) In this experiment diesel particulate matter (DPM) acted as pollution and was added to cultured keratinocytes together with the extracts of interest. The gene expression was then measured from cDNA using quantitative PCR (qPCR). Figure 10 shows an overview of the main steps of the assay and each step includes more specific steps described below. This experiment was only performed on four extracts due to limited access to the labs after the outbreak of SARS-CoV-2.

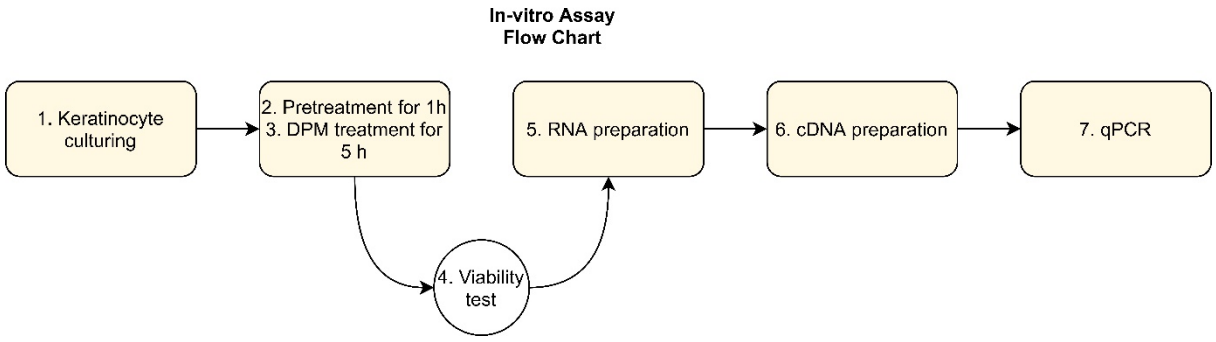


Figure 10. Overview of the main steps of pollution induced CYP1A1 gene expression experiment.

First, keratinocyte cells were cultured into a 48-well plate. Unfortunately, the cells on half of the plates did not survive, therefore only half of two plates were used, see figures 11 and 12. For each extract and each concentration, four replicates were prepared, two for the gene expression experiment and two for the viability experiment as can be seen in figures 11 and 12.

The wells used for compounds and control were pretreated with compound 1 hour prior to DPM treatment. DPM and vehicles were then added, and the cells were incubated for 5h at 37 ºC. After 5h the media was removed, and the wells were washed with PBS. 300 µL RLT buffer was added to the cells prepared for gene expression to lyse them. The lysate was then transferred to RNase free 1.5 mL Eppendorf tubes and put at -20 for RNA/qPCR analysis to be carried out another day.

Gene expression		Viability					
UT/Vehicle	UT/Vehicle	UT/Vehicle	UT/Vehicle				
DPM	DPM	DPM	DPM				
Kale brown juice 100 µM	Kale brown juice 100 µM	Kale brown juice 100 µM	Kale brown juice 100 µM				
Kale green juice 10 µM	Kale green juice 10 µM	Kale green juice 10 µM	Kale green juice 10 µM				
Lucerne white protein 100 µM	Lucerne white protein 100 µM	Lucerne white protein 100 µM	Lucerne white protein 100 µM				
Lucerne white protein 10 µM	Lucerne white protein 10 µM	Lucerne white protein 10 µM	Lucerne white protein 10 µM				

Figure 11. Lay-out for the first 48-well plate where the right half of the cells are dead. The two columns to the left are used for gene expression and each extract is done in duplicates. The two columns to the right are used for viability experiment and are done in duplicates. UT/Vehicle = not treated with DPM, DPM = treated with DPM but no extract is added, the rest are the two extracts Kale brown juice and Lucerne white protein that are added in two concentrations 100 µM and 10 µM.

Gene expression		Viability					
UT/Vehicle	UT/Vehicle	UT/Vehicle	UT/Vehicle				
DPM	DPM	DPM	DPM				
Lucerne Leaves 100 µM	Lucerne Leaves 100 µM	Lucerne Leaves 100 µM	Lucerne Leaves 100 µM				
Lucerne Leaves 10 µM	Lucerne Leaves 10 µM	Lucerne Leaves 10 µM	Lucerne Leaves 10 µM				
Quercetin 10 µM (control)	Quercetin 10 µM (control)	Quercetin 10 µM (control)	Quercetin 10 µM (control)				
Sugar beet fiber 10 µM	Sugar beet fiber 10 µM	Sugar beet fiber 10 µM	Sugar beet fiber 10 µM				

Figure 12. Lay-out for the second 48-well plate where the right half of the cells are dead. The two columns to the left are used for gene expression and each extract is done in duplicates. The two columns to the right are used for viability experiment and are done in duplicates. UT/Vehicle = not treated with DPM, DPM = treated with DPM but no extract is added, the rest are the extract Lucerne leaves that is added in two concentrations 100 µM and 10 µM, sugar beet fiber that is added in one concentration 10 µM due to lack of space, and the control Quercetin that is added in the concentration 10 µM..

Before continuing to RNA extraction, the cell viability test was performed (the same day as cell treatment) with a Cell Titer-Glo kit, see table A3 in Appendix 1. This was done to calculate the viability of the cells. The cells needed to have > 80% viability to be usable for the experiment. The media in the rest of the wells (the ones for viability) were first replaced with 100 µL PBS and 100 µL Cell Titer-Glo reagent that were mixed to induce cell lysis. The cells were incubated at room temperature for 10 minutes and then transferred to a 96-well plate before reading (200 µL of each well). The luminescent signal was measured using a spectrophotometer microplate reader.

RNA synthesis was done for the cells that had been exposed to the highest concentration of extract given they had viability > 80%. The cells were taken out from the freezer and 300 µL 70% ethanol was added to each tube and the cell suspension was carefully mixed before transferred to spin columns placed in 2 mL collection tubes. The tubes were centrifuged for 15 s at 18000 x g. The collected media was discarded and 350 µL RW1 buffer was added to remove biomolecules (e.g. carbohydrates, fatty acids and proteins). The tubes were centrifuged again at the same conditions, discarded and repeated once more. Then 500 µL RPE buffer was added to remove salts, the tubes were spun as before but for two minutes and the media discarded, this was also repeated once more. To make sure all liquid was gone, the spin column was placed in a new 1.5 mL Eppendorf tube and spun for 1 min. The Eppendorf tube was discarded, and the column placed in a new one. This time, 30 µL RNase-free water was added directly to the column membrane, it was centrifuged for 1 min and now the RNA had left the membrane and was placed in the Eppendorf tube. The column was discarded, and the Eppendorf tube saved and marked. Using Nanodrop the concentration of RNA was estimated, 2 µL of each sample was measured at absorbance ratio 260 nm/280 nm. This was done to see that the concentration was high enough to proceed to the next step.

In order to look at the CYP1A1 gene expression the RNA had to be converted to cDNA. From each sample of RNA two new samples were prepared by doing the following steps in duplicates. In a PCR plate, 15 µL RNA was transferred from each sample, 4 µL 5x iScript advanced reaction mix and 1 µL iScript advanced reverse transcriptase were then added to each well and mixed. The plate was sealed using a sticky film and then run through the qPCR. The reversed transcription in the qPCR ran at 42 ºC for 30 minutes and the procedure ended with inactivation at 85 ºC for 5 minutes.

After cDNA synthesis a BIORAD CFX Connect Real-Time qPCR System was used to monitor the DNA via fluorescent signals. qPCR enables real time monitoring while the DNA amplifies until it reaches a threshold where a Ct value is given and the lower amount of DNA, the higher the Ct value since it takes longer until the threshold is reached. In the last step, each sample was duplicated and for the two samples (same origin), two different mixes were prepared, one using the CYP1A1 primers to find the Ct value for that gene expression and one using primers for the housekeeping gene GAPDH. They were prepared in a PCR plate but first, the cDNA was diluted 1:2 in RNase free water. Then it proceeded as following: 10 µL of SYBR green mix (containing DNA polymerase required for reaction and fluorescent dye) was added to all used wells, 5 µL diluted cDNA was added to the corresponding wells, 1 µL primer (CYP1A1 or GAPDH) was added as well as 4 µL RNase free water before mixing. The qPCR plate was placed in the qPCR which was run as following: 95 ºC for 2 minutes for enzyme activation, then 40 cycles 95 ºC for 10 seconds and 60 ºC for 30 seconds.

3.1.5.1 Calculations

The fold change was calculated in several steps. First the Ct values were calculated for each gene replicate. The Ct value represents the number of cycles required for the fluorescent signal to cross the threshold representing the background level. Then the Ct value for the reference gene (GAPDH) was compared to the Ct of the target gene (CYP1A1) by calculating the difference, ΔCt. The Ct values and the ΔCt values were calculated for both treated and untreated cells so that the difference between the two ΔCt could be calculated, ΔΔCt. The fold change was then finally calculated by assuming that in each cycle, the transcript was doubled.

$$\Delta Ct_1 = Ct(CYP1A1_{treated} - GAPDH_{treated}) \quad (6)$$

$$\Delta Ct_2 = Ct(CYP1A1_{untreated} - GAPDH_{untreated}) \quad (7)$$

$$\Delta\Delta Ct = \Delta Ct_1 - \Delta Ct_2 \quad (8)$$

$$Fold\ change = 2^{-\Delta\Delta Ct} \quad (9)$$

3.1.6 In-Silico Target Prediction

Reaxys (https://www.reaxys.com/#/search/quick) is a digital platform with information about plants, substances and targets. For instance, if it is desired to know what compounds are present in kale that have a molecular weight between x and y and have biological activity in human or any other characteristics that might be of interest, Reaxys database have the tools to get results. The same way it can be used to find targets for a specific compound, e.g human target. In this project the Reaxys system was used to predict human targets for the compounds identified in the extracts and thereby predict possible activities and targets for the whole extract.

After a list of identified compounds had been made in section 3.2.3, that information could be used to look for interesting targets in human skin. Each compound was screened through Reaxys, limiting the data to activity of >3 pX[1] and human target only an excel file containing physical data and data of drug likeness (such as Lipinski's variables) was downloaded and compared to another file more specific to target. The second file was prepared by opening a so-called Heat map where all human targets were listed together with pX value, after downloading this file as well a tidy table was created with the following information:

- Plant species and chemical name of the compound
- Molar weight, log P, H-bond acceptor, H-bond donor and rotable bonds
- Target uniport ID
- Type of action
- Value and unit of activity

Analysing the target uniport ID for each target in the website "the human protein atlas" (https://www.proteinatlas.org/), the target acronym could be identified as well as the level of

[1] The value of pX is defined as a measurement of activity for the substrate to each target. The activity data that Reaxys uses is taken from literature and might therefore be described differently for different targets and substrates. The pX is therefore a way of comparing these activities between the targets and can therefore not be defined with a unit.

protein expression in the three different human skin cells: fibroblasts, keratinocytes and melanocytes. These results were then added to the tidy table resulting in a list of what target each compound is predicted to have and how active it is in the skin. Depending on what information that is desired, more specific parameters can be chosen such as the substance action on target (e.g. agonist, antagonist, inhibitor, stimulator etc), bioassay category (e.g in vitro, metabolism), type of activity (e.g. inhibition, IC50, EC50) or target species (e.g. human, mouse, mango).

Chapter 4: Results

This chapter includes the results of the identification of compounds, results of the activity from the primary screening assays as well as target prediction from the database study.

4.1 Identification

Results from LC/MS analysis show that there are interesting compounds found in the different fractions of each plant and some are found in more than one. In table 4 the compounds are shown as C1-C76 and figure 13 shows an example of what a chromatogram can look like. Each peak represents a compound present in the extract and the intensity of the peak (the area) is a measurement of the quantity of the compound. The latter was not calculated or used in this project.

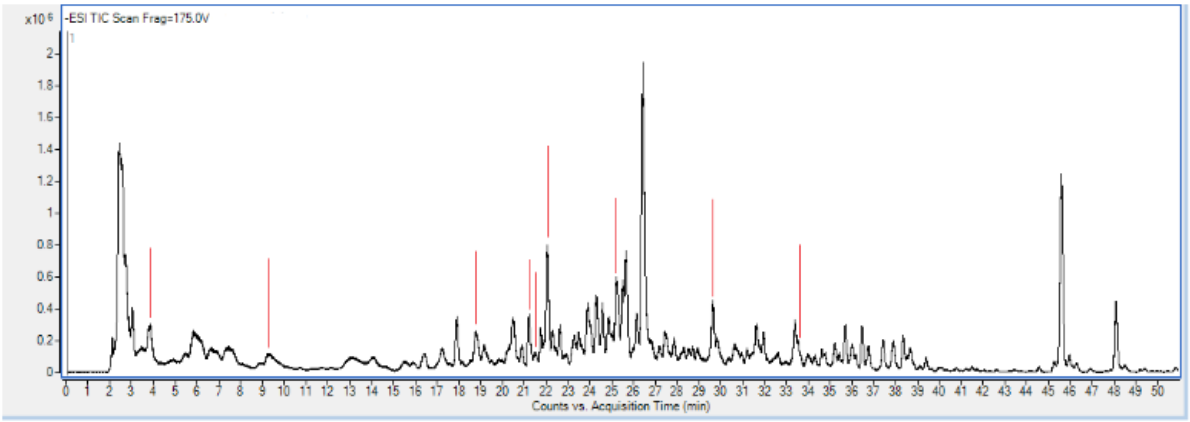
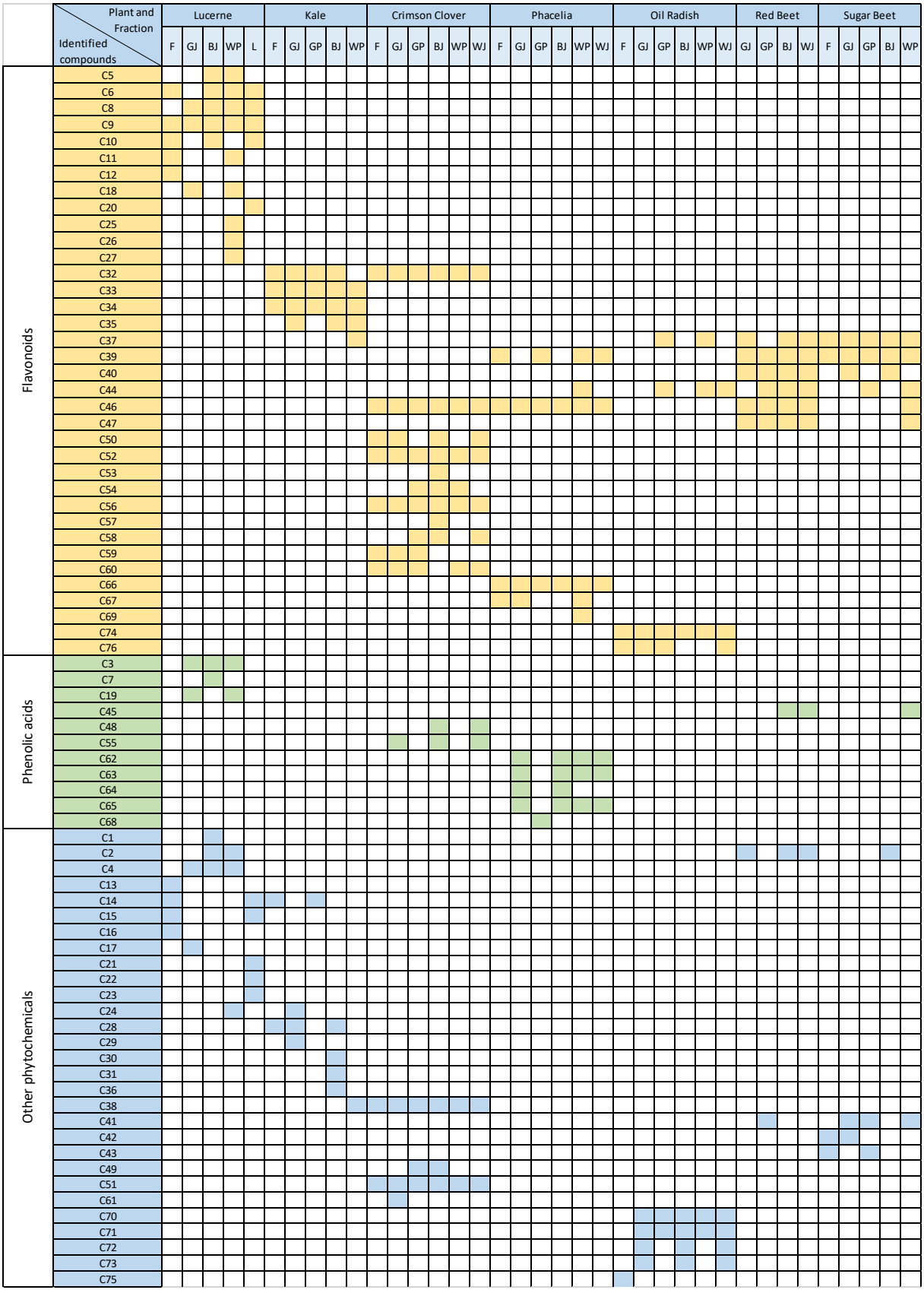


Figure 13. The figure shows a chromatogram of one of the extracts analyzed. This is an example of the results retrieved from LC/MS analysis. Some of the peaks are identified by mass and compound name.

The results in table 4 show that the composition in the fractions of the same plant are very similar whereas the plants show very different compositions compared to each other. Sugar beet and Red beet are the same species and have very similar composition. The compounds identified, C1-C76, were categorized into the different subgroups of polyphenols: flavonoids, phenolic acids and other phytochemicals, according to section 2.5. The matrix shows that every plant material is composed of flavonoids, the yellow boxes. Lucerne, Crimson clover and Phacelia are also composed of phenolic acids, green boxes, where Phacelia had most phenolic acids and then Sugar beet and Red beet included one phenolic acid for only a few fractions. Oil radish did not include any phenolic acids in the fractions. The blue boxes represent other phytochemicals and include for instance carbohydrates, lipids and terpenoids. These compounds were present in most plant material except for Phacelia.

Table 4. Matrix of compounds identified and where they are present. The compounds are named C1-C76 but represent actual compounds. F=Fiber, GJ=Green juice, GP =Green protein, BJ=Brown juice, WP=White protein, WJ=White juice and L=Leaves.



4.2 Antioxidant Activity Investigated Using the DPPH Method

The extracts in graph a and b were analysed simultaneously and the extracts in graph c were analysed at another time. %Radical inhibition of Vitamin C (the control) was 80% in the first experiment and 78% in the second experiment which is above the limit, 70%, which makes the experiments valid.

60% Radical inhibition or more was seen in three extracts: Phacelia green protein, Crimson clover fiber and Oil radish white protein. Some extracts (Phacelia fiber, Crimson clover green protein, Crimson clover white protein, Oil radish green protein and Red beet green protein, Red beet brown juice, Red beet white juice, Sugar beet brown juice, Lucerne white protein, Lucerne brown juice, Kale brown juice, Sugar beet green protein, Sugar beet green juice and Lucerne leaves) had medium radical inhibition (between 30-60%). The rest of the extracts had lower than 30% Radical inhibition or no radical inhibition at all. Figure 14 shows the average results for the assay, note that negative values do not imply activation of radical scavenging, but they imply there is no activity in the extracts.

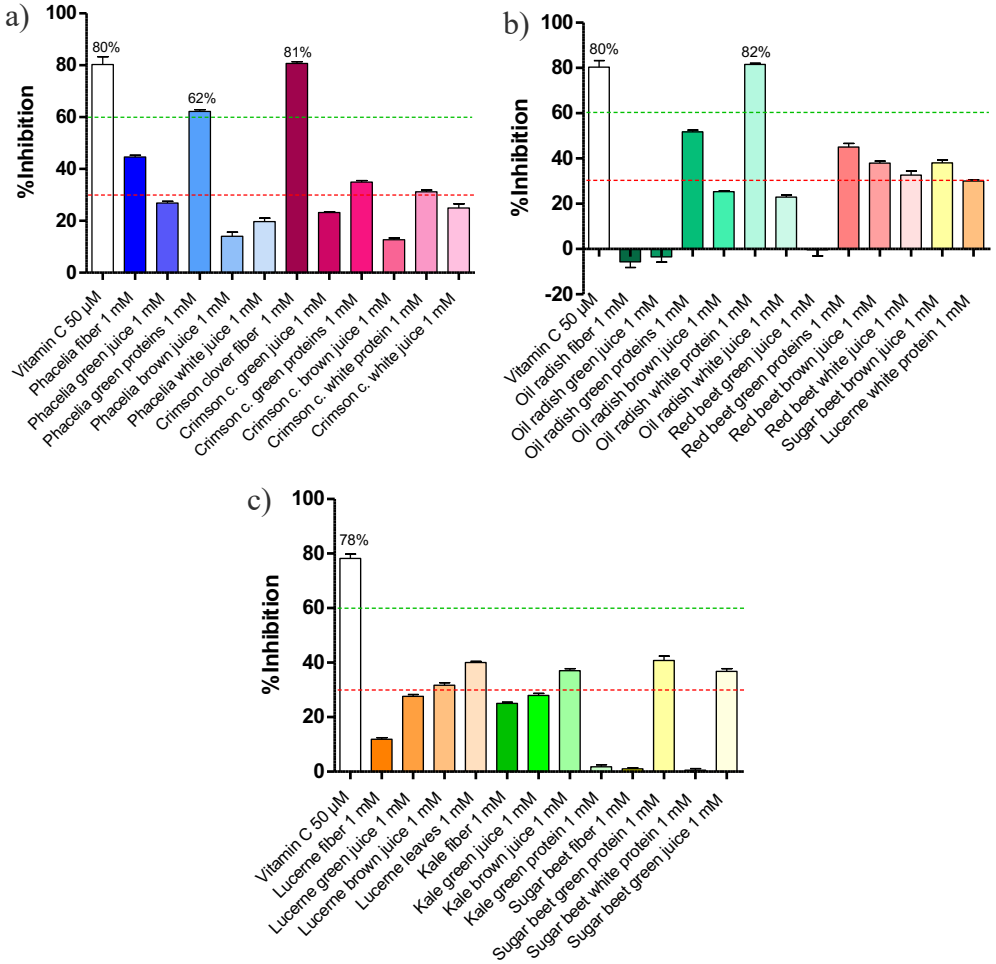


Figure 14. The three graphs a, b and c show the %radical inhibition for 35 extracts from the DPPH method. The values represent mean+/- SD. Each experiment was done n=3 times and each extract in the experiments was done in triplicates. Vitamin C, which is represented to the left in white in all three graphs, is the control. The red dashed line represents the 30% inhibition which is the lower limit for inhibitive effect and the green dashed line represents the 60% inhibition which is the lower limit for good inhibitive effect.

There were high activities for both Crimson clover fiber (80%) and for Oil radish white protein (80%) and the decision was made to continue analyzing the antioxidant activities of these two extracts with IC50. Although there was not enough extract to perform the assay on Oil radish white protein and more of it will have to be extracted for it to be done and therefore only IC50 was determined for Crimson clover fiber. The IC50 value for crimson clover fiber was calculated to 0.34 mM p. In this method a low value is what you strive for since a lower amount would be needed for higher inhibition. Figure 15 shows the IC50 curve along with the calculated IC50 value.

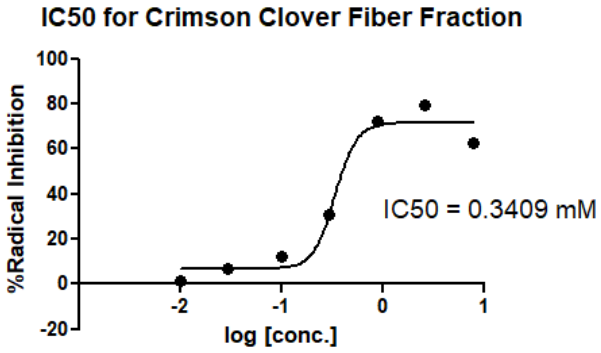


Figure 15. The graph shows the IC50 curve for the Crimson clover fiber extract. The IC50 value is calculated by plotting the logarithmic concentration against the %Radical inhibition and the value is the concentration where there is 50% inhibition.

4.3 Anti-Glycation Activity Using the Fluorescence of BSA-Ribose Reaction Products

The graphs in figure 16 show the percentage inhibition from the extracts from the experiments. Rutin 1 mM was used as a control for the assay and had 92%, 92% and 89% inhibition in the respective plates (1, 2 and 3) that are higher than 84% required for the validity of the assay. The anti-glycation assay shows that Kale brown juice, Lucerne white protein, Sugar beet brown juice, Crimson Clover fiber, Oil radish green juice, Oil radish white juice, Red beet brown juice and Lucerne leaves extracts had good effect on glycation inhibition. Phacelia fiber and Kale fiber had no effect on glycation inhibition and the rest of the extracts had medium effect on glycation inhibition. This is shown in figure 16.

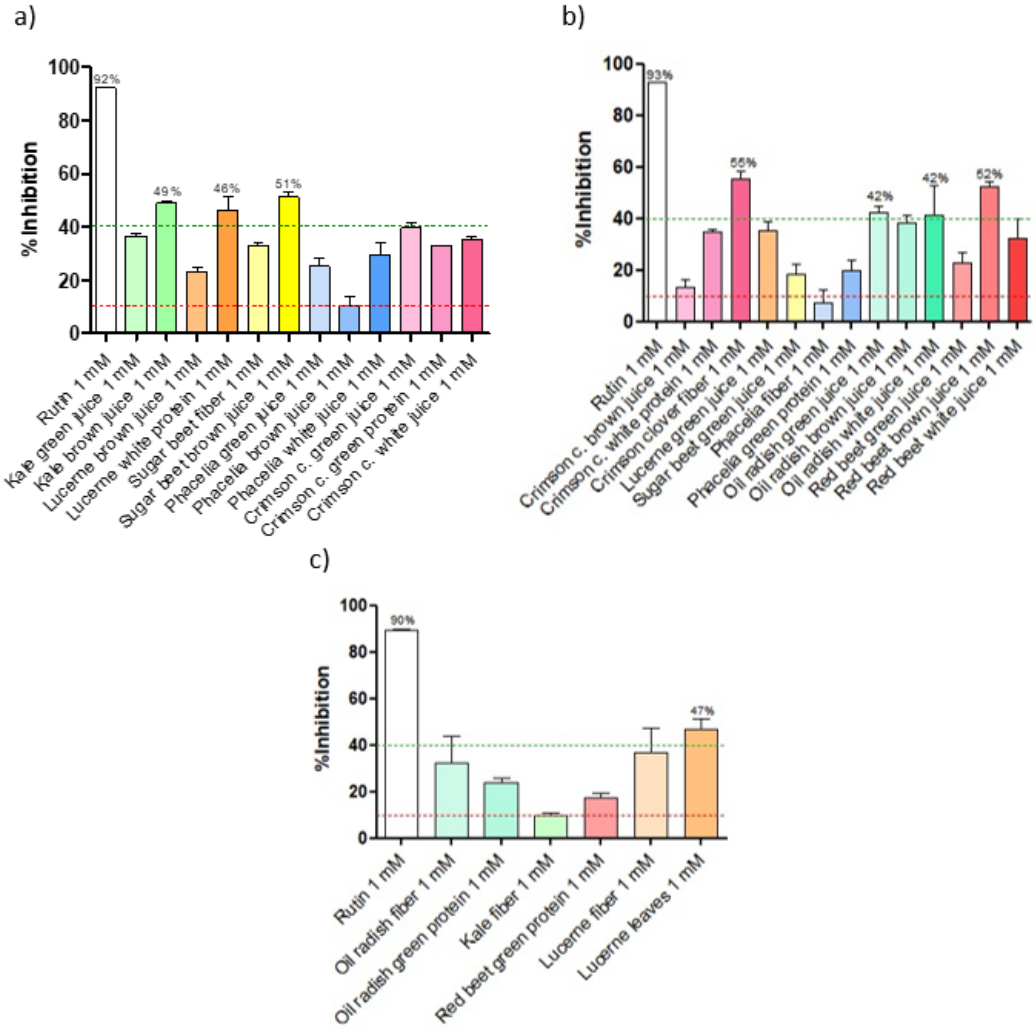


Figure 16. The three graphs a, b and c show the percentage inhibition of glycation for 31 extracts determined using the glycation inhibition method. The values represent mean+/- SD. Each extract in the experiment was done in triplicates. Rutin, which is represented to the left in white in all three graphs, is the control. The red dashed line represents the 10% inhibition which is the lower limit for inhibitive effect and the green dashed line represents the 40% inhibition which is the lower limit for good inhibitive effect.

The next step would be to examine the IC50 analysis of the extracts with %inhibition above 40%. As seen in the graphs, the %inhibition is around 50% for all eight extracts at 1 mM p which would mean that higher concentrations would need to be analyzed for the IC50. This could be difficult since the solutions were prepared 100 mM p in DMSO and higher concentrations could be hard to achieve due to small amount of extracts and difficulties to dissolve in higher concentration. This also indicates that the IC50 for the extracts are around 1 mM p.

4.4 Depigmentation Measured Using a Tyrosinase Inhibition Assay

The graphs in figure 17 show the percentage inhibition of Kojic acid and the extracts from the experiment. Kojic acid 156 µM was used as a control for the assay and had 92% inhibition that is higher than 90% required for the validity of the assay. Phacelia green juice extract had medium anti-tyrosinase activity with 45% inhibition. The rest of the extracts had no anti-tyrosinase activity. Again, note that negative values do not imply activation of the enzyme, but

they imply there is no activity in the extracts. Due to the lack of activity in these extracts the decision was made not to repeat the experiment n=3 times which normally is required for secure results. No IC50 analysis was performed since no extracts showed high activity.

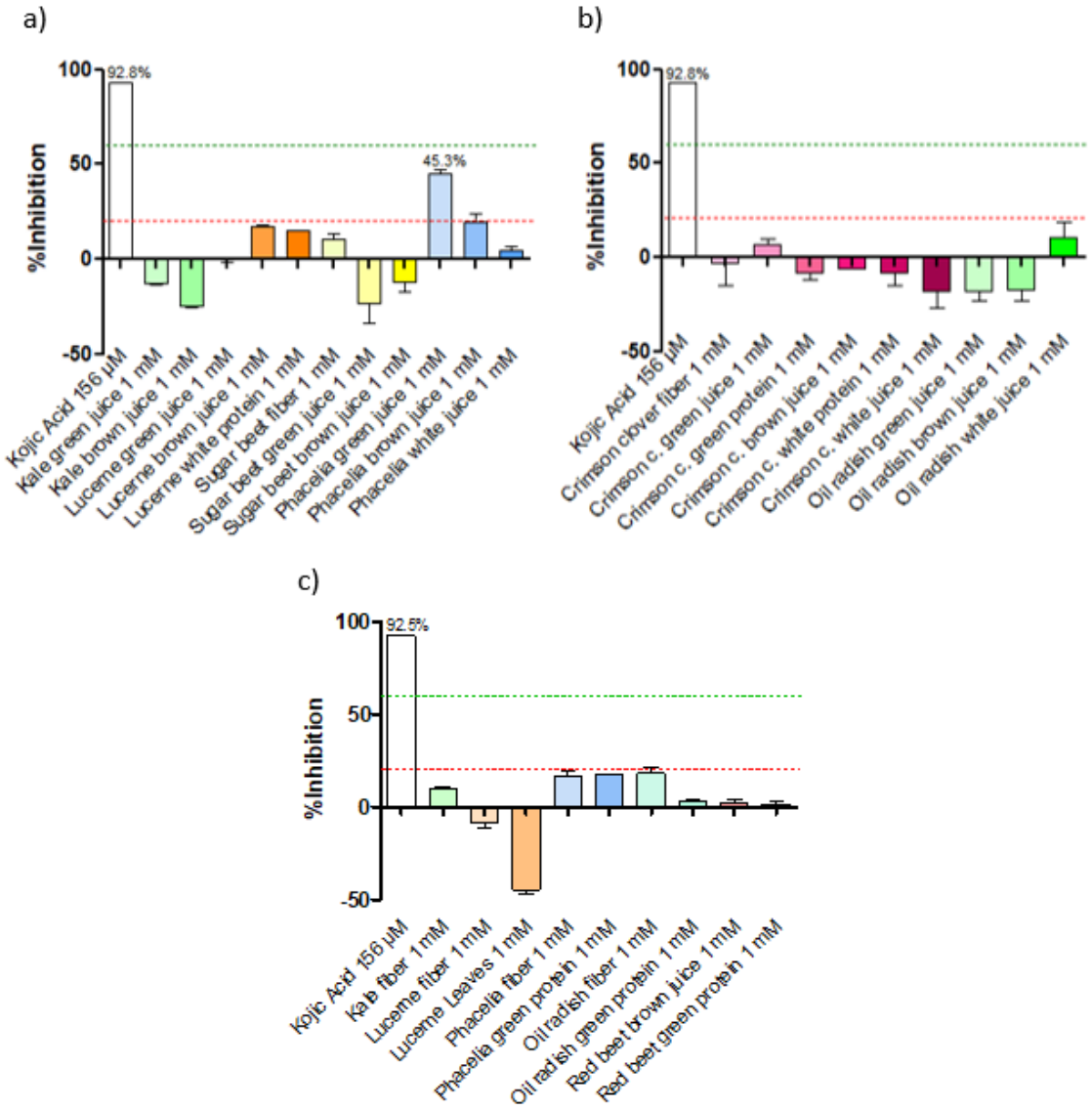


Figure 17. The graphs a, b and c show the percentage inhibition of the enzyme Tyrosinase for 29 extracts determined using the depigmentation assay. Graphs a and b represent plate 1 and graph c represent plate 2. The values represent mean+/- SD. Each extract in the experiment was done in triplicates. Kojic Acid, which is represented to the left in white in all three graphs, is the control. The red dashed line represents the 20% inhibition which is the lower limit for inhibitive effect and the green dashed line represents the 60% inhibition which is the lower limit for good inhibitive effect.

4.5 Kallikrein-Related Peptidase 5 Inhibition Assay

The graphs in figure 18 show the percentage inhibition of the SBTI and the extracts from the experiment. SBTI 1 µM was used as a control for the assay and had 99% inhibition that is higher than 80% required for the validity of the assay. Three extracts, Sugar beet brown juice, Lucerne fiber and Lucerne leaves had medium inhibition activity on KLK5. Many of the extracts had negative result as shown in the figure, note that this does not imply activation of

the enzyme, but they imply there is no activity in the extracts. As for the tyrosinase inhibition assay, the decision was made not to repeat the experiment n=3. No IC50 analysis was performed since no extracts showed high activity. Although, Lucerne fiber had relatively high activity and it could be interesting to do an IC50 on that extract.

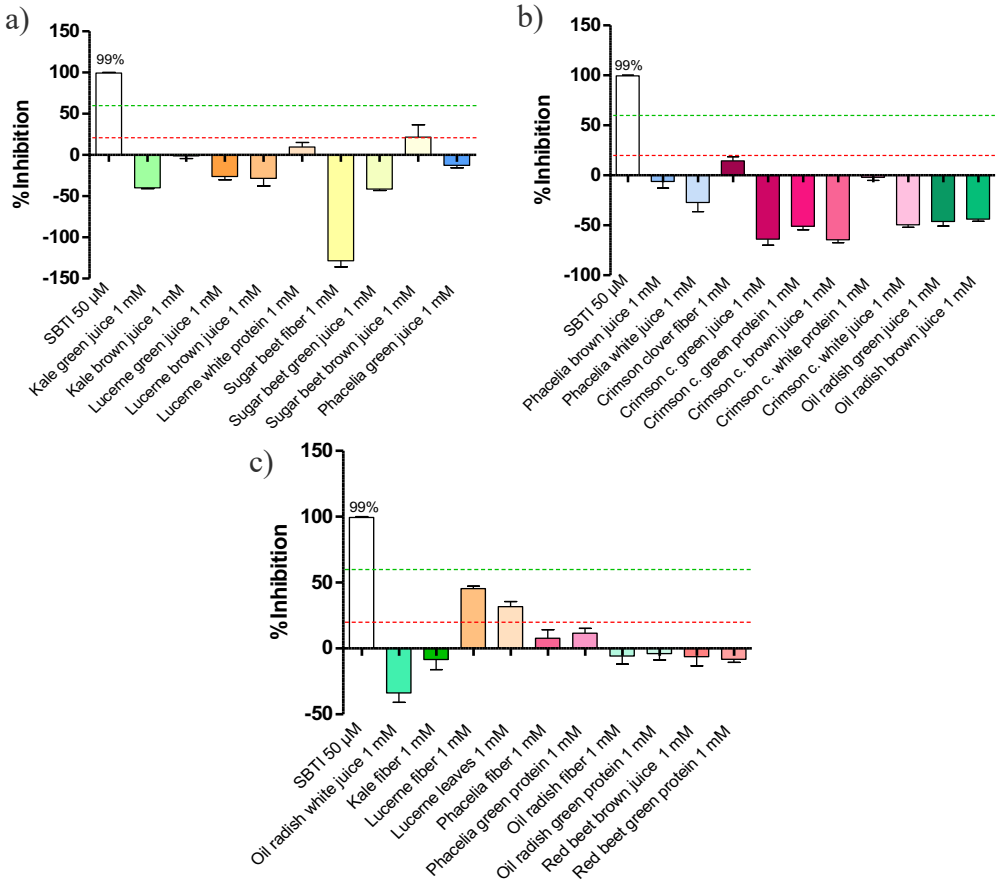


Figure 18. The three graphs a, b and c show the percentage inhibition of KLK5 for 29 extracts determined using the KLK% inhibition assay. The values represent mean+/- SD. Each extract in the experiment was done in triplicates. SBTI, which is represented to the left in white in all three graphs, is the control. The red dashed line represents the 20% inhibition which is the lower limit for inhibitive effect and the green dashed line represents the 60% inhibition which is the lower limit for good inhibitive effect.

4.6 Pollution Induced CYP1A1 Gene Expression

The viability test showed positive results, see figure 19. According to these results the highest concentration (100 µM) was chosen for RNA expression analysis for Kale green juice and Lucerne white protein. For Sugar beet fiber, only one concentration was used (10 µM) and therefore used for RNA analysis. The lowest concentration (10 µM) was chosen for Lucerne leaves since the higher one (100 µM) was below the accepted limit of 80%.

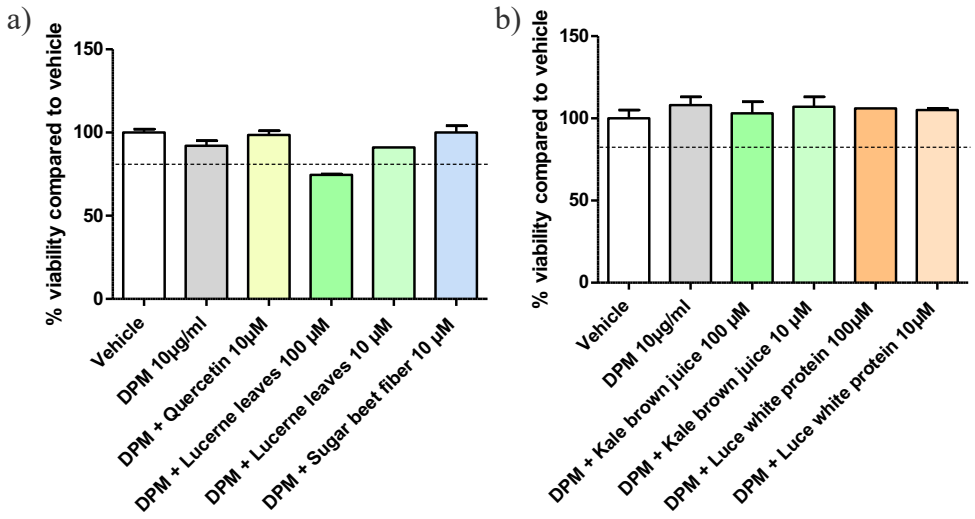


Figure 19. The diagrams show the % viability of the cells after 6 hours treatment with DPM and extracts. The dashed lines represent the lowest accepted % viability which is 80%.

Figure 20 shows the two graphs representing the gene expression in the cells for the different media. Vehicle did not have DPM or extract present and therefore the gene expression is low representing the normal CYP1A1 gene expression of the cells (untreated). DPM treatment of the cells significantly up regulated the gene expression of CYP1A1 on both plates (grey bars). The control compound Quercetin significantly downregulated the DPM induced gene expression back to the original level (yellow bars). Lucerne white protein extract also significantly downregulated the DPM induced CYP1A1 gene expression back to the original level (orange bar). The Kale brown juice and Sugar beet fiber extracts downregulated the gene expression, but not as significantly as Lucerne white protein (green and blue bars in graph b). The Lucerne leaves extract did not downregulate the DPM induced gene expression (green bar in graph a). Downregulation is desired for this assay since that implies the extracts protect the cells from DPM and prohibit the expression of the CYP1A1 gene.

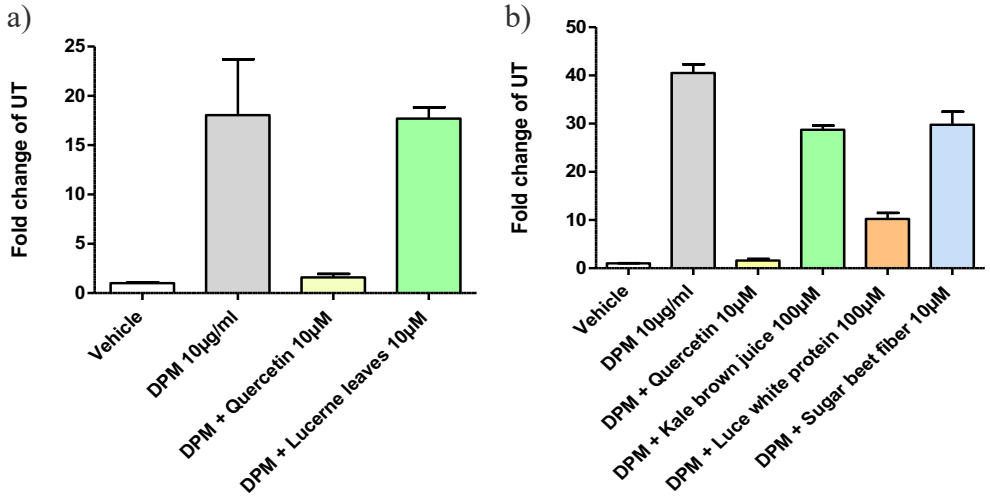


Figure 20. Graph a and b show the DPM induced CYP1A1 gene expression of the cells after 6 hours treatment with DPM and extracts. The white bars represent the vehicle where no DPM or extracts are added, the grey bars represent the DPM without extracts and therefore the gene expression is significantly upregulated compared to vehicle. The yellow bars are the control Quercetin that significantly downregulates the gene expression and validate the assay. The green, orange and blue bars represent the DPM + extracts.

4.7 Target Prediction

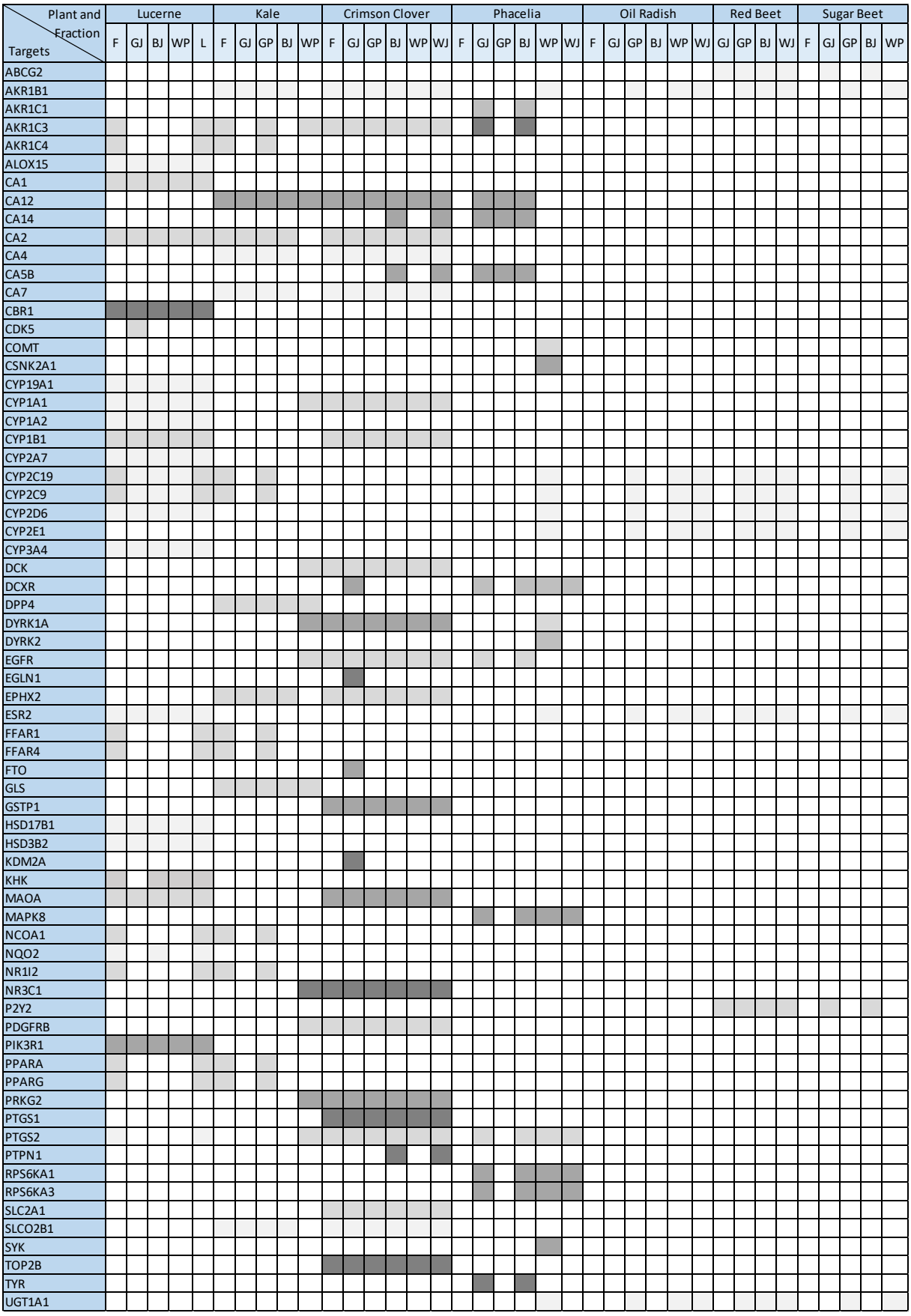
The target prediction via Reaxys gave results that can be found in table 5. In the table, the compounds (C1-C76), that were identified in 4.1, are matched to the targets that they were predicted to have activity on. The activities are ranked from low (light grey) to medium (grey) to high (dark grey) and only the 22 compounds that had targets in the skin are shown together with their corresponding phytochemical groups.

In table 6 the results from table 4 and 5 are compared resulting in a new table where the targets are identified for each extract. For Sugar beet, Red beet and Oil radish there were only a few targets identified and the skin expression was very low. This is seen by the lack of grey boxes to the right in the table. Sugar beet fiber, Phacelia fiber and Oil radish fiber, green juice and brown juice did not give any results. Kale matched some targets with low skin expression, Lucerne had similar results but also one target with medium skin expression and one with high skin expression. The rest of the Phacelia extracts matched some of the targets mainly with medium skin expression. The last crop, Crimson clover, had the strongest results where targets ranged from low to high skin expression and Crimson clover green juice stand out where it has seven targets with medium skin expression and five with high skin expression.

Table 5. Matrix of the compounds predicted to have biological activity. The x-axis shows the 22 compounds that are predicted to have activity and their corresponding phytochemical groups and the y-axis show the targets in the skin.

	Flavonoids												Phenolic acids				Other phytochemicals					
Compound / Target	C6	C9	C10	C20	C32	C33	C34	C40	C44	C50	C52	C69	C62	C63	C64	C68	C14	C15	C17	C38	C48	C61
ABCG2																						
AKR1B1																						
AKR1C1																						
AKR1C3																						
AKR1C4																						
ALOX15																						
CA1																						
CA12																						
CA14																						
CA2																						
CA4																						
CA5B																						
CA7																						
CBR1																						
CDK5																						
COMT																						
CSNK2A1																						
CYP19A1																						
CYP1A1																						
CYP1A2																						
CYP1B1																						
CYP2A7																						
CYP2C19																						
CYP2C9																						
CYP2D6																						
CYP2E1																						
CYP3A4																						
DCK																						
DCXR																						
DPP4																						
DYRK1A																						
DYRK2																						
EGFR																						
EGLN1																						
EPHX2																						
ESR2																						
FFAR1																						
FFAR4																						
FTO																						
GLS																						
GSTP1																						
HSD17B1																						
HSD3B2																						
KDM2A																						
KHK																						
MAOA																						
MAPK8																						
NCOA1																						
NQO2																						
NR1I2																						
NR3C1																						
P2Y2																						
PDGFRB																						
PIK3R1																						
PPARA																						
PPARG																						
PRKG2																						
PTGS1																						
PTGS2																						
PTPN1																						
RPS6KA1																						
RPS6KA3																						
SLC2A1																						
SLCO2B1																						
SYK																						
TOP2B																						
TYR																						
UGT1A1																						

Table 6. Matrix of the extracts that consist of compounds predicted to have biological activity. The x-axis shows the extracts that are predicted to have activity and the y-axis show the targets in the skin.



4.8 Summary of Results

The results from the primary screening assays are summarized in table 7 where the four investigated activities are marked for each fraction. The dark blue marks represent high activity, the light blue marks represent medium activity while the empty boxes represent no activity and the boxes with a line means these extracts weren't analyzed due to either lack of material or difficulties when dissolving. Inhibition of glycation was the most common activity among the extract and inhibition of tyrosinase and KLK5 were more rare activities found in only a few extracts. Lucerne leaves was the only extract showing three types of activities. Crimson clover fiber was the only extract showing high activity for more than one type. Kale fiber was the only extract lacking activity in all four categories. The results from the in vitro assay was not included since it was only performed on four extracts which is not enough to get an all covering picture.

Table 7. Summary of activity for each extract investigated from the primary screening assays. Dark blue mark = high activity, light blue mark = medium activity and empty box = no activity. The white protein fractions whit a line lacked material and Kale and Sugar beet green protein and Red beet green and white juice were unable to be dissolved and weren't analyzed.

Plant	Fraction	Antioxidant	Glycation inhibition	Tyrosinase inhibition	KLK5 inhibition
Lucrene	Fiber		✓		✓
	Green juice		✓		
	Brown juice	✓	✓		
	White protein	✓	✓		
	Leaves	✓	✓		✓
Kale	Fiber				
	Green juice		✓		
	Green protein		-	-	-
	Brown juice	✓	✓		
	White protein	-	-	-	-
Sugar beet	Fiber		✓		
	Green juice	✓	✓		
	Green protein	✓	-	-	-
	Brown juice		✓		✓
	White protein		-	-	-
Phacelia	Fiber	✓			
	Green juice		✓	✓	
	Green protein	✓	✓		
	Brown juice		✓		
	White protein	-	-	-	-
	White juice		✓		
Crimson clover	Fiber	✓	✓		
	Green juice		✓		
	Green protein	✓	✓		
	Brown juice		✓		
	White protein	✓	✓		
	White juice		✓		
Oilradish	Fiber		✓		
	Green juice		✓		
	Green protein	✓	✓		
	Brown juice		✓		
	White protein	✓	-	-	-
	White juice		✓		
Redbeet	Green juice		✓	-	-
	Green protein	✓	✓		
	Brown juice		✓		
	White protein	-	-	-	-
	White juice		✓	-	-

The graphs in figure 21 and 22 represent how many of each fraction showed activity for antioxidation and antiglycation. This was done to try to find any correlations between the fraction type and the activities. The dark grey bars represent the amount of extracts showing activity for each fraction type and the light grey bars represent the total amount of extracts examined for each fraction type.

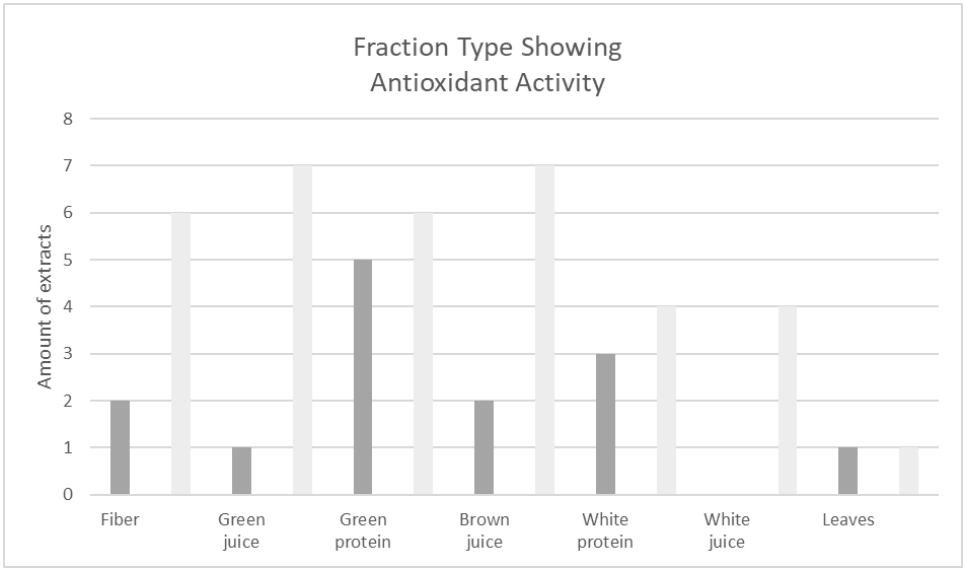


Figure 21. Seven different fraction types examined for antioxidant activity where the total amount of extracts analyzed are shown as light grey and the extracts with medium or high activity are shown as dark grey.

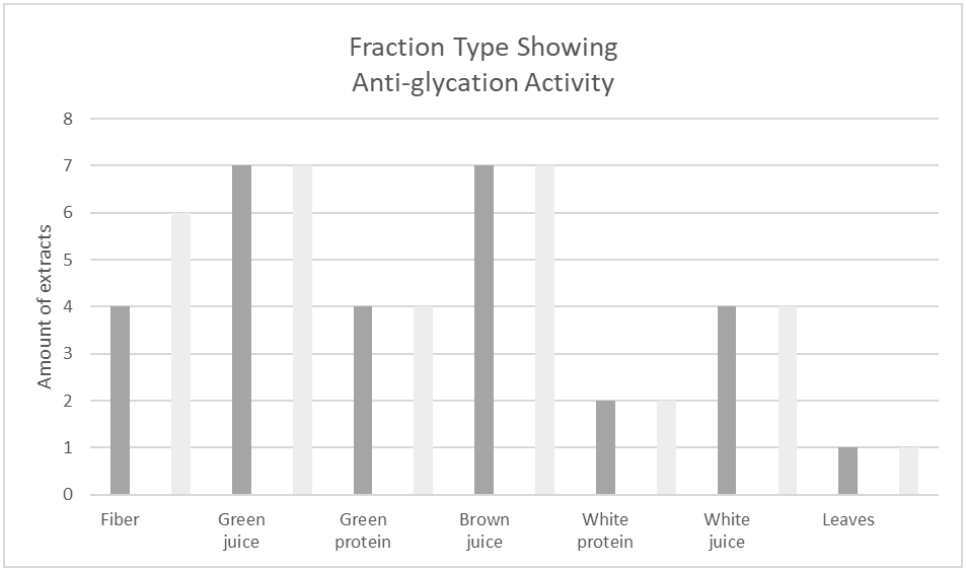


Figure 22. Seven different fraction types examined for antiglycation activity where the total amount of extracts analyzed are shown as light grey and the extracts with medium or high activity are shown as dark grey.

Chapter 5: Discussion

In this chapter, the results are interpreted and discussed, and their reliability, based on performance and error factors, is evaluated. The overall method of the project is assessed, and future aspects are discussed.

5.1 Phytochemical Content and Biological Activity

Looking at the compounds identified in the plant fractions, there is a clear pattern of the contents. The contents are very different between the plant species pointing out how unique the composition of phytochemicals is. It is also easily seen that the fractions within one species have similar compositions, and that is not surprising since they are from the same plants. However, it is interesting to see that there is a slight difference that indicates that different compounds ended up in different fractions which might be the cause that different fractions from the same crop showed different levels of activity in the primary screening assays. The compound compositions in Sugar beet and Red beet are as mentioned in section 4.1 very similar which might be since they are the same species and only different cultivar groups. Overall, looking at the phytochemical content of the fractions within each plant they are not significantly different and therefore it cannot be stated that fractionating the plant material split the phytochemicals in a certain way or that it will benefit the process of finding active ingredients.

There were three extracts that showed high antioxidant activity from the radical scavenging experiment, no extract had high activity for tyrosinase or KLK5 inhibition but eight had high activity for inhibition of the glycation mechanism. One extract showed high activity for both antioxidation and glycation inhibition: Crimson clover fiber that is composed of substances predicted to target 21 targets. All Crimson clover extracts showed promising activity for glycation inhibition and half of them for antioxidation. Some extracts lacked material or were insoluble in the solvents used (section 4.8) this means that all extracts were not analyzed for all assays. However, ten of the extracts showed high activity for at least one of the four categories. These ten extracts are therefore extra interesting in the search for active ingredients: Lucerne white protein and leaves, Kale brown juice, Sugar beet brown juice, Phacelia green protein, Crimson clover fiber, Oil radish green juice, white protein and white juice and Red beet brown juice. Looking at the seven plants, at least one extract from each of them showed some type of high activity and Oil radish was the plant that had most positive results, namely three extracts with activity higher than average, two for antiglycation and one for antioxidation and might therefore be interesting to further investigate.

The IC50 of Crimson clover fiber was calculated to 0.34 mM p and to evaluate this result it can be compared to Green tea extract which is an extract considered an excellent antioxidant. Green tea extract has an IC50 of around 0.1 mM which has been estimated in the SRI laboratory. The IC50 of Crimson clover fiber is more than three times higher indicating that to get the same antioxidant effect as for Green tea, three times more extract would be needed in the formulation. Depending on the cost for raw material and extraction of the two extracts, it can be discussed whether Crimson clover fiber is still a potential active ingredient for antioxidative purposes or not. It can also be discussed that the story behind the extract's origin, that it is retrieved from wasted plant material grown in Sweden and thereby supporting a sustainable environment, could benefit and compensate for the fact that the IC50 value is higher. This cannot be fully examined without looking further into the cost of large-scale production as well as comparing costs for antioxidants currently in use.

The in-vitro pollution induced CYP1A1 gene expression experiment was performed on four extracts where one of them showed promising results. Lucerne white protein significantly down regulated the DPM induced CYP1A1 gene expression and continuing the examination of its protective properties could lead to it being used as an active pollution protector in a skin care product.

5.1.1 Patterns Between Activity and Fraction Type

In section 4.8 the relationship between fraction type and activity for both antioxidation and antiglycation was examined and summed up in two graphs. In the examination the medium and high activities were used (light and dark blue marks). For antioxidation, there was no fraction type that was active for all included extracts except for leaves but that fraction was only existing for one plant type. For green protein and white protein 5/6 and 3/4 extracts showed activity respectively and for the rest of the fraction types less than half of the extracts showed activity, among the white juices no extract showed activity and among the green juices one showed activity.

For antiglycation, all extracts except two showed activity (two fiber extracts) and therefore it is not possible to really compare the different fraction types except that they are all showing high levels of activity. For the fiber fractions 4/6 extracts showed activity even though this is the fraction type with fewest extracts showing activity, it still has more than half showing activity.

5.1.2 Connection Between Compounds and Activity

The connection between the compounds identified and the activities examined in the primary screening experiments were examined for each of the four activities. The comparison was made for the higher activities (the dark blue marks in table 7) for antioxidation and antiglycation and for the medium activities (light blue marks in table 7) for tyrosinase and KLK5 inhibition.

Phacelia green juice was the only extract that showed activity for Tyrosinase inhibition, but it does not have any compound that other extracts do not and therefore the activity cannot be related to a specific compound.

Sugar beet brown juice, Lucerne fiber and leaves that had activity for KLK5 inhibition do not have any specific compound or compounds in common that could be responsible for the activity. Lucerne fiber and leaves have some compounds in common, flavonoids and other phytochemicals but no that other extracts of Lucerne or other plants do not have. Therefore, there is no clear connection between any specific compounds and the activity of KLK5 inhibition either.

5.2 Human Targets

The in-silico prediction of targets gave interesting results that made it possible to see which compounds of C1 to C76 that might have biological activity on human skin and it also gave indications on which plants and which fractions that are of interest. There were 68 identified targets and most of them are targeted by multiple substances, such as CA12 that is targeted by six compounds present in totally 14 extracts and all with medium skin expression. The results from the in-silico study were negative for three of the plant materials as mentioned in section 4.7, Sugar beet, Red beet and Oil radish. The three plants are therefore not as interesting for

further studies using these targets. However, it is interesting that Oil radish gave promising results for the primary screening assays and then three of the extracts showed no results at all in the in-silico study. This could be a result of some errors in the identification where maybe all compounds were not identified and therefore the compounds showing activity in the assays were not found in-silico. Instead Crimson clover was the crop that was more interesting and matched targets with quite high skin expression. This makes Crimson clover more interesting than Oil radish in this aspect. Phacelia was close behind Crimson clover and the results can be valuable for further studies. When the targets are more detailly identified and their effects known, these results will give helpful indications on what these plant materials can be used for. When looking at target prediction it is not very specific for the fractions but instead very similar within the specific plants and can therefore specify which plants are more interesting, leaving out the fractions to be examined and compared to each other in the assays.

5.3 Reliability of Results

The metabolites were extracted using methanol but there are many other solvents that can be used for extraction and depending on what solvent is used, different types and amounts of compounds might be extracted. Choosing a different extraction method could therefore result in different results for identification but also for activity screening. In order to find the optimal extraction solvent, different solvents need to be used and the assays performed need to be performed for the extracts and then the results can be compared between the extracts originating from the same plants and fractions. Therefore, the results for each assay is limited to the method of extraction and can only be interpreted with that in mind.

The identification method of the compounds is very reliable since the method used is approved by SRI and used by them continuously even though all compounds could not be identified, and some remain unknown. This will lead to some lacking information about the content and as a result to that, the in-silico evaluation is not fully complete.

The primary screening assays have also been approved and when followed accurately, they should be reliable. For each assay a control was used to see if the test was valid or not, the value for the control had to exceed a certain value (stated in table 3), this was the case for each experiment and from that aspect the experiments are valid and reliable. The methods also say to repeat the same experiment three times on three different days and retrieve a result based on data from all three of them. Since the lab access was limited in the later part of the project period, repeated experiments were not performed for all assays. Glycation inhibition, tyrosinase inhibition and KLK5 inhibition assays were only performed once and the results are not as reliable as if they could be completed with two more trials. Two more trials for each of the three assays must therefore be performed in order to state that the level of activity is proven. The antioxidant activity assay was the only assay performed three times and is therefore the only assay with results that can be said to be reliable and proven. Similar statements can be said for the in-vitro test where the assay should be repeated with different keratinocyte donors as well as with different concentrations of the extracts to be able to give reliable and accurate results.

The in-silico evaluation was based on prediction of targets in the human skin for the compounds identified. How reliable it is, is hard to know and the results from this evaluation is more meant to be a guide into further analysis. The results are meant to give an indication of what assays should be performed, which plants and fractions that should have higher priority or are of

greater interest in the area of skin care. Using these results, unnecessary experiments can hopefully be avoided and finding an active ingredient that is useful might be easier.

5.4 Evaluation of Method

Overall, the method used to find new active ingredients has been working as wanted and has successfully generated results. It is an excellent step by step procedure that investigates the crops potential as active ingredients in multiple ways. The secondary metabolites were extracted, and their content identified using a relatively fast method. The identification procedure where the results from the LC/MS were compared to the lists retrieved from Reaxys was done manually and might therefore be less reliable than if it was done automatically. On the other hand, the list where the two files were compared and matched was double-checked again using the LC/MS instrument.

The activity screening methods covered the most important areas of activity desired for skin care. The assays rely on manual preparation of the extracts and other reagents using micro pipettes, ultrasound baths and vortex. The micro pipettes used were calibrated in the beginning of the year and should therefore not contribute to any errors. The homogeneity of the samples after mixing is approved by the human eye and could therefore fail to detect smaller undissolved particles leading to inaccurate concentration of extract able to react inhibitory in the assays and give faulty results. By repeating the assays three times these errors are minimised and should not be a big concern.

5.5 Future Aspects

This project can be improved and continued. Firstly, the extraction method can be optimized by exploring different extraction solvents other than methanol, such as alkalic solvents. Secondly, extraction yields can be compared as well as looking at the phytochemicals present. Optimizing this method would mean repeating the extraction for the samples multiple times which would require time and material but could lead to better results in the end. The primary screening assays can also be repeated for stronger results as mentioned in section 5.2, and IC50 experiments can be performed on the extracts showing higher than average activity to establish the activity is something to further investigate. Other primary activity assays screening for collagenase inhibition, elastase inhibition, hyaluronidase inhibition and SIRT 1 activation and inhibition can also be performed to increase the possibility if finding biologically active extracts.

Further investigation of the targets (e.g. what they do and how the substances would affect them) can result in a great guide for choosing the next step in the analysis as well as for knowing what extracts that should be further investigated. To know how active a substance is on a predicted target and how it affects the aging of the skin, specific laboratory procedures must be designed to be able to test the activity on the skin cells in question. Before that, it is only possible to know that there is a potential activity. Also, the actual amount of the compound within the extract is not known and to get a more precise prediction on the activity of the extract, that must be examined. To better understand this prediction and not only see that there might be activity on the skin, the targets could be further investigated through research to find the true activity. That could also help rank the targets to see which are more interesting to create a product for.

By quantifying the compounds within the extracts, they can bring more information for the in-silico evaluation as well as making it possible to examine if the plant materials can be used as resources for a specific compound of interest. Some compounds might already have been investigated for the purpose or even used in existing products today and finding them in these extracts could lead to them being a new resource for that material. This could contribute to a more sustainable solution as well as be economically advantageous.

The process of finding an active ingredient suitable for being in a skin care product is long and screening for biological activity is an early stage of that process. Steps 1-5 in section 3.2 briefly describe this process of finding active ingredients and after primary screening there are two more steps: in-vitro assays where the activities found in step 4 are further investigated using skin cell-based assays such as anti-pollution, UV-protection and anti-inflammatory properties, and ex-vivo assays where the activities are confirmed using donated skin. When an extract or compound has gone through all these steps and has positive results and desired characteristics a formulation of a product (e.g. cream, serum or priming essence) can be created using the found substance as an active ingredient.

Chapter 6: Conclusion

The aim of this project to investigate if extracts from various sample fractions produced from agricultural waste material can be used as active ingredients in skin care products. The secondary metabolites were extracted from the agricultural waste material using methanol extraction which resulted in 37 extracts that were analysed for their contents that were identified using LC/MS system and Reaxys. The extracts were also investigated for biological activities using four primary screening methods and an in-vitro assay was used to look for anti-pollution properties for four extracts. Apart from this, human targets were found using an in-silico evaluation method that can be used to further investigate the extracts. This summarizes the events leading to fulfilling the aim and additional objectives of this project.

The results from this research are not enough to state that the extracts will provide protection of the skin from pollutants. More examination must be done in order to know the exact protective properties of them. However, the plant materials did not possess any outstanding activities and in the current situation, with the current knowledge, not many extracts show suitable as active ingredients. Nevertheless, it cannot be excluded that an outstanding result for one or more of the extracts might be found using these assays and the extracts could possibly be suitable as active ingredients. However, Lucerne white protein is highly potential to be used for anti-pollution approaches but will have to be further investigated. Crimson clover fiber could potentially act as an antioxidant and/or glycation inhibitor since it showed high activity for both assays. The rest of the Crimson clover extracts are also interesting as a result of the in-silico evaluation where they matched multiple targets with both medium and high skin expression.

In other words, there is a possibility for some of these extracts having biological activities valuable enough for them being used as active ingredients in skin care products. However, more research is needed to fully know which extracts and for what purpose. The method can be used to investigate the activity of other plant extracts to broaden the research and increase the chances of finding a new active ingredient for skin care.

No relationships were found between the identified compounds and the activities from the primary screenings. Likewise, no relationships were found between the fraction types and the activities from the primary screenings, but one could see that the phytochemical contents of the fractions within the same plants were very similar and therefore using different fractions might not be very beneficial.

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Appendices

Appendix 1: Material and Preparations

In this section, the fractions are listed in table A1 and the final concentration and solvent used for each extract for the LC/MS assay are listed in table A2. It also includes information about the material and chemicals used for the laboratory experiments of this project, in table A3 as well as preparation information of the reagents for the different assays in table A4.

Table A1. A complete list of the plants and fractions received from the PPF.

Plant	*Fraction*	*Plant*	*Fraction*
Lucerne	Fiber Green juice Brown juice Leaves White protein	*Crimson Clover*	Fiber Green juice Brown juice White juice Green protein White protein
Kale	Fiber Green juice Brown juice Green protein White protein	*Oil Radish*	Fiber Green juice Brown juice White juice Green protein White protein
Sugar Beet	Fiber Green juice Brown juice Green protein White protein	*Red Beet*	Green juice Brown juice White juice Green protein White protein
Phacelia	Fiber Green juice Brown juice White juice Green protein White protein		

*Table A2. Final concentration and solvent used for each extract for the LC/MS analysis. *After extraction there wasn't enough material to do an identification with LC/MS.*

Plant	*Fraction*	*Solvent*	*Concentration*
Lucerne	Fiber	H2O/ACN 30:70	5 mg/mL
	Green juice	H2O/ACN 95:5	10 mg/mL
	Brown juice	H2O/ACN 95:5	10 mg/mL
	Leaves	DMSO	8 mg/mL
	White protein	H2O/ACN 50:50	6 mg/mL
Kale	Fiber	H2O/ACN 25:75	5 mg/mL
	Green juice	H2O/ACN 95:5	10 mg/mL
	Brown juice	H2O/ACN 95:5	10 mg/mL
	Green protein	DMSO	8 mg/mL
	White protein	H2O/ACN 50:50	10 mg/mL
Sugar Beet	Fiber	H2O/ACN 31:69	5 mg/mL
	Green juice	H2O/ACN 95:5	10 mg/mL
	Brown juice	H2O/ACN 95:5	10 mg/mL
	Green protein	DMSO	5 mg/mL
	White protein	H2O/ACN 50:50	10 mg/mL
Phacelia	Fiber	H2O/ACN 25:75	10 mg/mL
	Green juice	H2O/ACN 95:5	10 mg/mL
	Brown juice	H2O/ACN 95:5	10 mg/mL
	White juice	H2O/ACN 95:5	10 mg/mL
	Green protein	DMSO	5 mg/mL
	White protein	H2O/ACN 50:50	5 mg/mL
Crimson Clover	Fiber	H2O/ACN 25:75	10 mg/mL
	Green juice	H2O/ACN 95:5	8 mg/mL
	Brown juice	H2O/ACN 95:5	10 mg/mL
	White juice	H2O/ACN 95:5	8 mg/mL
	Green protein	DMSO	10 mg/mL
	White protein	H2O/ACN 50:50	10 mg/mL
Oil Radish	Fiber	DMSO	8 mg/mL
	Green juice	H2O/ACN 95:5	10 mg/mL
	Brown juice	H2O/ACN 95:5	10 mg/mL
	White juice	H2O/ACN 95:5	10 mg/mL
	Green protein	DMSO	6 mg/mL
	White protein	H2O/ACN 50:50	5 mg/mL
Red Beet	Green juice	H2O/ACN 25:75	5 mg/mL
	Brown juice	H2O/ACN 95:5	10 mg/mL
	White juice	H2O/ACN 95:5	10 mg/mL
	Green protein	DMSO	6 mg/mL
	White protein	-*	-

Table A3. Material and chemicals used or prepared for each assay preformed.

Assay	*Material*	*Chemicals*
Methanol Extraction	• Free standing centrifuge tubes, 50 mL • Polypropylene conical tube, 50 mL (falcon tube) • Balance • Freeze dryer 1: Labconco 4.5 Plus FreeZone • Freeze dryer 2: VirTis, Benchtop K • Graduated cylinder • VWR Ultrasonic cleaner, USC-THD • Ultrasound bath - Fisher scientific, Branson 5510 • Eppendorf Centrifuge 5702 • Filter paper grade 1: Qualitative Circles, Cat No 1004-042 (Ø = 42.5 mm, pore size = 11 µm) • Filter paper grade 4: Qualitative Circles, Cat No 1004-042 (Ø = 42.5 mm, pore size = 20.25 µm) • Vacuum flask • Round-bottomed flask • Rotavapor - Buchi Switzerland • Separatory funnel • Fume hood • Disposable gloves	• Distilled water • Methanol – Sigma Aldrich – 34860N • Hexane
LC/MS	• 1,5 mL Eppendorf tubes • Micropipettes BIOHIT + tips Eppendorf • Vials • LC/MS - Agilent Technologies 1290 Infinity, 6520 Accurate-Mass Q-TOF LC/MS • Disposable gloves • Fume hood	• Distilled water • Ethanol • DMSO • H_2O/AcN 50/50
Radical Scavenging Activity by DPPH	• Balance • Beakers • 1,5 mL tubes Eppendorf • 96 wells plate - Costar 3595 - Corning Incorporated • Spectrophotometer Microplate Reader - Cytation 3 - BioTek • Ultrasound bath - Fisher scientific, Branson 5510 • Vortex - Genie2 - Scientific Industries • Micropipettes BIOHIT + tips Eppendorf • Disposable gloves	• Methanol – Sigma Aldrich – 34860N • DMSO Fisher Scientific • DPPH (2,2-diphenyl-1-pikryl-hydrazyl) – Aldrich cat. D913-2 • Ascorbic acid – Sigma Aldrich - 33034

Anti-Glycation	• 500 mL and 25 mL and 10 mL volumetric flask • Micropipettes BIOHIT + tips Eppendorf • 1.5 mL and 2 mL tubes Eppendorf 5 mL tubes • pH-meter (from plant stem cell lab) • Scale • Vortex - Genie2 - Scientific Industry • Ultrasound bath - Fisher scientific, Branson 5510 • 96 wells plate black + Aluminium cover • 37 ºC oven • Spectrophotometer microplate reader - Cytation 3 – BioTek • Disposable gloves	• Distilled water • DMSO - Fisher Scientific • Sodium phosphate • HCl (1M) • BSA - Bovine Serum Albumin – Sigma • D-Ribose – Aldrich • Rutin - Sigma
Tyrosinase inhibition	• Beakers • Glass pipettes • 10 mL and 50 mL volumetric flask • Micropipettes BIOHIT + tips Eppendorf • 1.5 mL, 2 mL and 5 mL tubes Eppendorf • Scale • Vortex - Genie2 - Scientific Industry • 96 wells plate • Spectrophotometer microplate reader - Cytation 3 – BioTek • Disposable gloves	• L-tyrosine - Sigma T3754-100G • Mushroom tyrosinase -Sigma T3824, Batch no: SLBM7158V (see details of units/mg solid attached) • Potassium phosphate monobasic - Sigma P8414-500G • DMSO Fisher Scientific • Kojic Acid – Sigma
Kalikrein 5 inhibition	• Beakers • Volumetric flasks 500ml • pH meter • Magnetic stirrer • 1.5 ml and 2 ml tubes Eppendorf • Micropipettes BIOHIT + tips Eppendorf • Vortex - Genie2 - Scientific Industry • 96-well plates black • Spectrophotometer Microplate Reader - Cytation 3 – Biotek • Disposable gloves	• Distilled water • DMSO Fisher Scientific • Sodium phosphate dibasic anhydrous - Fluka71636 • Rh Kallikrein 5 - R&D Systems - 1108-SE010 • Boc-VPR-AMC - R&D Systems - ES011 • SBTI - Trypsin inhibitor from Glycine max (Soybean) - Sigma - T6522-25MG
DMP induced CYP1A1 gene expression	• 48 well cell culture plates • Multichannel pipette • Sterile RNase-free pipet tips	• DPM • Vehicles • PBS -/- Mg2+ and Ca2+

	• Sterile RNase-free Eppendorf tubes 1,5 and 2 mL • Disposable gloves • 96-well plate • Spectrophotometer microplate reader - Cytation 3 – Biotek • Freezer -20 ºC • Laminar Air Flow cabinet • Fume hood • Centrifuge 10 000 g • Nanodrop • Micropipettes + tips Eppendorf • PCR Plate + seal • qPCR – BIORAD CFX Connect Real-Time System • Ice	• RLT buffer • RW1 buffer • RPE buffer • CellTiter-Glo Kit (including Buffer and Substrate) • 5x iScript advanced reaction mix (contains dNTPS, oligo(dt), random primers, buffer, MgCl2, enhancers and stabilizers) • iScript advanced reverse transcriptase (contain iScript MMLV-RT (RNaseH+) and RNase inhibitor) • RNase free water • Ethanol 70 % • SYBR green mix (containing DNA polymerase and fluorescent dye) • Housekeeping gene GAPDH primers • CYP1A1 primers

Table A4. Preparation description of all reagents and other solutions needed for each of the assays.

Assay	*Solution*	*Preparation*
Radical Scavenging Activity by DPPH	*DPPH*	m_{DPPH} = 7,9055 mg was first dissolved with help of sonication and vortex in a 1,5 mL tube and then poured in a 100 mL volumetric flask with MeOH and the volume was completed to 100 mL. The solution should be placed in the fridge and can be kept two weeks.
	Vitamin C	$m_{Ascorbic\ acid}$ = 1,7640 mg was dissolved in MeOH with help of sonication and vortex in a 1,5 mL tube (Vit C 10000 µM). 100 µL of that solution was then diluted with 900 µL MeOH with help of sonication and vortex in a new 1,5 mL tube (Vit C 1000 µM). 100 µL of the newly prepared solution was then diluted again with 900 µL MeOH with help of sonication and vortex in a new 1,5 mL tube (Vit C 100 µM). The solutions should be stored in freezer.
Anti-Glycation	*Buffer sodium phosphate (pH 7,4 at 50 mM)*	0,354 g was dissolved into ~400 mL H2O. The pH was adjusted to 7,405 with HCl 1M using a pH-meter. More H2O was added into the 500 mL volumetric flask until the total volume was 500 mL.
	BSA	10 mg/mL was prepared by dissolving 0,25044 g BSA in 25 mL Buffer. Aliquot 5 x 5 mL in 5 mL tube, stored in freezer.
	D-Ribose (0,5 M)	0,75031 g D-Ribose was dissolved in 10 mL Buffer. Aliquot 5 x 2 mL in 2 mL Eppendorf tube, stored in freezer.
	Rutin	Stock solution Rutin 100 mM: 61,18 mg was dissolve into 1000 µl DMSO in a 2 mL Eppendorf tube and vortexed for 30 sec then sonicated for 50 min Solution Rutin 10 mM: 100 µl stock solution Rutin 100 mM + 900 µl buffer *(This solution was newly prepared before each experiment)*
Tyrosinase inhibition	*Potassium Phosphate Buffer (50 mM)*	Preparation in EXP_018_GJ3505
	L-tyrosine	2 mM was prepared by dissolving 18,2424 mg in 50 mL Buffer. Aliquot 3 mL in 5 mL tubes, stored in freezer.
	Tyrosinase	The enzyme arrived from supplier as 2687 units/mg solid. Calculations: 333 units/mg solid * 1 / 2687 units/mg solid = 0,124 mg/mL --> 1,24 mg/10 mL 333 units/mL was prepared by dissolving 1,2345 mg in 10 mL Buffer. Aliquot 2 mL in 2 mL Eppendorf tubes, stored in freezer.

	Kojic Acid	Kojic Acid stock solution 150 mM was taken from freezer. Following ddilutions were newly prepared before each experiment the same day as reading. 23400 µM Kojic acid was prepared by diluting 15,6 µL of stock solution with 84,4 µL DMSO. 468 µM Kojic acid was finally prepared by diluting 20 µL of 23400 µM Kojic acid with 980 µL DMSO.
KLK5 inhibition	*Sodium Phosphate Buffer*	The buffer was already prepared.
	KLK5 enzyme	1 ng/µL was prepared by dissolving 18,13 µL of KLK5 enzyme (332ng/µl) in 6000 µL buffer in a 15 mL tube. Then mixed using Vortex.
	Boc-VPR-AMC	100 µM was prepared by dissolving 7,6 µL substrate (98,6 mM) from freezer in 7492,4 µL buffer in a 15 mL tube. Then mixed using Vortex.
	STBI	100 µM was prepared by dissolving 0,4258 mg STBI in 213 µL DMSO using vortex and ultrasound bath.
DMP induced CYP1A1 gene expression		All solutions used came in kits ready to use.

Appendix 2: Additional Data for Calculations and Results

In this section additional data is added to support calculations and results of the assays performed. Plate lay-outs, reading results and calculation tables are shown for each of the four primary screening assays as well as for the in-vitro assay.

Tables A5-A19 represent the radical scavenging experiments that were performed for Kale, Lucerne and Sugar beet (n=3 times) and then later for Crimson clover, Phacelia, Oil radish and Red beet (n=3 times). Table A5 shows the plate lay out for the three experiments done for Kale, Lucerne and Sugar beet, tables A6, A8 and A10 are absorption results retrieved from the spectrophotometer microplate reader and can be compared to the plate lay-out. Tables A7, A9 and A11 are calculations where the absorbances are converted to %inhibition using equation 1 in section 4.2. Table A12 shows the plate lay out for the three latter experiments done for Crimson clover, Phacelia, Oil radish and Red beet, tables A13, A15 and A17 are absorption results retrieved from the spectrophotometer microplate reader and can be compared to the plate lay-out. Tables A14, A16 and A18 are calculations where the absorbances are converted to %inhibition using equation 1 in section 4.2. Table A19 is a summary of the results for all seven plants (35 extracts) where the %inhibition is shown as an average from all three repeated experiments. Note that the plate lay outs also include the reagents added as well as their volumes (this goes for all plates in all the assays).

Table A5. Plate lay out of DPPH experiment for Kale, Lucerne and Sugar beet using methanol and DMSO as blanks and Vitamin C as reference. Three replicates of each sample were analyzed, and three replicates of the blanks and reference were also analyzed.

	1	2	3	4	5	6	7	8	9	10	11	12
A	100 µ MeOH + 100 µL DPPH	100 µ MeOH + 100 µL DPPH	100 µ MeOH + 100 µL DPPH	100 µL Kale green juice + 100 µL DPPH	100 µL Kale green juice + 100 µL DPPH	100 µL Kale green juice + 100 µL DPPH	100 µL Vit C (100 µM) + 100 µL DPPH	100 µL Vit C (100 µM) + 100 µL DPPH	100 µL Vit C (100 µM) + 100 µL DPPH			
B	2µL DMSO + 98 µL MeOH + 100 µL DPPH	2µL DMSO + 98 µL MeOH + 100 µL DPPH	2µL DMSO + 98 µL MeOH + 100 µL DPPH	100 µL Kale brown juice + 100 µL DPPH	100 µL Kale brown juice + 100 µL DPPH	100 µL Kale brown juice + 100 µL DPPH						
C	100 µL Lucerne fiber + 100 µL DPPH	100 µL Lucerne fiber + 100 µL DPPH	100 µL Lucerne fiber + 100 µL DPPH	2 µL Kale green protein in DMSO + 98 µL MeOH + 100 µL DPPH	2 µL Kale green protein in DMSO + 98 µL MeOH + 100 µL DPPH	2 µL Kale green protein in DMSO + 98 µL MeOH + 100 µL DPPH						
D	100 µL Lucerne green juice + 100 µL DPPH	100 µL Lucerne green juice + 100 µL DPPH	100 µL Lucerne green juice + 100 µL DPPH	100 µL Sugar beet fiber + 100 µL DPPH	100 µL Sugar beet fiber + 100 µL DPPH	100 µL Sugar beet fiber + 100 µL DPPH						
E	100 µL Lucerne brown juice + 100 µL DPPH	100 µL Lucerne brown juice + 100 µL DPPH	100 µL Lucerne brown juice + 100 µL DPPH	100 µL Sugar beet green protein + 100 µL DPPH	100 µL Sugar beet green protein + 100 µL DPPH	100 µL Sugar beet green protein + 100 µL DPPH						
F				100 µL Sugar beet white protein + 100 µL DPPH	100 µL Sugar beet white protein + 100 µL DPPH	100 µL Sugar beet white protein + 100 µL DPPH						
G	100 µL Kale fiber + 100 µL DPPH	100 µL Kale fiber + 100 µL DPPH	100 µL Kale fiber + 100 µL DPPH	100 µL Sugar beet green juice + 100 µL DPPH	100 µL Sugar beet green juice + 100 µL DPPH	100 µL Sugar beet green juice + 100 µL DPPH						
H				100 µL Lucerne leaves + 100 µL DPPH	100 µL Lucerne leaves + 100 µL DPPH	100 µL Lucerne leaves + 100 µL DPPH						

Table A6. Results for inhibition from DPPH screening (n=1) according to the plate lay-out in table A5.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,508	0,504	0,513	0,392	0,365	0,368	0,146	0,102	0,093	0,048	0,048	0,048	516
B	0,505	0,509	0,518	0,344	0,328	0,319	0,462	0,471	0,459	0,048	0,048	0,048	516
C	0,464	0,457	0,459	0,513	0,507	0,508	0,459	0,454	0,452	0,05	0,049	0,049	516
D	0,386	0,376	0,378	0,513	0,507	0,509	0,461	0,457	0,454	0,049	0,048	0,051	516
E	0,377	0,355	0,355	0,343	0,323	0,321	0,47	0,461	0,456	0,05	0,05	0,049	516
F	0,431	0,432	0,431	0,516	0,51	0,519	0,464	0,464	0,468	0,05	0,048	0,05	516
G	0,392	0,384	0,384	0,35	0,33	0,334	0,473	0,465	0,468	0,051	0,049	0,05	516
H	0,434	0,431	0,45	0,314	0,301	0,301	0,457	0,451	0,46	0,051	0,048	0,048	516

Table A7. Calculation of %Radical inhibition for Kale, Lucerne and Sugar beet (n=1).

	Abs 1	Abs 2	Abs 3	Abs average	% Radical inhibition 1	% Radical inhibition 2	% Radical inhibition 3	Average% Radical inhibition	Std Dev
MeOH	0,508	0,504	0,513	0,508					
DMSO + MeOH	0,505	0,509	0,518	0,511					
Lucerne fiber	0,464	0,457	0,459		8,7	10,1	9,7	9,5	0,7
Lucerne green juice	0,386	0,376	0,378		24,1	26,0	25,6	25,2	1,0
Lucerne brown juice	0,377	0,355	0,355		25,8	30,2	30,2	28,7	2,5
Kale fiber	0,392	0,384	0,384		22,9	24,5	24,5	23,9	0,9
Kale green juice	0,392	0,365	0,368		22,9	28,2	27,6	26,2	2,9
Kale brown juice	0,344	0,328	0,319		32,3	35,5	37,2	35,0	2,5
Kale green protein	0,513	0,507	0,508		-0,5	0,7	0,5	0,3	0,6
Sugar beet fiber	0,513	0,507	0,509		-0,9	0,3	-0,1	-0,3	0,6
Sugar beet green protein	0,343	0,323	0,321		32,5	36,5	36,9	35,3	2,4
Sugar beet white protein	0,516	0,51	0,519		-1,5	-0,3	-2,1	-1,3	0,9
Sugar beet green juice	0,35	0,33	0,334		31,1	35,1	34,3	33,5	2,1
Lucerne leaves	0,314	0,301	0,301		38,2	40,8	40,8	39,9	1,5
Vitamin C	0,146	0,102	0,093		71,3	79,9	81,7	77,6	5,6

Table A8. Results for inhibition from DPPH screening (n=2) according to the plate lay-out in table A5.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,486	0,492	0,495	0,36	0,341	0,35	0,138	0,092	0,101	0,048	0,048	0,047	516
B	0,479	0,477	0,464	0,31	0,293	0,308	0,436	0,442	0,442	0,048	0,049	0,048	516
C	0,428	0,427	0,429	0,484	0,482	0,488	0,429	0,437	0,438	0,05	0,048	0,048	516
D	0,358	0,347	0,352	0,482	0,482	0,484	0,433	0,44	0,427	0,048	0,048	0,048	516
E	0,327	0,326	0,326	0,287	0,265	0,275	0,439	0,439	0,434	0,048	0,048	0,048	516
F	0,432	0,434	0,429	0,486	0,478	0,492	0,436	0,443	0,436	0,048	0,048	0,048	516
G	0,375	0,364	0,359	0,318	0,301	0,302	0,44	0,445	0,436	0,049	0,048	0,047	516
H	0,415	0,436	0,434	0,301	0,291	0,289	0,439	0,439	0,433	0,049	0,049	0,048	516

Table A9. Calculation of %Radical inhibition for Kale, Lucerne and Sugar beet (n=2).

	Abs 1	Abs 2	Abs 3	Abs average	% Radical inhibition 1	% Radical inhibition 2	% Radical inhibition 3	Average% Radical inhibition	Std Dev
MeOH	0,486	0,492	0,495	0,491					
DMSO + MeOH	0,479	0,477	0,464	0,473					
Lucerne fiber	0,428	0,427	0,429		12,8	13,0	12,6	12,8	0,2
Lucerne green juice	0,358	0,347	0,352		27,1	29,3	28,3	28,2	1,1
Lucerne brown juice	0,327	0,326	0,326		33,4	33,6	33,6	33,5	0,1
Kale fiber	0,375	0,364	0,359		23,6	25,9	26,9	25,5	1,7
Kale green juice	0,36	0,341	0,35		26,7	30,5	28,7	28,6	1,9
Kale brown juice	0,31	0,293	0,308		36,9	40,3	37,3	38,2	1,9
Kale green protein	0,484	0,482	0,488		1,4	1,8	0,6	1,3	0,6
Sugar beet fiber	0,482	0,482	0,484		1,8	1,8	1,4	1,7	0,2
Sugar beet green protein	0,287	0,265	0,275		41,5	46,0	44,0	43,9	2,2
Sugar beet white protein	0,486	0,478	0,492		1,0	2,6	-0,2	1,2	1,4
Sugar beet green juice	0,318	0,301	0,302		35,2	38,7	38,5	37,5	1,9
Lucerne leaves	0,301	0,291	0,289		38,7	40,7	41,1	40,2	1,3
Vitamin C	0,138	0,092	0,101		71,9	81,3	79,4	77,5	5,0

Table A10. Results for inhibition from DPPH screening (n=3) according to the plate lay-out in table A5.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,503	0,503	0,507	0,369	0,353	0,353	0,133	0,092	0,084	0,048	0,048	0,047	516
B	0,497	0,499	0,506	0,323	0,309	0,307	0,448	0,451	0,446	0,048	0,048	0,047	516
C	0,438	0,435	0,44	0,472	0,486	0,495	0,447	0,438	0,441	0,048	0,048	0,048	516
D	0,361	0,348	0,359	0,495	0,496	0,496	0,445	0,443	0,444	0,048	0,048	0,048	516
E	0,347	0,331	0,338	0,298	0,281	0,278	0,447	0,449	0,451	0,048	0,048	0,048	516
F	0,427	0,429	0,423	0,496	0,495	0,495	0,449	0,442	0,438	0,048	0,048	0,049	516
G	0,376	0,37	0,378	0,314	0,301	0,301	0,442	0,441	0,439	0,049	0,048	0,048	516
H	0,414	0,429	0,434	0,313	0,3	0,296	0,438	0,436	0,431	0,048	0,048	0,048	516

Table A11. Calculation of %Radical inhibition for Kale, Lucerne and Sugar beet (n=3).

	Abs 1	Abs 2	Abs 3	Abs average	% Radical inhibition 1	% Radical inhibition 2	% Radical inhibition 3	Average% Radical inhibition	Std Dev
MeOH	0,503	0,503	0,507	0,504					
DMSO + MeOH	0,497	0,499	0,506	0,501					
Lucerne fiber	0,438	0,435	0,44		13,2	13,7	12,8	13,2	0,5
Lucerne green juice	0,361	0,348	0,359		28,4	31,0	28,8	29,4	1,4
Lucerne brown juice	0,347	0,331	0,338		31,2	34,4	33,0	32,8	1,6
Kale fiber	0,376	0,37	0,378		25,4	26,6	25,0	25,7	0,8
Kale green juice	0,369	0,353	0,353		26,8	30,0	30,0	28,9	1,8
Kale brown juice	0,323	0,309	0,307		36,0	38,7	39,1	37,9	1,7
Kale green protein	0,472	0,486	0,495		6,4	3,6	1,9	4,0	2,3
Sugar beet fiber	0,495	0,496	0,496		1,9	1,7	1,7	1,7	0,1
Sugar beet green protein	0,298	0,281	0,278		40,9	44,3	44,9	43,4	2,1
Sugar beet white protein	0,496	0,495	0,495		1,7	1,9	1,9	1,8	0,1
Sugar beet green juice	0,314	0,301	0,301		37,7	40,3	40,3	39,5	1,5
Lucerne leaves	0,313	0,3	0,296		37,9	40,5	41,3	39,9	1,8
Vitamin C	0,133	0,092	0,084		73,6	81,8	83,3	79,6	5,2

Table A12. Plate lay out of DPPH experiment for Crimson clover, Phacelia, Oil radish and Red beet using methanol and DMSO as blanks and Vitamin C as reference. Three replicates of each sample were analyzed, and three replicates of the blanks and reference were also analyzed.

	1	2	3	4	5	6	7	8	9	10	11	12
A	100 µ MeOH + 100 µL DPPH	100 µ MeOH + 100 µL DPPH	100 µ MeOH + 100 µL DPPH	100 µL Crimson clover green juice + 100 µL	100 µL Crimson clover green juice + 100 µL	100 µL Crimson clover green juice + 100 µL	100 µL Oil radish brown juice + 100 µL DPPH	100 µL Oil radish brown juice + 100 µL DPPH	100 µL Oil radish brown juice + 100 µL DPPH	100 µL Lucerne white protein + 100 µL DPPH	101 µL Lucerne white protein + 100 µL DPPH	102 µL Lucerne white protein + 100 µL DPPH
B	2µL DMSO + 98 µL MeOH + 100 µL DPPH	2µL DMSO + 98 µL MeOH + 100 µL DPPH	2µL DMSO + 98 µL MeOH + 100 µL DPPH	100 µL Crimson clover green protein + 100	100 µL Crimson clover green protein + 100	100 µL Crimson clover green protein + 100	100 µL Oil radish white protein + 100 µL DPPH	100 µL Oil radish white protein + 100 µL DPPH	100 µL Oil radish white protein + 100 µL DPPH	100 µL Vit C (100 µM) + 100 µL DPPH	100 µL Vit C (100 µM) + 100 µL DPPH	100 µL Vit C (100 µM) + 100 µL DPPH
C	100 µL Phacelia fiber + 100 µL DPPH	101 µL Phacelia fiber + 100 µL DPPH	102 µL Phacelia fiber + 100 µL DPPH	100 µL Crimson clover brown juice + 100 µL	100 µL Crimson clover brown juice + 100 µL	100 µL Crimson clover brown juice + 100 µL	100 µL Oil radish white juice + 100 µL DPPH	100 µL Oil radish white juice + 100 µL DPPH	100 µL Oil radish white juice + 100 µL DPPH			
D	100 µL Phacelia green juice + 100 µL DPPH	100 µL Phacelia green juice + 100 µL DPPH	100 µL Phacelia green juice + 100 µL DPPH	100 µL Crimson clover white protein + 100	100 µL Crimson clover white protein + 100	100 µL Crimson clover white protein + 100	2 µL Red beet green juice + 98 µL MeOH + 100 µL DPPH	2 µL Red beet green juice + 98 µL MeOH + 100 µL DPPH	2 µL Red beet green juice + 98 µL MeOH + 100 µL DPPH			
E	100 µL Phacelia green protein + 100 µL DPPH	100 µL Phacelia green protein + 100 µL DPPH	100 µL Phacelia green protein + 100 µL DPPH	100 µL Crimson clover white juice + 100 µL	100 µL Crimson clover white juice + 100 µL	100 µL Crimson clover white juice + 100 µL	100 µL Red beet green protein + 100 µL DPPH	100 µL Red beet green protein + 100 µL DPPH	100 µL Red beet green protein + 100 µL DPPH			
F	100 µL Phacelia brown juice + 100 µL DPPH	100 µL Phacelia brown juice + 100 µL DPPH	100 µL Phacelia brown juice + 100 µL DPPH	2 µL Oil radish fiber + 98 µL MeOH + 100 µL DPPH	2 µL Oil radish fiber + 98 µL MeOH + 100 µL DPPH	2 µL Oil radish fiber + 98 µL MeOH + 100 µL DPPH	100 µL Red beet brown juice + 100 µL DPPH	100 µL Red beet brown juice + 100 µL DPPH	100 µL Red beet brown juice + 100 µL DPPH			
G	100 µL Phacelia white juice + 100 µL DPPH	100 µL Phacelia white juice + 100 µL DPPH	100 µL Phacelia white juice + 100 µL DPPH	2 µL Oil radish green juice + 98 µL MeOH + 100 µL DPPH	2 µL Oil radish green juice + 98 µL MeOH + 100 µL DPPH	2 µL Oil radish green juice + 98 µL MeOH + 100 µL DPPH	100 µL Red beet white juice + 100 µL DPPH	100 µL Red beet white juice + 100 µL DPPH	100 µL Red beet white juice + 100 µL DPPH			
H	100 µL Crimson clover fiber + 100 µL	100 µL Crimson clover fiber + 100 µL	100 µL Crimson clover fiber + 100 µL	100 µL Oil radish green protein + 100 µL DPPH	101 µL Oil radish green protein + 100 µL DPPH	102 µL Oil radish green protein + 100 µL DPPH	100 µL Sugar beet brown juice + 100 µL DPPH	100 µL Sugar beet brown juice + 100 µL DPPH	100 µL Sugar beet brown juice + 100 µL DPPH			

Table A13. Results for inhibition from DPPH screening (n=1) according to the plate lay-out in table A12.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,547	0,553	0,561	0,433	0,423	0,424	0,414	0,406	0,406	0,378	0,379	0,378	516
B	0,542	0,562	0,564	0,377	0,36	0,357	0,097	0,1	0,093	0,094	0,057	0,056	516
C	0,315	0,293	0,302	0,48	0,475	0,476	0,425	0,409	0,414	0,503	0,508	0,501	516
D	0,399	0,389	0,397	0,39	0,373	0,372	0,537	0,546	0,492	0,495	0,504	0,5	516
E	0,229	0,201	0,207	0,398	0,403	0,4	0,298	0,274	0,278	0,506	0,508	0,502	516
F	0,461	0,457	0,468	0,569	0,566	0,53	0,337	0,337	0,335	0,493	0,506	0,502	516
G	0,432	0,425	0,436	0,549	0,551	0,566	0,36	0,349	0,345	0,496	0,5	0,497	516
H	0,109	0,093	0,095	0,279	0,254	0,256	0,341	0,325	0,323	0,484	0,5	0,491	516

Table A14. Calculation of %Radical inhibition for Crimson clover, Phacelia, Oil radish and Red beet (n=1) using equation 1 in section 4.2

	Abs 1	Abs 2	Abs 3	Abs average	% Radical inhibition 1	% Radical inhibition 2	% Radical inhibition 3	Average% Radical inhibition	Std Dev
MeOH	0,547	0,553	0,561	0,554					
DMSO+MeOH	0,542	0,562	0,564	0,556					
Phacelia Fiber	0,315	0,293	0,302		43,1	47,1	45,5	45,2	2,0
Phacelia Green juice	0,399	0,389	0,397		27,9	29,7	28,3	28,7	1,0
Phacelia Green proteins	0,229	0,201	0,207		58,6	63,7	62,6	61,6	2,7
Phacelia Brown juice	0,461	0,457	0,468		16,7	17,5	15,5	16,6	1,0
Phacelia White juice	0,432	0,425	0,436		22,0	23,2	21,3	22,2	1,0
Crimson clover Fiber	0,109	0,093	0,095		80,3	83,2	82,8	82,1	1,6
Crimson clover Green juice	0,433	0,423	0,424		21,8	23,6	23,4	22,9	1,0
Crimson clover Green proteins	0,377	0,36	0,357		31,9	35,0	35,5	34,1	1,9
Crimson clover Brown juice	0,48	0,475	0,476		13,3	14,2	14,0	13,8	0,5
Crimson clover White protein	0,39	0,373	0,372		29,6	32,6	32,8	31,7	1,8
Crimson clover White juice	0,398	0,403	0,4		28,1	27,2	27,8	27,7	0,5
Oil radish Fiber	0,569	0,566	0,53		-2,3	-1,8	4,7	0,2	3,9
Oil radish Green juice	0,549	0,551	0,566		1,3	0,9	-1,8	0,1	1,7
Oil radish Green proteins	0,279	0,254	0,256		49,6	54,1	53,8	52,5	2,5
Oil radish Brown juice	0,414	0,406	0,406		25,2	26,7	26,7	26,2	0,8
Oil radish White protein	0,097	0,1	0,093		82,5	81,9	83,2	82,5	0,6
Oil radish White juice	0,425	0,409	0,414		23,2	26,1	25,2	24,9	1,5
Red beet Green juice	0,537	0,546	0,492		3,4	1,8	11,5	5,6	5,2
Red beet Green proteins	0,298	0,274	0,278		46,2	50,5	49,8	48,8	2,3
Red beet Brown juice	0,337	0,337	0,335		39,1	39,1	39,5	39,3	0,2
Red beet White juice	0,36	0,349	0,345		35,0	37,0	37,7	36,5	1,4
Sugar beet Brown juice	0,341	0,325	0,323		38,4	41,3	41,7	40,5	1,8
Lucerne White protein	0,378	0,379	0,378		31,7	31,5	31,7	31,7	0,1
Vitamin C	0,094	0,057	0,056		83,0	89,7	89,9	87,5	3,9

Table A15. Results for inhibition from DPPH screening (n=2) according to the plate lay-out in table A12.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,544	0,543	0,549	0,431	0,412	0,415	0,416	0,403	0,401	0,393	0,377	0,378	516
B	0,538	0,538	0,553	0,368	0,352	0,345	0,094	0,099	0,097	0,21	0,128	0,132	516
C	0,307	0,302	0,304	0,473	0,465	0,466	0,422	0,405	0,409	0,498	0,498	0,505	516
D	0,402	0,39	0,393	0,387	0,365	0,357	0,514	0,52	0,54	0,546	0,547	0,553	516
E	0,211	0,191	0,195	0,399	0,387	0,383	0,309	0,283	0,285	0,495	0,491	0,499	516
F	0,447	0,449	0,45	0,551	0,564	0,548	0,336	0,322	0,322	0,491	0,488	0,492	516
G	0,427	0,419	0,423	0,531	0,535	0,532	0,363	0,349	0,342	0,487	0,482	0,494	516
H	0,104	0,098	0,099	0,279	0,246	0,252	0,338	0,32	0,316	0,485	0,489	0,488	516

Table A16. Calculation of %Radical inhibition for Crimson clover, Phacelia, Oil radish and Red beet (n=2) using equation 1 in section 4.2.

	Abs 1	Abs 2	Abs 3	Abs average	% Radical inhibition 1	% Radical inhibition 2	% Radical inhibition 3	Average% Radical inhibition	Std Dev
MeOH	0,544	0,543	0,549	0,545					
DMSO+MeOH	0,538	0,538	0,553	0,543					
Phacelia Fiber	0,307	0,302	0,304		43,7	44,6	44,3	44,2	0,5
Phacelia Green juice	0,402	0,39	0,393		26,3	28,5	27,9	27,6	1,1
Phacelia Green proteins	0,211	0,191	0,195		61,3	65,0	64,2	63,5	1,9
Phacelia Brown juice	0,447	0,449	0,45		18,0	17,7	17,5	17,7	0,3
Phacelia White juice	0,427	0,419	0,423		21,7	23,2	22,4	22,4	0,7
Crimson clover Fiber	0,104	0,098	0,099		80,9	82,0	81,8	81,6	0,6
Crimson clover Green juice	0,431	0,412	0,415		21,0	24,4	23,9	23,1	1,9
Crimson clover Green proteins	0,368	0,352	0,345		32,5	35,5	36,7	34,9	2,2
Crimson clover Brown juice	0,473	0,465	0,466		13,3	14,7	14,5	14,2	0,8
Crimson clover White protein	0,387	0,365	0,357		29,0	33,1	34,5	32,2	2,8
Crimson clover White juice	0,399	0,387	0,383		26,8	29,0	29,8	28,5	1,5
Oil radish Fiber	0,551	0,564	0,548		-1,5	-3,9	-0,9	-2,1	1,6
Oil radish Green juice	0,531	0,535	0,532		2,2	1,5	2,0	1,9	0,4
Oil radish Green proteins	0,279	0,246	0,252		48,8	54,9	53,8	52,5	3,2
Oil radish Brown juice	0,416	0,403	0,401		23,7	26,1	26,5	25,4	1,5
Oil radish White protein	0,094	0,099	0,097		82,8	81,8	82,2	82,3	0,5
Oil radish White juice	0,422	0,405	0,409		22,6	25,7	25,0	24,4	1,6
Red beet Green juice	0,514	0,52	0,54		5,3	4,2	0,6	3,4	2,5
Red beet Green proteins	0,309	0,283	0,285		43,3	48,1	47,7	46,4	2,7
Red beet Brown juice	0,336	0,322	0,322		38,4	41,0	41,0	40,1	1,5
Red beet White juice	0,363	0,349	0,342		33,4	36,0	37,3	35,6	2,0
Sugar beet Brown juice	0,338	0,32	0,316		38,0	41,3	42,1	40,5	2,1
Lucerne White protein	0,393	0,377	0,378		27,9	30,9	30,7	29,8	1,6
Vitamin C	0,21	0,128	0,132		61,5	76,5	75,8	71,3	8,5

Table A17. Results for inhibition from DPPH screening (n=3) according to the plate lay-out in table A12.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,447	0,453	0,459	0,347	0,347	0,345	0,352	0,34	0,337	0,335	0,315	0,321	516
B	0,437	0,443	0,469	0,295	0,293	0,284	0,092	0,09	0,09	0,105	0,075	0,063	516
C	0,266	0,252	0,238	0,406	0,407	0,41	0,372	0,363	0,36	0,476	0,47	0,46	516
D	0,343	0,339	0,344	0,322	0,316	0,317	0,491	0,503	0,491	0,477	0,473	0,466	516
E	0,18	0,167	0,177	0,374	0,365	0,366	0,291	0,267	0,257	0,478	0,474	0,468	516
F	0,412	0,42	0,42	0,512	0,518	0,522	0,298	0,299	0,294	0,434	0,473	0,464	516
G	0,381	0,391	0,388	0,501	0,508	0,508	0,352	0,328	0,329	0,478	0,477	0,468	516
H	0,094	0,099	0,1	0,232	0,222	0,222	0,314	0,3	0,296	0,481	0,473	0,463	516

Table A18. Calculation of %Radical inhibition for Crimson clover, Phacelia, Oil radish and Red beet (n=3) using equation 1 in section 4.2.

	Abs 1	Abs 2	Abs 3	Abs average	% Radical inhibition 1	% Radical inhibition 2	% Radical inhibition 3	Average% Radical inhibition	Std Dev
MeOH	0,447	0,453	0,459	0,453					
DMSO+MeOH	0,437	0,443	0,469	0,450					
Phacelia Fiber	0,266	0,252	0,238		41,3	44,4	47,5	44,4	3,1
Phacelia Green juice	0,343	0,339	0,344		24,3	25,2	24,1	24,5	0,6
Phacelia Green proteins	0,18	0,167	0,177		60,3	63,1	60,9	61,4	1,5
Phacelia Brown juice	0,412	0,42	0,42		9,1	7,3	7,3	7,9	1,0
Phacelia White juice	0,381	0,391	0,388		15,9	13,7	14,3	14,6	1,1
Crimson clover Fiber	0,094	0,099	0,1		79,2	78,1	77,9	78,4	0,7
Crimson clover Green juice	0,347	0,347	0,345		23,4	23,4	23,8	23,5	0,3
Crimson clover Green proteins	0,295	0,293	0,284		34,9	35,3	37,3	35,8	1,3
Crimson clover Brown juice	0,406	0,407	0,41		10,4	10,2	9,5	10,0	0,5
Crimson clover White protein	0,322	0,316	0,317		28,9	30,2	30,0	29,7	0,7
Crimson clover White juice	0,374	0,365	0,366		17,4	19,4	19,2	18,7	1,1
Oil radish Fiber	0,512	0,518	0,522		-13,9	-15,2	-16,1	-15,0	1,1
Oil radish Green juice	0,501	0,508	0,508		-11,4	-13,0	-13,0	-12,5	0,9
Oil radish Green proteins	0,232	0,222	0,222		48,8	51,0	51,0	50,3	1,3
Oil radish Brown juice	0,352	0,34	0,337		22,3	24,9	25,6	24,3	1,8
Oil radish White protein	0,092	0,09	0,09		79,7	80,1	80,1	80,0	0,3
Oil radish White juice	0,372	0,363	0,36		17,9	19,9	20,5	19,4	1,4
Red beet Green juice	0,491	0,503	0,491		-9,2	-11,9	-9,2	-10,1	1,5
Red beet Green proteins	0,291	0,267	0,257		35,8	41,1	43,3	40,0	3,9
Red beet Brown juice	0,298	0,299	0,294		34,2	34,0	35,1	34,4	0,6
Red beet White juice	0,352	0,328	0,329		22,3	27,6	27,4	25,8	3,0
Sugar beet Brown juice	0,314	0,3	0,296		30,7	33,8	34,7	33,0	2,1
Lucerne White protein	0,335	0,315	0,321		26,0	30,5	29,1	28,6	2,3
Vitamin C	0,105	0,075	0,063		76,8	83,4	86,1	82,1	4,8

Table A19. Summary of results from the radical scavenging analyses where the average %radical inhibition is shown next to the average standard deviation.

	% Radical inhibition average	Std Dev average
Lucerne fiber	11,9	1,8
Lucerne green juice	27,6	2,1
Lucerne brown juice	31,7	2,7
Kale fiber	25,0	1,3
Kale green juice	27,9	2,4
Kale brown juice	37,0	2,3
Kale green protein	1,8	2,1
Sugar beet fiber	1,1	1,0
Sugar beet green protein	40,8	4,6
Sugar beet white protein	0,5	1,7
Sugar beet green juice	36,8	3,1
Lucerne leaves	40,0	1,3
Phacelia Fiber	44,6	1,9
Phacelia Green juice	26,9	2,0
Phacelia Green proteins	62,2	2,1
Phacelia Brown juice	14,1	4,7
Phacelia White juice	19,7	3,9
Crimson clover Fiber	80,7	2,0
Crimson clover Green juice	23,2	1,1
Crimson clover Green proteins	35,0	1,8
Crimson clover Brown juice	12,7	2,1
Crimson clover White protein	31,2	2,1
Crimson clover White juice	25,0	4,8
Oil radish Fiber	-5,7	7,4
Oil radish Green juice	-3,5	6,8
Oil radish Green proteins	51,8	2,4
Oil radish Brown juice	25,3	1,5
Oil radish White protein	81,6	1,3
Oil radish White juice	22,9	2,9
Red beet Green juice	-0,4	7,9
Red beet Green proteins	45,1	4,7
Red beet Brown juice	37,9	2,8
Red beet White juice	32,6	5,5
Sugar beet Brown juice	38,0	4,1
Lucerne White protein	30,0	2,0

Tables A20-A29 represent the glycation inhibition experiment that was performed (n=1 time) using three different plates (plate 1, 2 and 3). Table A20 shows the plate 1 lay out, table A21 is the absorption results retrieved from the spectrophotometer microplate reader and can be compared to plate 1 lay-out. Table A22 is the calculations where the absorbances are converted to %inhibition using equations 2 and 3 in section 4.3. Table A23 shows the plate 1 lay out, table A24 is the absorption results retrieved from the spectrophotometer microplate reader and can be compared to plate 1 lay-out. Table A25 is the calculations where the absorbances are converted to %inhibition using equation 2 and 3 in section 4.3. Table A26 shows the plate 1 lay out, table A27 is the absorption results retrieved from the spectrophotometer microplate reader and can be compared to plate 1 lay-out. Table A28 is the calculations where the absorbances are converted to %inhibition using equation 2 and 3 in section 4.3. Table A29 is a summary of the results for all seven plants (31 extracts) where the %inhibition is shown as an average.

Table A20. Plate lay-out of plate 1 for anti-glycation assay where everything is done in triplicates. The blank control = 100% inhibition, control = no inhibition and then there are two preparations for each extract: blank extract = BSA + extract and extract = BSA + D-ribose + extract. The reference is Rutin 1 mM. When pipetting sample 12 and 13 in plate 1, there was a mix-up and the extracts were re-analyzed in plate 2.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Blank control:** 50 µL BSA + 50 µL Buffer	**Blank control:** 50 µL BSA + 50 µL Buffer	**Blank control:** 50 µL BSA + 50 µL Buffer	**Blank Lucerne white protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne white protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne white protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample
B	**Control:** 50 µL BSA + 10 µL Buffer (10% DMSO) +40 µL D-ribose	**Control:** 50 µL BSA + 10 µL Buffer (10% DMSO) +40 µL D-ribose	**Control:** 50 µL BSA + 10 µL Buffer (10% DMSO) +40 µL D-ribose	**Lucerne white protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Lucerne white protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Lucerne white protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample
C	**Blank Kale green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Kale green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Kale green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Sugar beet fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Sugar beet fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Sugar beet fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. white protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. white protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. white protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample
D	**Kale green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Kale green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Kale green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Sugar beet fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Sugar beet fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Sugar beet fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. white protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. white protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. white protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample
E	**Blank Kale brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Kale brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Kale brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Sugar beet brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Sugar beet brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Sugar beet brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample
F	**Kale brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Kale brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Kale brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Sugar beet brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Sugar beet brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Sugar beet brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample
G	**Blank Lucerne brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank reference:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank reference:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank reference:** 50 µL BSA + 40 µL Buffer + 10 µL Sample
H	**Lucerne brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Lucerne brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Lucerne brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Reference:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Reference:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Reference:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample

Table A21. Results for inhibition from anti-glycation screening (n=1) according to the plate lay-out in table A20.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	4472	4777	4796	36067	34485	36964	10240	11025	11200	14579	20496	20014	370,44
B	24364	23693	24792	46646	46756	45770	28987	27491	28804	30781	31089	32765	370,44
C	11345	11470	12076	11435	11215	11509	14589	13613	13355	14950	4607	15299	370,44
D	23738	23664	24784	24110	24379	25112	26912	27369	28610	27403	26631	25642	370,44
E	9567	9589	9065	11397	12086	12017	12310	11935	11844	12711	12524	12999	370,44
F	19898	19298	19042	20711	21268	22251	23696	24487	23490	25485	25759	25214	370,44
G	14766	14063	14436	10605	10425	11241	12126	12448	11874	756	761	659	370,44
H	30007	29589	28938	25822	25704	24794	25398	25514	25158	2336	2190	2182	370,44

Table A22. Calculation of %Inhibition for the sample analyzed in plate 1 (n=1), see table A20. The %inhibition that are colored light brown represent the extracts showing medium activity and the %inhibition that are colored brown represent the extracts showing good activity.

						%AGEs 1	%AGEs 2	%AGEs 3	%Inhibition 1	%Inhibition 2	%Inhibition 3	Average	Stdv
Blank control		4472	4777	4796	4681,67	101,5	96,5	102,0	- 1,5	3,5	- 2,0	- 0,0	3,0
Control		24364	23693	24792	24283								
Blank sample	Kale green juice	11345	11470	12076		63,2	62,2	64,8	36,8	37,8	35,2	36,6	1,3
Sample		23738	23664	24784									
Blank sample	Kale brown	9567	9589	9065		52,7	49,5	50,9	47,3	50,5	49,1	49,0	1,6
Sample	juice	19898	19298	19042									
Blank sample	Lucerne brown	14766	14063	14436		77,8	79,2	74,0	22,2	20,8	26,0	23,0	2,7
Sample	juice	30007	29589	28938									
Blank sample	Lucerne white	36067	34485	36964		54,0	62,6	44,9	46,0	37,4	55,1	46,2	8,8
Sample	protein	46646	46756	45770									
Blank sample	Sugar beet fiber	11435	11215	11509		64,7	67,2	69,4	35,3	32,8	30,6	32,9	2,4
Sample		24110	24379	25112									
Blank sample	Sugar beet	11397	12086	12017		47,5	46,8	52,2	52,5	53,2	47,8	51,1	2,9
Sample	brown juice	20711	21268	22251									
Blank sample	Phacelia green	10605	10425	11241		77,6	77,9	69,1	22,4	22,1	30,9	25,1	5,0
Sample	juice	25822	25704	24794									
Blank sample	Phacelia brown	10240	11025	11200		95,6	84,0	89,8	4,4	16,0	10,2	10,2	5,8
Sample	juice	28987	27491	28804									
Blank sample	Phacelia white	14589	13613	13355		62,9	70,2	77,8	37,1	29,8	22,2	29,7	7,5
Sample	juice	26912	27369	28610									
Blank sample	Crison clover	12310	11935	11844		58,1	64,0	59,4	41,9	36,0	40,6	39,5	3,1
Sample	green juice	23696	24487	23490									
Blank sample	Crimson clover	12126	12448	11874		67,7	66,7	67,8	32,3	33,3	32,2	32,6	0,6
Sample	green protein	25398	25514	25158									
Blank sample	Crimson clover												
Sample	brown juice												
Blank sample	Crimson clover												
Sample	white protein												
Blank sample	Crimson clover	12711	12524	12999		65,2	67,5	62,3	34,8	32,5	37,7	35,0	2,6
Sample	white juice	25485	25759	25214									
Blank sample	Rutin	756	761	659		8,1	7,3	7,8	91,9	92,7	92,2	92,3	0,4
Sample		2336	2190	2182									

Table A23. Plate lay-out of plate 2 for anti-glycation assay where everything is done in triplicates. The blank control = 100% inhibition, control = no inhibition and then there are two preparations for each extract: blank extract = BSA + extract and extract = BSA + D-ribose + extract. The reference is Rutin 1 mM. The blue coloured extracts were prepared in DMSO whereas the green extracts were prepared in buffer since they were not soluble in DMSO. They correspond to the controls with the same colours.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Blank control:** 50 µL BSA + 40 µL Buffer + 10 µL Buffer (10% DMSO)	**Blank control:** 50 µL BSA + 40 µL Buffer + 10 µL Buffer (10% DMSO)	**Blank control:** 50 µL BSA + 40 µL Buffer + 10 µL Buffer (10% DMSO)	**Blank Crimson C. white protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. white protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. white protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample
B	**Control:** 50 µL BSA + 10 µL Buffer (10% DMSO) +40 µL D-ribose	**Control:** 50 µL BSA + 10 µL Buffer (10% DMSO) +40 µL D-ribose	**Control:** 50 µL BSA + 10 µL Buffer (10% DMSO) +40 µL D-ribose	**Crimson C. white protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. white protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. white protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample
C	**Blank control:** 50 µL BSA + 50 µL Buffer	**Blank control:** 50 µL BSA + 50 µL Buffer	**Blank control:** 50 µL BSA + 50 µL Buffer	**Blank Crimson C. fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Phacelia green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample
D	**Control:** 50 µL BSA + 10 µL Buffer +40 µL D-ribose	**Control:** 50 µL BSA + 10 µL Buffer +40 µL D-ribose	**Control:** 50 µL BSA + 10 µL Buffer +40 µL D-ribose	**Crimson C. fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Phacelia green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample
E	**Blank reference:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank reference:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank reference:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample
F	**Reference:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Reference:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Reference:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Luverne green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Luverne green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Luverne green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample
G	**Blank Crimson C. brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Crimson C. brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Sugar beet green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Sugar beet green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Sugar beet green juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish brown juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet white juice:** 50 µL BSA + 40 µL Buffer + 10 µL Sample
H	**Crimson C. brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Crimson C. brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Sugar beet green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Sugar beet green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Sugar beet green juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish brown juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet white juice:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample

Table A24. Results for inhibition from anti-glycation screening (n=1) according to the plate lay-out in table A23.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	5959	6074	6619	19702	20822	20454	20361	20614	20527	25606	30486	23136	370,44
B	27487	28871	29679	34717	34986	35097	43300	40945	39604	40528	38714	39410	370,44
C	5910	6584	7007	22443	21747	21991	12920	12514	12332	13444	13488	13545	370,44
D	22789	23844	24636	31838	31124	33400	29226	31260	31343	27592	27256	25541	370,44
E	773	837	901	19708	19987	19705	17651	17030	16563	13134	12559	13714	370,44
F	2337	2453	2486	33088	34127	35853	31172	28846	30107	23878	23893	23692	370,44
G	13394	13533	13357	10838	10257	10877	16279	15969	15857	12425	11017	11581	370,44
H	31658	33119	33809	30339	29220	27486	31206	29200	29086	24433	24834	20810	370,44

Table A25. Calculation of %Inhibition for the sample analyzed in plate 2 (n=1), see table A23. The %inhibition that are colored light brown represent the extracts showing medium activity and the %inhibition that are colored brown represent the extracts showing good activity.

						%AGEs 1	%AGEs 2	%AGEs 3	%Inhibition 1	%Inhibition 2	%Inhibition 3	Average	Stdv
Blank control		5959	6074	6619	6217,33	95,8	101,5	102,7	4,2	- 1,5	- 2,7	0,0	3,6
Control		27487	28871	29679	28679								
Blank control		5910	6584	7007	6500,33	97,8	100,0	102,2	2,2	- 0,0	- 2,2	-	2,2
Control		22789	23844	24636	23756,3								
Blank control	Reference: Rutin	773	837	901		7,0	7,2	7,1	93,0	92,8	92,9	92,9	0,1
Control		2337	2453	2486									
Blank control	Crimson clover	13394	13533	13357		81,3	87,2	91,1	18,7	12,8	8,9	13,5	4,9
Control	brown juice	31658	33119	33809									
Blank control	Crimson clover	19702	20822	20454		66,8	63,1	65,2	33,2	36,9	34,8	35,0	1,9
Control	white protein	34717	34986	35097									
Blank control	Crimson clover	22443	21747	21991		41,8	41,7	50,8	58,2	58,3	49,2	55,2	5,2
Control	fiber	31838	31124	33400									
Blank control	Lucerne green	19708	19987	19705		59,6	63,0	71,9	40,4	37,0	28,1	35,2	6,4
Control	juice	33088	34127	35853									
Blank control	Sugar beet green	10838	10257	10877		86,8	84,4	73,9	13,2	15,6	26,1	18,3	6,8
Control	juice	30339	29220	27486									
Blank control	Phacelia fiber	20361	20614	20527		102,1	90,5	84,9	- 2,1	9,5	15,1	7,5	8,8
Control		43300	40945	39604									
Blank control	Phacelia green	12920	12514	12332		72,6	83,5	84,6	27,4	16,5	15,4	19,8	6,6
Control	protein	29226	31260	31343									
Blank control	Oil radish green	17651	17030	16563		60,2	52,6	60,3	39,8	47,4	39,7	42,3	4,4
Control	juice	31172	28846	30107									
Blank control	Oil radish	16279	15969	15857		66,5	58,9	58,9	33,5	41,1	41,1	38,6	4,4
Control	brown juice	31206	29200	29086									
Blank control	Oil radish white	25606	30486	23136		66,4	36,6	72,5	33,6	63,4	27,5	41,5	19,2
Control	juice	40528	38714	39410									
Blank control	Red beet green	13444	13488	13545		82,0	79,8	69,5	18,0	20,2	30,5	22,9	6,7
Control	juice	27592	27256	25541									
Blank control	Red beet brown	13134	12559	13714		47,8	50,5	44,4	52,2	49,5	55,6	52,4	3,0
Control	juice	23878	23893	23692									
Blank control	Red beet white	12425	11017	11581		69,6	80,1	53,5	30,4	19,9	46,5	32,3	13,4
Control	juice	24433	24834	20810									

Table A26. Plate lay-out of plate 2 for anti-glycation assay where everything is done in triplicates. The blank control = 100% inhibition, control = no inhibition and then there are two preparations for each extract: blank extract = BSA + extract and extract = BSA + D-ribose + extract. The reference is Rutin 1 mM.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Blank control:** 50 µL BSA + 40 µL Buffer + 10 µL Buffer (10% DMSO)	**Blank control:** 50 µL BSA + 40 µL Buffer + 10 µL Buffer (10% DMSO)	**Blank control:** 50 µL BSA + 40 µL Buffer + 10 µL Buffer (10% DMSO)	**Blank Lucerne leaves:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne leaves:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne leaves:** 50 µL BSA + 40 µL Buffer + 10 µL Sample						
B	**Control:** 50 µL BSA + 10 µL Buffer (10% DMSO) +40 µL D-ribose	**Control:** 50 µL BSA + 10 µL Buffer (10% DMSO) +40 µL D-ribose	**Control:** 50 µL BSA + 10 µL Buffer (10% DMSO) +40 µL D-ribose	**Lucerne leaves:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Lucerne leaves:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Lucerne leaves:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample						
C	**Blank reference:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank reference:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank reference:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample						
D	**Reference:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Reference:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Reference:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample						
E	**Blank Kale fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Kale fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Kale fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Oil radish green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample						
F	**Kale fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Kale fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Kale fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Oil radish green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample						
G	**Blank Lucerne fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Lucerne fiber:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample	**Blank Red beet green protein:** 50 µL BSA + 40 µL Buffer + 10 µL Sample						
H	**Lucerne fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Lucerne fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Lucerne fiber:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample	**Red beet green protein:** 50 µL BSA + 40 µL D-ribose + 10 µL Sample						

Table A27. Results for inhibition from anti-glycation screening (n=1) according to the plate lay-out in table A26.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	2891	3156	3449	8472	8770	8765	67	73	66	69	72	68	370,44
B	15260	16082	15687	19496	20122	20327	65	95	75	66	72	68	370,44
C	538	534	561	10919	11250	11331	69	76	77	72	76	78	370,44
D	1797	1928	1847	20822	21556	22119	88	70	80	74	80	68	370,44
E	11679	14478	14507	36799	41763	42549	73	70	81	75	80	79	370,44
F	22903	21373	21700	47247	48773	48738	76	67	80	87	80	86	370,44
G	10248	11116	11533	17910	19229	19511	84	70	79	83	73	71	370,44
H	19225	20971	21348	25214	26374	25054	72	66	88	70	73	59	370,44

Table A28. Calculation of %Inhibition for the sample analyzed in plate 3 (n=1), see table A26. The %inhibition that are colored light brown represent the extracts showing medium activity and the %inhibition that are colored brown represent the extracts showing good activity.

						%AGEs 1	%AGEs 2	%AGEs 3	%Inhibition 1	%Inhibition 2	%Inhibition 3	Average	Stdv
Blank control		2891	3156	3449	3165,333	98,9	103,3	97,8	1,1	3,3	2,2	-	2,9
Control		15260	16082	15687	15676,33								
Blank control	Reference:	538	534	561		10,1	11,1	10,3	89,9	88,9	89,7	89,5	0,6
Control	Rutin	1797	1928	1847									
Blank control	Oil radish fiber	11679	14478	14507		89,7	55,1	57,5	10,3	44,9	42,5	32,6	19,3
Control		22903	21373	21700									
Blank control	Oil radish green	10248	11116	11533		71,8	78,8	78,5	28,2	21,2	21,5	23,7	4,0
Control	protein	19225	20971	21348									
Blank control	Kale fiber	8472	8770	8765		88,1	90,7	92,4	11,9	9,3	7,6	9,6	2,2
Control		19496	20122	20327									
Blank control	Red beet green	10919	11250	11331		79,2	82,4	86,2	20,8	17,6	13,8	17,4	3,5
Control	protein	20822	21556	22119									
Blank control	Lucerne fiber	36799	41763	42549		83,5	56,0	49,5	16,5	44,0	50,5	37,0	18,1
Control		47247	48773	48738									
Blank control	Lucerne leaves	17910	19229	19511		58,4	57,1	44,3	41,6	42,9	55,7	46,7	7,8
Control		25214	26374	25054									

Table A29. Summary of results from the glycation inhibition analyses where the average %inhibition is shown next to the average standard deviation. The %inhibition that are colored light brown represent the extracts showing medium activity and the %inhibition that are colored brown represent the extracts showing good activity.

	Extracts	Average %inhibition	Stdv
1	Kale green juice	36,6	1,3
2	Kale brown juice	49,0	1,6
3	Lucerne brown juice	23,0	2,7
4	Lucerne white protein	46,2	8,8
5	Sugar beet fiber	32,9	2,4
6	Sugar beet brown juice	51,1	2,9
7	Phacelia green juice	25,1	5,0
8	Phacelia brown juice	10,2	5,8
9	Phacelia white juice	29,7	7,5
10	Crimson clover green juice	39,5	3,1
11	Crimson clover green protein	32,6	0,6
12	Crimson clover brown juice	13,5	4,9
13	Crimson clover white protein	35,0	1,9
14	Crimson clover white juice	35,0	2,6
15	Crimson clover fiber	55,2	5,2
16	Lucerne green juice	35,2	6,4
17	Sugar beet green juice	18,3	6,8
18	Phacelia fiber	7,5	8,8
19	Phacelia green protein	19,8	6,6
20	Oil radish green juice	42,3	4,4
21	Oil radish brown juice	38,6	4,4
22	Oil radish white juice	41,5	19,2
23	Red beet green juice	22,9	6,7
24	Red beet brown juice	52,4	3,0
25	Red beet white juice	32,3	13,4
26	Oil radish fiber	32,6	19,3
27	Oil radish green protein	23,7	4,0
28	Kale fiber	9,6	2,2
29	Red beet green protein	17,4	3,5
30	Lucerne fiber	37,0	18,1
31	Lucerne leaves	46,7	7,8

Tables A30-A36 represent the tyrosinase inhibition experiment that was performed (n=1 time) using two different plates (plate 1 and 2). Table A30 shows the plate 1 lay out, table A31 is the absorption results retrieved from the spectrophotometer microplate reader and can be compared to plate 1 lay-out. Table A32 is the calculations where the absorbances are converted to %inhibition using equation 4 in section 4.4. Table A33 shows the plate 2 lay out, table A34 is the absorption results retrieved from the spectrophotometer microplate reader and can be compared to plate 2 lay-out. Table A35 is the calculations where the absorbances are converted to %inhibition using equation 4 in section 4.4. Table A36 is a summary of the results for all seven plants (29 extracts) where the %inhibition is shown as an average.

Table A30. Plate 1 lay-out for tyrosinase assay where everything is done in triplicates. The blank has no enzyme = 0% inhibition and max activity = 100% inhibition. The blank and max activity are done for both 2% DMSO (to be used for the reference) and for 10% DMSO (to be used for the extracts). The reference is Kojic acid 156 µM.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Blank:** 70 µL blank (2% DMSO) + 30 µL buffer + 110 µL Sub	**Blank:** 70 µL blank (2% DMSO) + 30 µL buffer + 110 µL Sub	**Blank:** 70 µL blank (2% DMSO) + 30 µL buffer + 110 µL Sub	2,1 µL S4 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S4 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S4 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S12 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S12 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S12 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S20 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S20 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S20 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub
B	**Blank:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL buffer + 110 µL Sub	**Blank:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL buffer + 110 µL Sub	**Blank:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL buffer + 110 µL Sub	2,1 µL S5 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S5 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S5 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S13 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S613 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S613 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub			
C	**Max Activity:** 70 µL blank (2% DMSO) + 30 µL enzyme + 110 µL Sub	**Max Activity:** 70 µL blank (2% DMSO) + 30 µL enzyme + 110 µL Sub	**Max Activity:** 70 µL blank (2% DMSO) + 30 µL enzyme + 110 µL Sub	2,1 µL S6 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S6 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S6 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S14 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S614 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S614 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub			
D	**Max Activity:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	**Max Activity:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	**Max Activity:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S7 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S7 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S7 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S15 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S615 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S615 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub			
E	**Reference:** 70 µL Kojic Acid + 30 µL enzyme + 110 µL Sub	**Reference:** 70 µL Kojic Acid + 30 µL enzyme + 110 µL Sub	**Reference:** 70 µL Kojic Acid + 30 µL enzyme + 110 µL Sub	2,1 µL S8 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S8 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S8 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S16 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S16 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S16 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub			
F	2,1 µL S1 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S1 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S1 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S9 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S9 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S9 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S17 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S17 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S17 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub			
G	2,1 µL S2 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S2 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S2 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S10 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S10 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S10 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S18 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S18 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S18 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub			
H	2,1 µL S3 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S3 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S3 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S11 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S11 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S11 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S19 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S19 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S19 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub			

Table A31. Results for inhibition from tyrosinase screening (n=1) according to the plate lay-out in table A30.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,04	0,041	0,04	0,45	0,449	0,456	0,496	0,488	0,676	0,43	0,565	0,464	492
B	0,041	0,041	0,042	0,47	0,466	0,461	0,53	0,483	0,503	0,048	0,048	0,048	492
C	0,522	0,476	0,508	0,477	0,469	0,519	0,618	0,565	0,556	0,048	0,048	0,048	492
D	0,508	0,547	0,563	0,671	0,561	0,735	0,569	0,563	0,57	0,048	0,048	0,048	492
E	0,072	0,074	0,075	0,589	0,56	0,646	0,513	0,603	0,625	0,049	0,048	0,048	492
F	0,599	0,608	0,597	0,311	0,3	0,33	0,71	0,566	0,614	0,048	0,048	0,048	492
G	0,662	0,666	0,665	0,45	0,402	0,47	0,577	0,657	0,651	0,049	0,048	0,048	492
H	0,551	0,537	0,535	0,512	0,535	0,501	0,613	0,582	0,682	0,048	0,049	0,049	492

Table A32. Calculation of %Inhibition for the extracts analyzed in plate 1 (n=1), see table A30. The %inhibition that are colored light brown represent the extracts showing medium activity.

	Abs 1	Abs 1 - Average blank	Abs 2	Abs 2 - Average blank	Abs 3	Abs 3 - Average blank	Average buffer and average abs - blank	StdDev	%Inhibition 1 calculated from equation 4	%Inhibition 2 calculated from equation 4	%Inhibition 3 calculated from equation 4	%Inhibition Average	StdDev
Blank ref	0,04		0,041		0,04		0,040		91,7	90,6	91,4	91,2	0,6
Blank samples	0,041		0,041		0,042		0,041		91,2	91,9	91,9	91,7	0,4
Max Activity ref	0,522	0,482	0,476	0,436	0,508	0,468	0,462						
Max Activity samples	0,508	0,467	0,547	0,506	0,563	0,522	0,498						
Reference: Kojic Acid	0,072	0,032	0,074	0,034	0,075	0,035	0,033	0,00	93,1	92,7	92,5	92,8	0,3
Kale green juice	0,599	0,558	0,608	0,567	0,597	0,556	0,560	0,01	-12,0	-13,8	-11,6	-12,4	1,2
Kale brown juice	0,662	0,621	0,666	0,625	0,665	0,624	0,623	0,00	-24,6	-25,4	-25,2	-25,1	0,4
Lucerne green juice	0,551	0,510	0,537	0,496	0,535	0,494	0,500	0,01	-2,3	0,5	0,9	-0,3	1,8
Lucerne brown juice	0,45	0,409	0,449	0,408	0,456	0,415	0,410	0,00	17,9	18,1	16,7	17,6	0,8
Lucerne white protein	0,47	0,429	0,466	0,425	0,461	0,420	0,424	0,00	13,9	14,7	15,7	14,8	0,9
Sugar beet fiber	0,477	0,436	0,469	0,428	0,519	0,478	0,447	0,03	12,5	14,1	4,1	10,2	5,4
Sugar beet green juice	0,671	0,630	0,561	0,520	0,735	0,694	0,614	0,09	-26,4	-4,4	-39,3	-23,4	17,7
Sugar beet brown juice	0,589	0,548	0,56	0,519	0,646	0,605	0,557	0,04	-10,0	-4,1	-21,4	-11,8	8,8
Phacelia green juice	0,311	0,270	0,3	0,259	0,33	0,289	0,272	0,02	45,9	48,1	42,0	45,3	3,0
Phacelia brown juice	0,45	0,409	0,402	0,361	0,47	0,429	0,399	0,03	17,9	27,6	13,9	19,8	7,0
Phacelia white juice	0,512	0,471	0,535	0,494	0,501	0,460	0,475	0,02	5,5	0,9	7,7	4,7	3,5
Crimson clover fiber	0,496	0,455	0,488	0,447	0,676	0,635	0,512	0,11	8,7	10,3	-27,4	-2,8	21,3
Crimson clover green juice	0,53	0,489	0,483	0,442	0,503	0,462	0,464	0,02	1,9	11,3	7,3	6,8	4,7
Crimson clover green protei	0,618	0,577	0,565	0,524	0,556	0,515	0,538	0,03	-15,8	-5,2	-3,3	-8,1	6,7
Crimson clover brown juice	0,569	0,528	0,563	0,522	0,57	0,529	0,526	0,00	-6,0	-4,8	-6,2	-5,6	0,8
Crimson clover white protei	0,513	0,472	0,603	0,562	0,625	0,584	0,539	0,06	5,3	-12,8	-17,2	-8,2	11,9
Crimson clover white juice	0,71	0,669	0,566	0,525	0,614	0,573	0,589	0,07	-34,3	-5,4	-15,0	-18,2	14,7
Oil radish green juice	0,577	0,536	0,657	0,616	0,651	0,610	0,587	0,04	-7,6	-23,6	-22,4	-17,9	8,9
Oil radish brown juice	0,613	0,572	0,582	0,541	0,682	0,641	0,584	0,05	-14,8	-8,6	-28,6	-17,3	10,3
Oil radish white juice	0,43	0,389	0,565	0,524	0,464	0,423	0,445	0,07	22,0	-5,2	15,1	10,6	14,1

Table A33. Plate 2 lay-out for tyrosinase assay where everything is done in triplicates. The blank has no enzyme = 0% inhibition and max activity = 100% inhibition. The blank and max activity are done for both 2% DMSO (to be used for the reference) and for 10% DMSO (to be used for the extracts). The reference is Kojic acid 156 µM.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Blank:** 70 µL blank (2% DMSO) + 30 µL buffer + 110 µL Sub	**Blank:** 70 µL blank (2% DMSO) + 30 µL buffer + 110 µL Sub	**Blank:** 70 µL blank (2% DMSO) + 30 µL buffer + 110 µL Sub	2,1 µL S24 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S24 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S24 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub						
B	**Blank:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL buffer + 110 µL Sub	**Blank:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL buffer + 110 µL Sub	**Blank:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL buffer + 110 µL Sub	2,1 µL S25 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S25 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S25 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub						
C	**Max Activity:** 70 µL blank (2% DMSO) + 30 µL enzyme + 110 µL Sub	**Max Activity:** 70 µL blank (2% DMSO) + 30 µL enzyme + 110 µL Sub	**Max Activity:** 70 µL blank (2% DMSO) + 30 µL enzyme + 110 µL Sub	2,1 µL S26 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S26 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S26 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub						
D	**Max Activity:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	**Max Activity:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	**Max Activity:** 2,1 µL DMSO + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S27 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S27 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S27 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub						
E	**Reference:** 70 µL Kojic Acid + 30 µL enzyme + 110 µL Sub	**Reference:** 70 µL Kojic Acid + 30 µL enzyme + 110 µL Sub	**Reference:** 70 µL Kojic Acid + 30 µL enzyme + 110 µL Sub	2,1 µL S28 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S28 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S28 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub						
F	2,1 µL S21 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S21 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S21 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S29 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S29 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S29 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub						
G	2,1 µL S22 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S22 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S22 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub									
H	2,1 µL S23 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S23 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub	2,1 µL S23 + 67,9 µL Buffer + 30 µL enzyme + 110 µL Sub									

Table A34. Results for inhibition from tyrosinase screening (n=1) according to the plate lay-out in table A33.

	1	2	3	4	5	6	7	8	9	10	11	12	
A	0,04	0,041	0,041	0,493	0,508	0,555	0,049	0,048	0,048	0,048	0,048	0,048	492
B	0,04	0,04	0,041	0,515	0,52	0,515	0,048	0,049	0,048	0,048	0,048	0,048	492
C	0,705	0,478	0,609	0,491	0,488	0,55	0,051	0,049	0,048	0,049	0,048	0,048	492
D	0,666	0,672	0,504	0,592	0,59	0,612	0,049	0,048	0,048	0,048	0,048	0,048	492
E	0,087	0,082	0,078	0,626	0,582	0,602	0,048	0,048	0,048	0,049	0,048	0,048	492
F	0,559	0,548	0,563	0,59	0,598	0,628	0,049	0,048	0,048	0,048	0,048	0,048	492
G	0,625	0,666	0,691	0,048	0,048	0,048	0,049	0,048	0,048	0,05	0,049	0,047	492
H	0,858	0,855	0,898	0,047	0,048	0,048	0,048	0,049	0,048	0,048	0,048	0,048	492

Table A35. Calculation of %Inhibition for the extracts analyzed in plate 2 (n=1), see table A33. The %inhibition that are colored light brown represent the extracts showing medium activity although no extract has medium activity when looking at the average values.

	Abs 1	Abs 1 - Average blank	Abs 2	Abs 2 - Average blank	Abs 3	Abs 3 - Average blank	Average buffer and average abs - blank	StdDev	%Inhibition 1 calculated from equation 4	%Inhibition 2 calculated from equation 4	%Inhibition 3 calculated from equation 4	%Inhibition Average	StdDev
Blank ref	0,04		0,041		0,041		0,041						
Blank samples	0,04		0,04		0,041		0,040						
Max Activity ref	0,705	0,664	0,478	0,437	0,609	0,568	0,557						
Max Activity samples	0,666	0,626	0,672	0,632	0,504	0,464	0,574						
Reference: Kojic Acid	0,087	0,046	0,082	0,041	0,078	0,037	0,042	0,005	91,7	92,6	93,3	92,5	0,8
Kale fiber	0,559	0,519	0,548	0,508	0,563	0,523	0,516	0,008	9,6	11,5	8,9	10,0	1,4
Lucerne fiber	0,625	0,585	0,666	0,626	0,691	0,651	0,620	0,033	-1,9	-9,1	-13,4	-8,1	5,8
Lucerne Leaves	0,858	0,818	0,855	0,815	0,898	0,858	0,830	0,024	-42,5	-42,0	-49,5	-44,7	4,2
Phacelia fiber	0,493	0,453	0,508	0,468	0,555	0,515	0,478	0,032	21,1	18,5	10,3	16,6	5,6
Phacelia green protein	0,515	0,475	0,52	0,480	0,515	0,475	0,476	0,003	17,3	16,4	17,3	17,0	0,5
Oil radish fiber	0,491	0,451	0,488	0,448	0,55	0,510	0,469	0,035	21,4	22,0	11,2	18,2	6,1
Oil radish green protein	0,592	0,552	0,59	0,550	0,612	0,572	0,558	0,012	3,8	4,2	0,3	2,8	2,1
Red beet brown juice	0,626	0,586	0,582	0,542	0,602	0,562	0,563	0,022	-2,1	5,6	2,1	1,9	3,8
Red beet green protein	0,59	0,550	0,598	0,558	0,628	0,588	0,565	0,020	4,2	2,8	-2,4	1,5	3,5

Table A36. Summary of results from the tyrosinase inhibition analysis where the average %inhibition is shown next to the average standard deviation. The %inhibition that is colored light brown represent the extract showing medium activity.

Extracts	%Inhibition Average	StdDev
Kale green juice	-12,4	1,2
Kale brown juice	-25,1	0,4
Lucerne green juice	-0,3	1,8
Lucerne brown juice	17,6	0,8
Lucerne white protein	14,8	0,9
Sugar beet fiber	10,2	5,4
Sugar beet green juice	-23,4	17,7
Sugar beet brown juice	-11,8	8,8
Phacelia green juice	45,3	3,0
Phacelia brown juice	19,8	7,0
Phacelia white juice	4,7	3,5
Crimson clover fiber	-2,8	21,3
Crimson clover green juice	6,8	4,7
Crimson clover green protei	-8,1	6,7
Crimson clover brown juice	-5,6	0,8
Crimson clover white protei	-8,2	11,9
Crimson clover white juice	-18,2	14,7
Oil radish green juice	-17,9	8,9
Oil radish brown juice	-17,3	10,3
Oil radish white juice	10,6	14,1
Kale fiber	10	1,4
Lucerne fiber	-8,1	5,8
Lucerne Leaves	-44,7	4,2
Phacelia fiber	16,6	5,6
Phacelia green protein	17	0,5
Oil radish fiber	18,2	6,1
Oil radish green protein	2,8	2,1
Red beet brown juice	1,9	3,8
Red beet green protein	1,5	3,5

Tables A37-A40 represent the KLK5 inhibition experiment that was performed for all seven plants (29 extracts) n=1 time. Table A37 shows the plate lay out, table A38 and A39 are the absorption results for the pre- and full readings retrieved from the spectrophotometer microplate reader and can be compared to plate lay-out. Table A40 is the calculations where the absorbances are converted to %inhibition using equation 5 in section 4.5.

Table A37. Plate lay-out for KLK5 assay where everything is done in triplicates. The blank has no enzyme = 0% inhibition and max activity = 100% inhibition. The reference is SBTI 50 µM.

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Blank:** 1 µL DMSO + 50 µL buffer + 50 µL Sub	**Blank:** 1 µL DMSO + 50 µL buffer + 50 µL Sub	**Blank:** 1 µL DMSO + 50 µL buffer + 50 µL Sub	**Extract 6** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 6** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 6** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 14** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 14** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 14** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 22** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 22** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 22** 1 µL extract + 50 µL enzyme + 50 µL Sub
B	**Max Activity:** **1** µL DMSO + 50 µL enzyme + 50 µL Sub	**Max Activity:** 1 µL DMSO + 50 µL enzyme + 50 µL Sub	**Max Activity:** 1 µL DMSO + 50 µL enzyme + 50 µL Sub	**Extract 7** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 7** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 7** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 15** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 15** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 15** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 23** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 23** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 23** 1 µL extract + 50 µL enzyme + 50 µL Sub
C	**Reference:** 1 µL SBTI + 50 µL enzyme + 50 µL Sub	**Reference:** 1 µL SBTI + 50 µL enzyme + 50 µL Sub	**Reference:** 1 µL SBTI + 50 µL enzyme + 50 µL Sub	**Extract 8** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 8** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 8** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 16** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 16** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 16** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 24** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 24** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 24** 1 µL extract + 50 µL enzyme + 50 µL Sub
D	**Extract 1** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 1** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 1** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 9** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 9** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 9** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 17** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 17** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 17** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 25** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 25** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 25** 1 µL extract + 50 µL enzyme + 50 µL Sub
E	**Extract 2** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 1** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 1** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 10** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 10** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 10** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 18** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 18** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 18** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 26** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 26** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 26** 1 µL extract + 50 µL enzyme + 50 µL Sub
F	**Extract 3** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 3** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 3** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 11** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 11** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 11** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 19** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 19** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 19** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 27** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 27** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 27** 1 µL extract + 50 µL enzyme + 50 µL Sub
G	**Extract 4** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 4** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 4** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 12** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 12** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 12** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 20** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 20** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 20** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 28** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 28** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 28** 1 µL extract + 50 µL enzyme + 50 µL Sub
H	**Extract 5** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 5** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 5** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 13** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 13** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 13** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 21** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 21** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 21** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 29** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 29** 1 µL extract + 50 µL enzyme + 50 µL Sub	**Extract 29** 1 µL extract + 50 µL enzyme + 50 µL Sub

Table A38. Results for pre-reading from KLK5 screening (n=1) according to the plate lay-out in table A37.

					Pre-reading								
	1	2	3	4	5	6	7	8	9	10	11	12	
A	49	57	58	69	73	76	84	81	92	142	149	151	380,46
B	58	49	56	87	102	83	96	89	98	111	105	100	380,46
C	59	59	52	94	74	87	92	74	81	76	90	81	380,46
D	85	72	80	70	73	76	103	85	104	64	67	73	380,46
E	68	61	65	70	70	61	93	77	92	73	62	77	380,46
F	112	115	104	77	81	86	85	71	81	76	73	74	380,46
G	127	111	94	67	76	80	101	91	102	64	75	82	380,46
H	176	185	161	72	91	89	68	65	72	98	99	86	380,46

Table A39. Results for full-reading from KLK5 screening (n=1) according to the plate lay-out in table A37.

					Full reading								
	1	2	3	4	5	6	7	8	9	10	11	12	
A	72	73	74	3410	3181	3070	2109	2267	2140	913	956	871	380,46
B	1424	1500	1405	2008	2048	2086	2287	2408	2405	1154	1068	958	380,46
C	70	82	97	787	1286	1460	1513	1563	1416	1187	1502	1407	380,46
D	2032	1983	2028	1583	1604	1717	2172	2209	2109	1287	1390	1221	380,46
E	1558	1417	1436	1640	1359	1619	2193	2141	1994	1502	1692	1421	380,46
F	1929	1760	1884	1589	1986	1951	2112	2080	2009	1396	1615	1542	380,46
G	1669	2089	1902	1337	1294	1161	1928	2123	1794	1365	1710	1569	380,46
H	1401	1329	1567	2183	2481	2382	1778	1425	1516	1613	1647	1528	380,46

Table A40. Calculation of %inhibition of KLK5 from pre-reading and full reading. The average %inhibition and standard deviation for each extract is shown to the right.

	Sample	FI from pre-reading			FI from full reading			FI from full reading - FI from pre reading (x)			Average	Values from (x) - Average from blank			Average	%Inhibition 1 calculated from equation 5	%Inhibition 2 calculated from equation 5	%Inhibition 3 calculated from equation 5	Average %Inhibition	StDev
	Blank	49	57	58	72	73	74	23	16	16	18,3									
	Max activity	58	49	56	1424	1500	1405	1366	1451	1349		1348	1433	1331	1370					
	Control	59	59	52	70	82	97	11	23	45		-7	5	27		100,5	99,7	98,1	99,4	1,3
S1	Kale green juice	85	72	80	2032	1983	2028	1947	1911	1948		1929	1893	1930		-40,7	-38,1	-40,8	-39,9	1,5
S2	Kale brown juice	68	61	65	1558	1417	1436	1490	1356	1371		1472	1338	1353		-7,4	2,4	1,3	-1,2	5,4
S3	Lucerne green juice	112	115	104	1929	1760	1884	1817	1645	1780		1799	1627	1762		-31,3	-18,7	-28,6	-26,2	6,6
S4	Lucerne brown juice	127	111	94	1669	2089	1902	1542	1978	1808		1524	1960	1790		-11,2	-43,0	-30,6	-28,3	16,0
S5	Lucerne white protein	176	185	161	1401	1329	1567	1225	1144	1406		1207	1126	1388		11,9	17,9	-1,3	9,5	9,8
S6	Sugar beet fiber	69	73	76	3410	3181	3070	3341	3108	2994		3323	3090	2976		-142,5	-125,5	-117,1	-128,4	12,9
S7	Sugar beet green juice	87	102	83	2008	2048	2086	1921	1946	2003		1903	1928	1985		-38,8	-40,7	-44,8	-41,4	3,1
S8	Sugar beet brown juice	94	74	87	787	1286	1460	693	1212	1373		675	1194	1355		50,8	12,9	1,1	21,6	25,9
S9	Phacelia green juice	70	73	76	1583	1604	1717	1513	1531	1641		1495	1513	1623		-9,1	-10,4	-18,4	-12,6	5,1
S10	Phacelia brown juice	70	70	61	1640	1359	1619	1570	1289	1558		1552	1271	1540		-13,2	7,3	-12,4	-6,1	11,6
S11	Phacelia white juice	77	81	86	1589	1986	1951	1512	1905	1865		1494	1887	1847		-9,0	-37,7	-34,8	-27,1	15,8
S12	Crimson clover fiber	67	76	80	1337	1294	1161	1270	1218	1081		1252	1200	1063		8,7	12,5	22,5	14,5	7,1
S13	Crimson clover green juice	72	91	89	2183	2481	2382	2111	2390	2293		2093	2372	2275		-52,7	-73,1	-66,0	-63,9	10,3
S14	Crimson clover green protein	84	81	92	2109	2267	2140	2025	2186	2048		2007	2168	2030		-46,4	-58,2	-48,1	-50,9	6,4
S15	Crimson clover brown juice	96	89	98	2287	2408	2405	2191	2319	2307		2173	2301	2289		-58,6	-67,9	-67,0	-64,5	5,2
S16	Crimson clover white protein	92	74	81	1513	1563	1416	1421	1489	1335		1403	1471	1317		-2,4	-7,3	3,9	-1,9	5,6
S17	Crimson clover white juice	103	85	104	2172	2209	2109	2069	2124	2005		2051	2106	1987		-49,6	-53,7	-45,0	-49,4	4,3
S18	Oil radish green juice	93	77	92	2193	2141	1994	2100	2064	1902		2082	2046	1884		-51,9	-49,3	-37,5	-46,2	7,7
S19	Oil radish brown juice	85	71	81	2112	2080	2009	2027	2009	1928		2009	1991	1910		-46,6	-45,3	-39,4	-43,7	3,8
S20	Oil radish white juice	101	91	102	1928	2123	1794	1827	2032	1692		1809	2014	1674		-32,0	-46,9	-22,1	-33,7	12,5
S21	Kale fiber	68	65	72	1778	1425	1516	1710	1360	1444		1692	1342	1426		-23,4	2,1	-4,0	-8,5	13,3
S22	Lucerne fiber	142	149	151	913	956	871	771	807	720		753	789	702		45,1	42,4	48,8	45,4	3,2
S23	Lucerne Leaves	111	105	100	1154	1068	958	1043	963	858		1025	945	840		25,2	31,1	38,7	31,7	6,8
S24	Phacelia fiber	76	90	81	1187	1502	1407	1111	1412	1326		1093	1394	1308		20,3	-1,7	4,6	7,7	11,3
S25	Phacelia green protein	64	67	73	1287	1390	1221	1223	1323	1148		1205	1305	1130		12,1	4,8	17,6	11,5	6,4
S26	Oil radish fiber	73	62	77	1502	1692	1421	1429	1630	1344		1411	1612	1326		-2,9	-17,6	3,3	-5,8	10,7
S27	Oil radish green protein	76	73	74	1396	1615	1542	1320	1542	1468		1302	1524	1450		5,0	-11,2	-5,8	-4,0	8,2
S28	Red beet brown juice	64	75	82	1365	1710	1569	1301	1635	1487		1283	1617	1469		6,4	-18,0	-7,2	-6,3	12,2
S29	Red beet green protein	98	99	86	1613	1647	1528	1515	1548	1442		1497	1530	1424		-9,2	-11,6	-3,9	-8,2	4,0