DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Volatile organosulfur compounds from plants:
biological properties"

verfasst von / submitted by:

Augustin Seremet

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of:

Magister der Pharmazie (Mag. Pharm.)

Wien, 2020 / Vienna, 2020

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	*UA 449*
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	*Diplomstudium Pharmazie*
Betreut von / Supervisor:	*Univ.-Prof. Mag. pharm. Dr. Gerhard Buchbauer*

Danksagung

An erster Stelle möchte ich meinem Betreuer, Herr Prof. Dr. Buchbauer danken, der mich richtungsweisend und mit Geduld während meiner Arbeit betreut hat.

Danke auch an meine Freunde bzw. Studienkollegen für die gegenseitige Unterstützung und Zusammenarbeit im Labor.

Besonderer Dank kommt meinen Eltern zu, nicht nur für ihre finanzielle Unterstützung, sondern auch für die gegebene Zuversicht, auch in schwierigen Phasen nicht aufzugeben.

Genauso viel Lob und Dank verdient meine liebevolle Ehefrau für ihren Beistand auch an den nicht so schönen Tagen, und die konsequente Unterstützung, ohne die ein solcher Weg nicht möglich wäre.

Abstract

Volatile sulfur compounds (VSC), from a biological point of view, are an exciting group of compounds that originate from different sources in nature. Of great interest from the pharmaceutical and medical point of view are the VSCs that originate from plants. In part, the positive effects of various medicinal plants are explained by the effects of VSC. So far, a large number of VSC from plants are known and found in a large number of plant families and species. However, not all VSC possess significant biological effects. The the most productive plants when considering the concentration and number of different VSC are plants from the Brassicaceae Burnett and Amaryllidaceae families. In addition to these two families, biologically active sulfur compounds are also isolated from other families. This paper systematizes the most important plant families with the corresponding VSC and a depiction of the important biological effects for each compound. These substances have been shown to have antioxidant, antiproliferative, proapoptotic, as well as direct anticancer effects.

Zusammenfassung

Flüchtige Schwefelverbindungen (VSC) sind aus biologischer Sicht eine aufregende Gruppe von Verbindungen, die aus verschiedenen Quellen in der Natur stammen. Aus pharmazeutischer und medizinischer Sicht von großem Interesse sind VSCs, die aus Pflanzen stammen. Zum Teil werden die positiven Wirkungen verschiedener Heilpflanzen durch die Wirkungen von VSC erklärt. Bisher ist eine große Anzahl von VSC aus Pflanzen bekannt und kommt in einer großen Anzahl von Pflanzenfamilien und -arten vor. Allerdings besitzen nicht alle VSC signifikante biologische Wirkungen. Die produktivsten Pflanzen unter Berücksichtigung der Konzentration und Anzahl der verschiedenen VSC sind Pflanzen aus den Familien Brassicaceae Burnett und Amaryllidaceae. Neben diesen beiden Familien werden auch biologisch aktive Schwefelverbindungen aus anderen Familien isoliert. In diesem Artikel werden die wichtigsten Pflanzenfamilien mit dem entsprechenden VSC und einer Schilderung der wichtigen biologischen Wirkungen für jede Verbindung systematisiert. Es wurde gezeigt, dass diese Substanzen antioxidative, antiproliferative, proapoptotische sowie direkte Antikrebseffekte haben.

CONTENTS

1. General part 6
1.1 Sulfur compounds 6
1.2 Volatile sulfur compounds (VSC) 6
1.3 Chemical structure of volatile sulfur compounds 7
1.4 Classification of volatile sulfur compounds 8
1.5 Glucosinolates (GLS) 11
1.6 Methods of isolation of volatile compounds 12
1.6.1 Distillation 13
1.6.2 Extraction 14
1.7 Methods for identifying volatile sulfur compounds 15
1.7.1 GC-MS 15
1.7.2 Fourier Transform InfraRed (FTIR) 16
1.7.3 Other methods 17
2. Biological properties of volatile sulfur compounds isolated from plants 18
2.1 Brassicaceae Burnett family 19
2.1.1 Isothiocyanates 21
2.2 Amaryllidaceae family 27
2.3 Moringaceae family 32
2.4 Rosaceas family 33
2.5 Caricaceae family 34
2.6 Capparaceae family 34
2.7 Rutaceae family 36
2.8 Resedaceae family 36
2.9 Solanaceae family 37
3. Conclusion 38
References 39

1. General part

1.1 Sulfur compounds

Sulfur compounds are widespread in the kingdom of animals and plants; they are found in fungi as well as in microorganisms, primarily bacteria. Bacteria, among other things, convert inorganic sulfur into hydrogen sulfide (H_2S), which shows many effects [1]. Interestingly, over a hundred genes found in *E. coli* are involved in sulfur metabolism [1, 2], which goes to show how vital sulfur compounds are in the Bacteria domain. Sulfur compounds also play an essential role in the realm of fungi, and several sulfur compounds can be isolated from them [3].

Despite its prevalence in almost all kingdoms, sulfur compounds are at the most prevalent in plants. In some plants, they are only found in traces, and on the other hand, there are plants in which a considerable amount of these compounds is present. An example of such plants is *Allium sativum*. Sulfur compounds are contained in different parts of the plant - leaves, flowers, seeds, stems, but also in the roots.[4]. The concentration is influenced by the way the plants are grown, as well as by the climate zone in which they grow.

1.2 Volatile sulfur compounds (VSC)

In plants, sulfur-containing metabolites have a structure varying from the primary (sulfur-containing amino acids) to secondary metabolites like glucosinolates [4]. Some of these metabolites are non-polar with low molecular weight, and are therefore often volatile. Like other sulfur compounds, volatile compounds of this element are widely distributed in the living world. It should be noted that, for the most part, these compounds are found in the plant world. It is estimated that as many as 43 plant families (173 genera) contain some of the volatile sulfur compounds [4]. Also, the highest variability in chemical structure is observed in terrestrial plants [4].

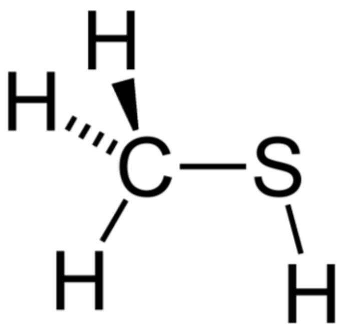
As noted at the beginning of this paper, sulfur compounds and thus, VSC can be isolated from all parts of the plant.

VSC play a significant role in maintaining homeostasis in the human body [4]. Among other things, these compounds have antibacterial (e.g., allicin which inhibits DNA gyrase in bacteria) [5], antioxidant (e.g., thiosulphates which can also have antibacterial effects) [6], antiproliferative (e.g., diallyl disulfide which, among other things, possesses antiproliferative effects on HL-60 cells) [7], apoptotic (e.g., diallyl disulfide and HL-60 cells) [7], as well as an anticancer role (e.g., isothiocyanate prevents recurrence of prostate cancer) [8].

1.3 Chemical structure of volatile sulfur compounds

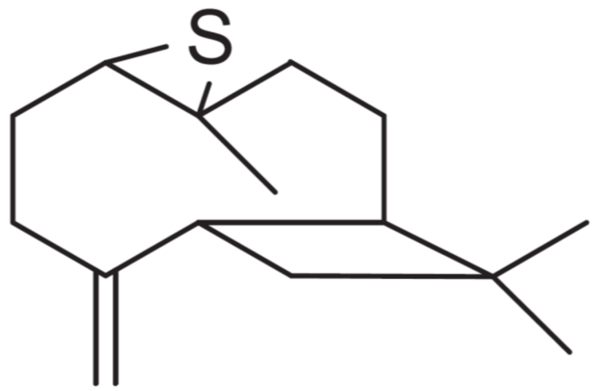
VSC can be simple chemical structures, but there are also compounds with complex structures. Compounds with more complex structures usually have other heteroatoms incorporated into the structure (e.g., nitrogen). The chemical structure of simple, essential VSC can best be understood by the example of methanethiol (Figure 1) or dimethyl sulfide.

Fig 1. Chemical structure of methanethiol



Examples of VSC with a more complex chemical structure are 5-(4-acetoxy-1-butynyl)-2,2’-bithiophene and 4,5-epithiocaryophyllene (Figure 2). Various VSC are found in different concentrations in different plants. Thus, compounds such as dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide are very often present in different plants, while on the other hand, compounds such as ethyl disulfide are found only in a small number of plants [4].

Fig 2. Chemical Structure of 4,5-epithiocaryophyllene [4]



1.4 Classification of volatile sulfur compounds

There are different classifications of VSC in the scientific literature. The most common one is based on the number of sulfur atoms in a given compound [4]. In simplest terms, VSC can be divided into four groups:

1. **S1 – containing one sulfur atom (S1)**

2. **S2 – containing two sulfur atoms (S2)**

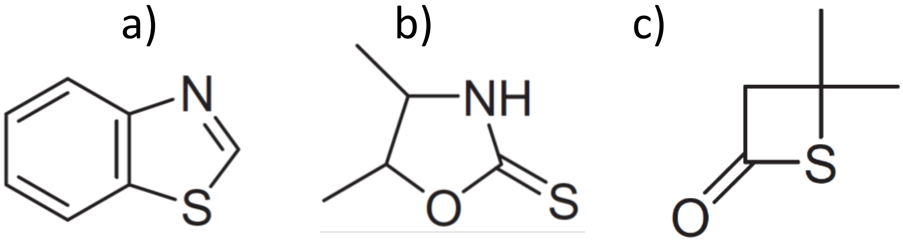
3. **S3 – containing three sulfur atoms (S3)**

4. **S4 – four sulfur atoms or more (S4)**

Additionally, the S1 and S2 groups are divided into subgroups depending on the number of oxygen and nitrogen atoms that make up this compound. Thus, the S1 group is divided into three subgroups (Figure 3):

a) Compounds with one / two **N** atoms (e.g., benzothiazole)
b) Compounds with one **N** atom and one **O** atom (e.g., 4,5-dimethyl oxazolidine-2-thione)
c) Compounds with one / two **O** atoms (e.g., 4,4-dimethyl thiacyclobutane-2-one)

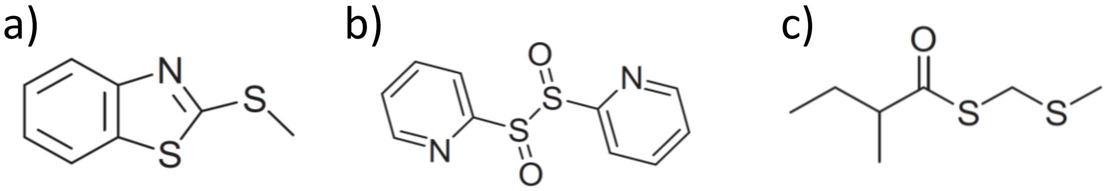
Fig 3. Volatile sulfur compounds group S1 [4]



Group S2 subgroups (Fig 4):

a) Compounds with one / two **N** atoms (e.g., 2-methyl thiobenzothiazole)
b) Compounds with one **N** atom and one **O** atom (e.g., 2-dipyridyl disulfinate)
c) Compounds with one / two **O** atoms (e.g., methyl thiomethyl 2-methyl butanethioate)

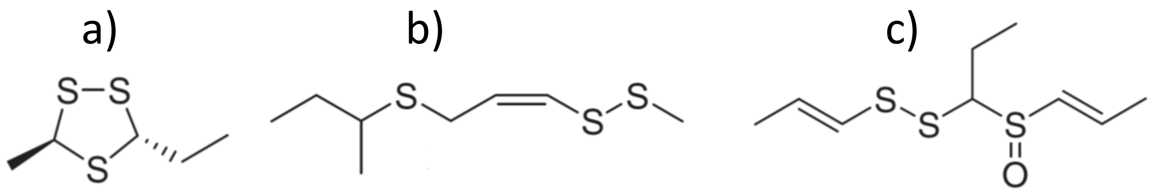
Fig 4. VSC of group S2 [4]



Group S3 is further subdivided into subgroups based on the presence of oxygen in the structure of these compounds, as well as on their structure. Thereby, the S3 group is divided into the following subgroups (Figure 5):

a) Compounds with cyclic structure (eg, trans-3-ethyl-5-methyl-1,2,4-trithiolane)
b) Compounds with a linear structure (eg 1- (methyldithio) -3- (sec-butylthio) -(*Z*) -propane)
c) Compounds with different numbers of **O** atoms (eg 1- (1- (*E*) -propenylsulfinyl) -1- (1- (*E*) -propenyldithio) propane)

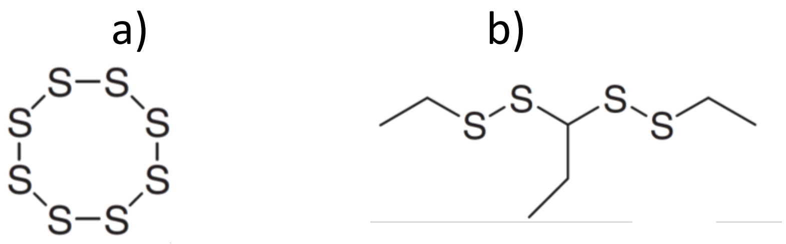
Fig 5. VSCs of group S3 [4]



Like the previous groups, the S4 group is also subdivided. There are two subgroups here that are formed based on the structure of the compounds. The VSC of group S4 are divided into the following subgroups (Figure 6):

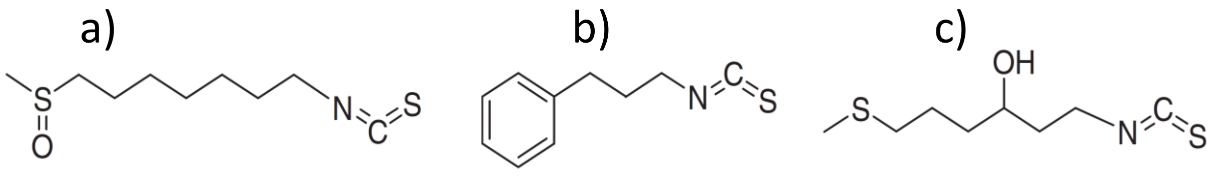
a) Compounds with a cyclic structure (e.g., cyclo-S8 (cyclo-octasulfur)
b) Compounds with a linear structure (e.g., 1- (ethyldithio) -1- (ethylthio) propane)

Fig 6. VSC of group S4 [4]



In addition to the above four groups, which are formed based on the number of sulfur atoms, there is a fifth group that includes thiocyanate and isothiocyanate (Figure 7).

Fig 7. Thiocyanate and isothiocyanate sulfur compounds of group 5 [4]



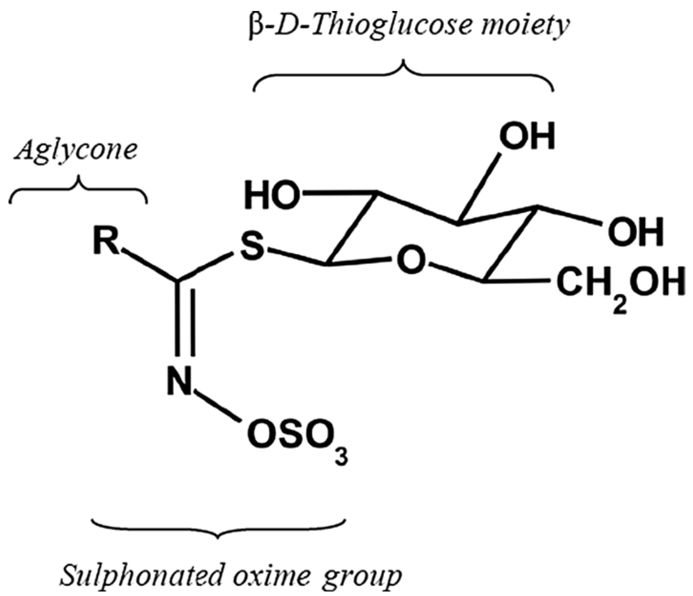
a) 7-(methyl sulfinyl) heptyl isothiocyanate; b) 3-phenylpropyl isothiocyanate; c) 3-hydroxy-6-(methylthio) hexyl isothiocyanate.

1.5 Glucosinolates (GLS)

GLS (β-thioglucoside-N-hydroxysulfates) are water-soluble organic anions formed during amino acid metabolism. All the compounds in this group share a similar structure (Figure 8) and contain sulfur in their structure [9]. But, they are not VSC. However, the breakdown products of many GLS are VSC [9]. To date, over 130 different GLS have been detected from sixteen different dicotyledonous plants [9, 10], and a great deal of information is available in the literature on the isolation of GLS from plants. To a large extent, GLS are found in the Brassicaceae family (e.g. cabbage) [9, 10].

As seen in Figure 8, the GLS are composed of three basic parts: β-*D*-thioglucose, sulfonated oxime groups, and a variable aglycone group. The variable aglycone group, or GLS sidechain, is derived from the metabolites of one of eight amino acids (alanine, leucine, isoleucine, methionine, valine, tryptophan, phenylalanine and tyrosine) [10, 11]. Based on the sidechain, GLS are separated into three main groups: aliphatic (metabolites formed from alanine, leucine, isoleucine, methionine, and valine), aromatic (metabolites formed from phenylalanine and tyrosine) and indole (metabolite formed from tryptophan) [10, 11].

Fig 8. Basic chemical structure of GLS [10]



Interest in these chemical compounds has increased significantly in recent years, as these substances and their degradation products have been found to have fungicidal, bactericidal, and nematocidal effects [11]. Also, research has shown that these substances exert anticancer effects [12, 13].

GLS can be isolated from a large number of plants. As noted above, over 120 compounds from this group have been detected so far, grouped into a large number of different chemical subgroups based on similarities in the constitution. The structure of GLS consists of three distinct parts:

1. β-D-glucopyranose structure

2. O-sulfated anomeric (*Z*) –thiohydroxamate

3. Sidechain

In this overview, only those GLS will be described whose decomposition releases VSC.

1.6 Methods of isolation of volatile compounds

The isolation of VSC is carried out from the roots, stems, leaves, and flowers of the plant. Essential oils, which contain VSC, reach the surface of the plants by a slow process called hydrodiffusion. Therefore, in preparation for isolation, it is necessary to chop the parts of the plants from which we want to isolate the VSC. Also, the plants must be adequately stored before the isolation process. If the plants are treated improperly, the concentration of isolated substances may be lower. Besides, changes in the chemical structure of the substance may occur. As one might imagine, parts that have more water, such as flowers and leaves, will lose more essential oil than seeds, roots, or woody parts that contain less water.

In practice, there are different isolation methods used when trying to isolate different chemical substances from plants. The most commonly used methods for the isolation of VSC are distillation and extraction using organic solvents.

It should be noted that a large number of different VSC can be found in plants and that these compounds may be present in low concentrations. This is very important from a practical point of view since, in addition to isolation, it is necessary to concentrate these chemical substances in order to be able to perform various physical and chemical tests on them. Concentration is performed by simultaneous distillation and extraction where the first VSC are isolated during the distillation process and simultaneously concentrated by the extraction process using an organic solvent or solvent combination.

1.6.1 Distillation

Distillation is one widespread method of isolating various volatile substances. During the distillation, separation of substances from the initial mixture occurs due to the difference in the boiling point. During the distillation process, the organic compounds, which will be in the composition of the essential oils, which are not miscible with water, are evaporated along with the water at temperatures below their boiling point (temperature below 100 ° C). Further, the water vapor condenses, that is, the cooling of the water vapor occurs, and then it becomes a drop. The resulting distillate resulting from the condensation process is collected in another vessel.

The advantage of distillation over other methods of isolation is that distillation does not produce non-volatile substances. In this way, the possibility of the influence of non-volatile substances on further analysis is reduced.

Distillation speed is affected by two processes - hydrodiffusion and hydrolysis. Hydrodiffusion is the process by which essential oils exit the surface of a plant. Oils that are closer to the surface will be distilled faster than those found deep in plants. Therefore, it is necessary to grind the plant well before the distillation process. Hydrolysis is a process that occurs at elevated temperatures when unstable components degrade.

There are several types of distillation, such as simple distillation, fractional distillation, steam distillation, and vacuum distillation. Depending on the contact of plants with water, distillation is divided into several subtypes: water distillation, steam distillation, water-steam distillation.

Of all the subtypes mentioned, the most common and most used method is steam distillation. In this process, the plant material is mixed with water and heated to the boiling point. A variety of apparatus is used for this process. Among others, the Clevenger apparatus, introduced by this scientist back in 1928, should be noted. [14] Many pharmacopoeia now have various diagrams and instructions on how to extract essential oils from plants. The distillers themselves are made up of several parts. First of all, there is a container in which one puts the plants together with water. Then each appliance must have a condenser in which water vapor to liquid is converted. Lastly, there must be a vessel in which distillation products are collected.

1.6.2 Extraction

Extraction is the process by which matter is extracted from plant matter based on the difference in solubility in specific solvents. This procedure can be used to extract oils from the plant material, as well as to isolate volatile compounds from the aqueous solution formed after the distillation process. This process uses volatile and non-volatile solvents that must be selective for what we want to extract, have a low and ideally unique boiling point, be able to and should be dissolved in water, be inert, and preferably cheap and non-flammable.

The following solvents are commonly used for the extraction process: diethyl ether, pentane, chloroform, dichloromethane, as well as other low boiling solvents.

Currently, modern extraction techniques are preferred, which include:

1. Ultrasonic extraction (ultrasonic waves assist the extraction process)
2. Solvent microwave extraction (microwave assist extraction process)
3. Solvent-free microwave extraction (microwave application only)
4. Accelerated solvent extraction
5. CO_2 extraction

1.7 Methods for identifying volatile sulfur compounds

1.7.1 GC-MS

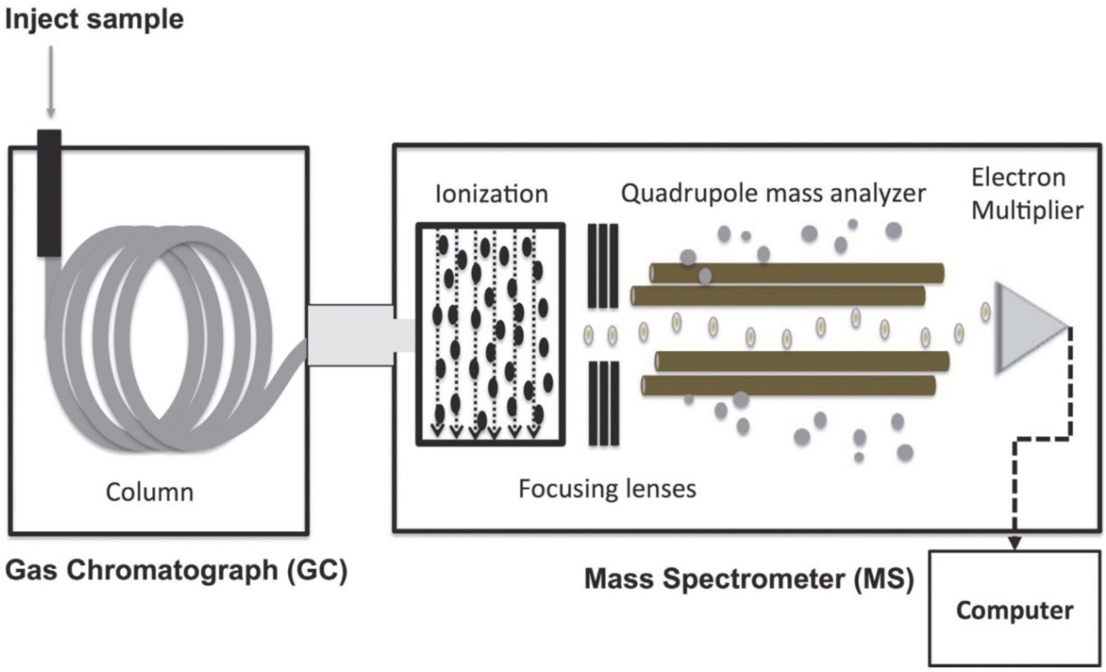
Gas chromatography – mass spectometry is a combination of two methods used to identify different chemical compounds and, among other things, VSC. Notably, MS is a method by which the relative molecular weight of a molecule or chemical compound is determined. In this analytical method, the molecules are converted to ions, which are grouped based on mass. In this way, the mass spectrum is formed, and the name of this method is derived from this. GC is a rapid analytical method by which different constituents are extracted from an existing mixture based on the stationary phase affinity.

Due to their complementarity, the two methods are combined into one. GC is a method of separation and quantification that is not reliable for the identification of complex organic compounds (qualitative determination). MS, on the other hand, supplements GC by being precise in identifying complex organic compounds. With this combined apparatus, maximum data can be obtained using a minimum amount of sample. The fusion of the two methods into one was possible since both works with gas samples.

For each compound identified by this method, characteristic data is obtained, such as the retention time of this compound, i.e., the time required to detect the compound (time of passage through columns), and the peak area that provides information on the amount of this compound. Knowledge of its mass spectrum is necessary to identify a chemical compound.

Usually, the device has a four-pole mass analyzer that quickly captures spectra and monitors compounds coming out of chromatographic columns [15]. The part of the apparatus called collector generates an electronic signal which is amplified and by which we obtain the data that the processor records during the whole process. (Figure 9)

Fig 9. GC-MS [15]



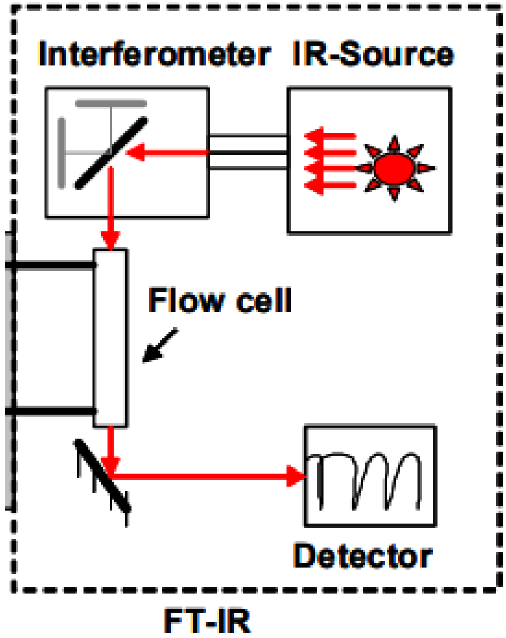
Data shows that VSC originating from plants can be detected by this combined method and that this method is extensively used to detect named compounds [16-18].

1.7.2 Fourier Transform InfraRed (FTIR)

This method is one of the methods used in infrared spectrometry. The basis is the measurement of the electromagnetic spectrum, which is in the range between visible and microwave radiation [19]. Infrared rays have a spectrum of 4000 to 200 cm^{-1} and do not have the energy required to excite electrons. However, infrared radiation causes vibrations, bending, and rotations of covalent bonds, which can be detected by spectrometry.

There is an interferometer in this apparatus, in which the radiation beams are separated into two rays. These two rays are reflected on two mirrors, one fixed and the other continually moving for the length expressed in mm. As the mirror moves, the wavelength of the second beam is continually changing. After reflection, the rays reconnect, and the final ray will be a combination of the two rays (Figure 10).

Fig 10. FTIR schematic [19]



Fourier transform is essentially a mathematical procedure by which the obtained interferogram is decoded and interpreted. The frequencies detected by this method are displayed in cm^{-1} and are called wavenumbers. Frequency spectra are required for the analysis, that is, to identify an individual molecule it is necessary to know the absorption intensity (emission or reflection) for each individual frequency. Therefore, the interferogram cannot be interpreted directly but must be "decoded", which is achieved by a known mathematical technique called Fourier transform. Transformation is performed by a computer that ultimately shows the user the desired spectrum.

This method is used to identify various VSC from plant samples [20, 21]. It is often used in combination with other methods such as GC-MS [20, 21].

1.7.3 Other methods

In addition to these methods, other methods for detecting VSC originating from plants are GC-NMR, multidimensional GC-MS, GS-TOF-MS, and other techniques.

GC-NMR is a technique in which GC is combined with NMR [19]. In this combination, NMR is used for identification, while GC is used for substance separation. The main problem with this technique is the physical condition of the specimen. More specifically, the samples must be in a liquid or solid state [19]. If an analysis were performed with the sample in the gas state, the results obtained would be poorly read. This problem is overcome by introducing into the system computers that increase sensitivity - they can reduce the noise produced while analyzing the results.

Multidimensional GC-MS serves to obtain better peak resolution after gas chromatography. The use of multidimensional gas chromatography will adequately separate the components of the analyzed sample, allowing mass spectrometry to detect even trace compounds [19].

With the development in optical design, electronics, as well as improvements in the processing of received signals, new technologies, and detection techniques have been developed, including the GC-TOF-MS [19]. This method is excellent for identifying organic compounds from a complex medium. Despite its excellent precision and sensitivity, this method has some drawbacks; it is not as good for quantification due to its limited dynamic range.

2. Biological properties of volatile sulfur compounds isolated from plants

In recent years, VSC isolated from plants have been increasingly tested. More specifically, their biological effects are examined. Several studies have been designed to investigate the effect of VSC on the functioning of the immune system, to determine what effects these compounds have on oxidative stress, whether they show antiproliferative, apoptotic activity, and whether these substances can exert direct effects on the onset and progression of tumor disease. In addition to these effects, the effects of VSC on bacterial and fungal diseases are also being investigated. These tests are conducted to identify a chemical compound that will be able to be used in the process of creating future drugs. However, as the most significant attention is paid to investigating the antioxidant, antiproliferative, and antitumor effects of these substances, it is clear that the highest interest of the scientific community is directed towards the detection of substances that could potentially be used in the treatment of cancer patients.

The methods mentioned above can isolate VSC from a large number of plant families. These substances may be present in trace and may also be present in larger quantities. Medicinal plants such as plants from the Brassicaceae and Amaryllidaceae families are abundant in VSC. In addition to these plants, VSC can be found in many other plants.

2.1 Brassicaceae Burnett family

The Brassicaceae Burnett family, or as they are called cabbage family, is one large family comprising about 4000 species of plants from about 350-400 genera [10, 11]. These herbs are widely used, can be used as edible, spicy, or ornamental plants [9-11].

Plants from the Brassicaceae family are among the oldest cultivated crops. The production of plants from this family in Europe is 70 million tons per year [10, 11]. The most known plants of this family are *Brassica oleracea* (broccoli, cabbage cauliflower), *Brassica rapa* (turnips), *Brassica nigra* (black mustard), *Brassica napus* (rapeseed), *Sinapis alba* (white licorice), as well as many other plants.

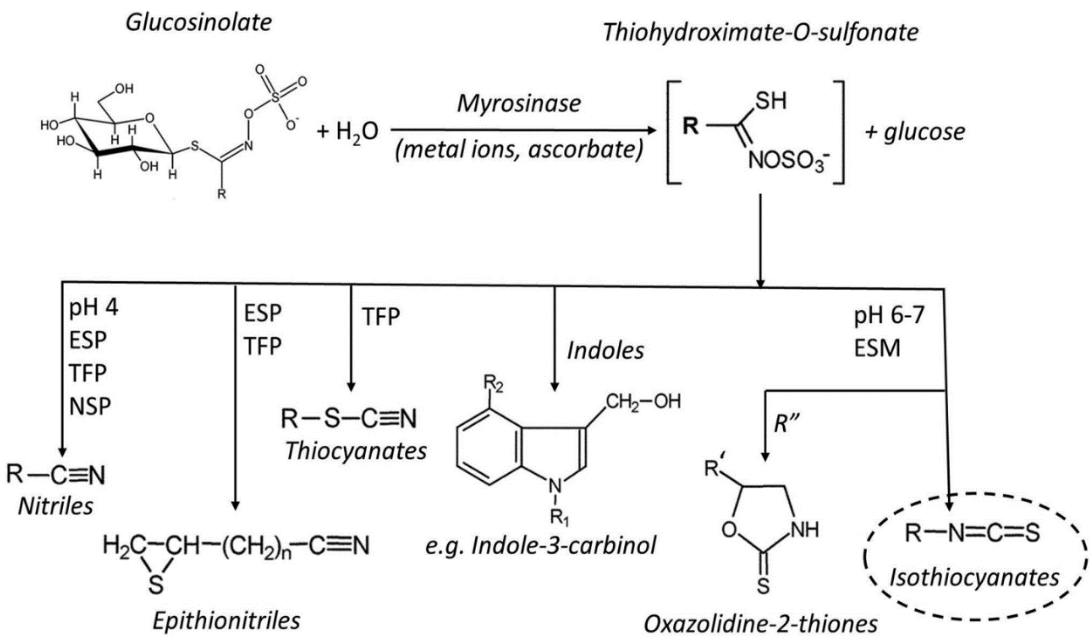
Plants of this family are rich in glucosinolates (GLS), glucoside compounds that contain sulfur [9-11]. When these plants are ground or powdered, the GLS, which become free and exit to the surface of the plant, begin to hydrolyze and release a specific, "burning," sharp component that gives a specific taste and aroma.[9-11].

Although plants of this genus also possess other significant micronutrients, such as tocopherols and carotenoids, most attention is given to GLS. GLS are considered to be a significant component of these plants. GLS, originating from these plants, can influence the occurrence of goiter, affect growth, as well as liver damage in experimental animals. However, epidemiological studies have shown that the intake of these compounds is associated with a reduced risk of tumor formation [9-11] [22]. The greatest protective effect was observed in lung, stomach, colon and rectal cancers [23-28].

Plants of the genus Brassicaceae mainly produce GLS for protection against different pathogens [9-11]. The GLS from these plants are not biologically active [29].

It is necessary to change their structure in order to turn biologically active. Most GLS are not affected by the increase in temperature and are chemically stable. When they undergo hydrolysis, biologically active products are formed [29]. Under normal circumstances, GLS in plants do not decompose, but after damage to the cell membrane, the β-thioglucosidase enzyme is released [29]. This enzyme breaks down glycosides, resulting in the formation of compounds that play a defensive role against plant pathogens, insects, and herbivores, which also damage the cell membrane [9-11] As a consequence of the breakdown of GLS, various compounds such as thiocyanate, isothiocyanate, nitrile, epithionitrile, and oxazolidine are formed. Of the compounds mentioned, three are from the group of VSC (thiocyanate, isothiocyanate, and oxazolidines). GLS metabolism and the formation of these compounds are shown in Figure 11 [30]. Isothiocyanate is usually formed as a product of GLS degradation. However, what chemical compounds will form during GLS degradation depends mainly on the GLS structure itself, the pH of the medium, as well as the presence of different proteins and cofactors [31, 32].

Fig 11. VSC formed by the breakdown of glucosinolates [30]



***ESP**, epithiospecifier protein; **TFP**, thiocyanate-forming protein; **ESM**, epithiospecifier modifier protein; **NSP**, nitrile-specifier proteins; R, variable side chain; R', alkenyl side chain; R', R''-CH(OH)CH2- side chain*

When plants from this family are introduced into the body, during the process of chewing and ingestion, damage to cell membranes and release of the enzyme β-thioglucosidase(myrosinase), which further degrades glycosides, occurs. Cooking leads to the inactivation of the enzyme β-thioglucosidase. However, bacterial microflora localized in the gut can degrade glucosinolate by the already mentioned β-thioglucosidase enzyme, which is found in these microorganisms [9-11]. Then, GLS are metabolized to biologically active compounds such as indoles, nitriles, thiocyanates, and isothiocyanates. Isocyanates, for example, are then conjugated with glutathione before entering the systemic circulation, after which it can exert its biological effects. [9-11].

2.1.1 Isothiocyanates

There are over 100 isothiocyanates in nature; chemical compounds in plants from this group have been found mainly in the genus Brassicaceae (Table 1) [4, 29, 37]. Isothiocyanates are formed after GLS metabolism, show anticancer effects, and are considered candidates for cancer therapy [29, 34]. In addition to this, isothiocyanates possess other biological effects. Isothiocyanates affect the systemic reduction of oxidative stress levels, and may also affect cytokine activity [29, 34]. These substances can also affect the induction of apoptosis and inhibit the cell cycle [29, 34]. Additionally, these substances also affect the inhibition of angiogenesis. All these effects together enhance the anticancer effects attributed to isothiocyanates. In addition to anticancer effects, isothiocyanates support the function of the immune system as they have antibacterial effects as well as antiviral effects [29, 38, 62].

Tab 1. Some isothiocyanates in plants of the family Brassicaceae

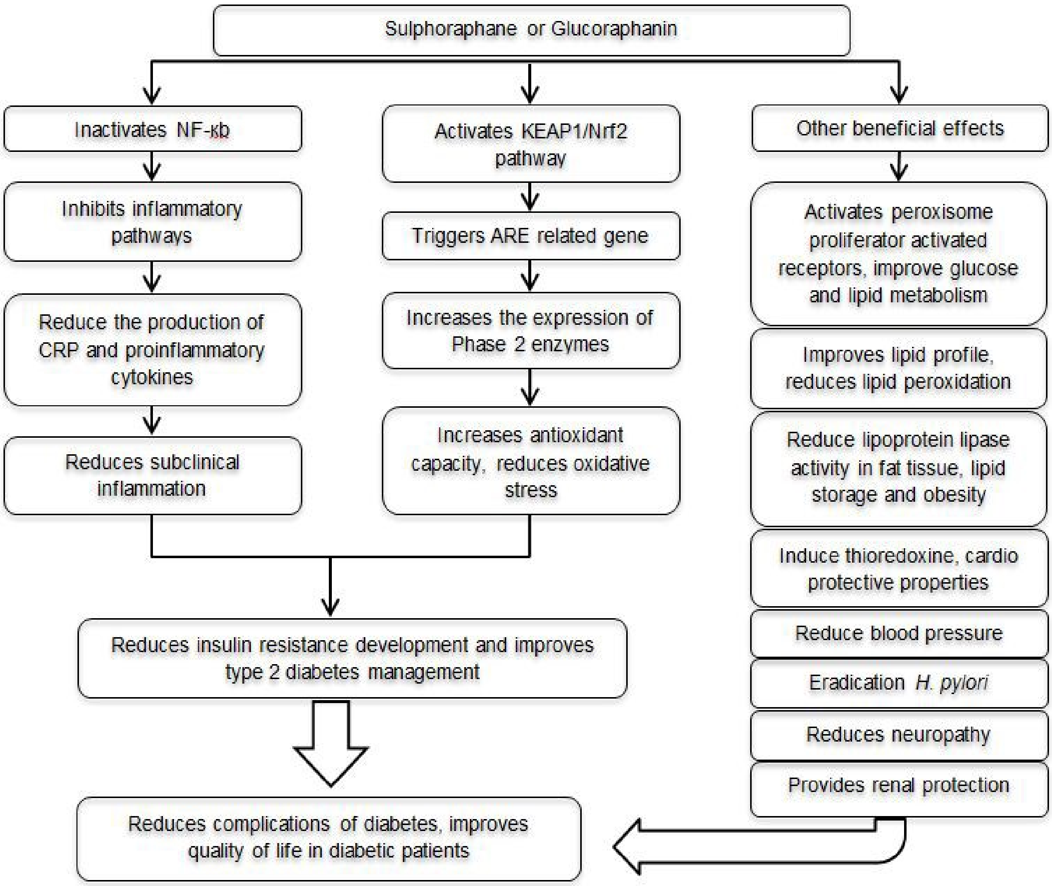
Isothiocyanates	*Brassicaceae*
Allyl isothiocyanate	broccoli, cauliflower, kohlrabi, brussel sprouts
2-Butyl isothiocyanate	broccoli, kohlrabi
Isobutyl isothiocyanate	broccoli, cauliflower, kohlrabi
Hexane, 1-isocyanate-	broccoli, kohlrabi, brussel sprouts
3-Butenyl isothiocyanate	broccoli, cauliflower, kohlrabi
Cyclopentyl isothiocyanate	broccoli,cauliflower
Butane, 1-isothiocyanato-	broccoli, kohlrabi
2-Methylbutyl isothiocyanate	broccoli, cauliflower, kohlrabi, brussel sprouts
Phenethyl isothiocyanate	broccoli
Propane, 1-isothiocyanato-	broccoli, kohlrabi
Cyclopropane, isothiocyanato-	cauliflower, brussel sprouts
Propane, 1-isocyanato-2-methyl-	cauliflower
Propane, 1-isothiocyanato-	cauliflower
Methane, isothiocyanato-	kohlrabi
Isopropyl isothiocyanate	kohlrabi, brussel sprouts
Butane, 2-isothiocyanato-	kohlrabi
Butane, 1-isothiocyanato-	kohlrabi
n-Pentyl isothiocyanate	kohlrabi
4-Methylpentyl isothiocyanate	kohlrabi
Heptane, 1-isothiocyanato-	kohlrabi
Propane, 1-isothiocyanato-	kohlrabi
Octane, 1-isothiocyanato-	kohlrabi

Nonetheless, regardless of other effects, the anticancer effects of these substances are of predominant importance. So far, two principal mechanisms by which these substances exert anticancer effects have been proposed. The first mechanism is the presumed interaction of isothiocyanate and enzymes from the first phase of drug metabolism, namely cytochrome P450, when these enzymes are inactivated. Also, enzyme activity from the second phase reaction are affected. Another mechanism is reflected in the induction of apoptosis of genetically engineered cells, as well as the effect on the cell cycle of these cells [33-35].

The most significant and most commonly formed isothiocyanate, formed from the precursor GLS glucoraphanine, is 1-isothiocyanato-4-methylsulfinylbutane or as it is called sulforaphane. This compound is found in plants of the genus Brassicaceae and is a highly active substance with antioxidant and antitumor effects. The National Cancer Institute in the US has classified sulforaphane among 40 substances that have the potential to be used in clinical practice as drugs, due to their anticancer effects. Studies have shown that a diet in which Brassicaceae plants, which are rich in isothiocyanate and sulforaphane, are highly prevalent, prevent the development of various cancers, most notably cancer of the colon, rectum and thyroid gland [29]. Also, if used in larger quantities in the diet, plants of this genus can have a protective effect against other types of cancer [29].

For the most part, sulforaphane can be found in broccoli. There is a difference in the amount of this substance in young and old plants. Young plants have a higher amount of sulforaphane. Broccoli sprouts have the highest amount, and during the ripening process, the amount of this substance is reduced. The difference between the sprout and the mature form of this plant in the amount of sulforaphane is up to ten times. More specifically, in mature broccoli, the amount of sulforaphane ranges from 4 mg to 71mg per 100g of dry, dry matter. In broccoli sprouts, the amount of sulforaphane averages 1153mg per 100 g of dry matter. With this in mind, and to increase sulforaphane intake, nutrition experts recommend broccoli for human consumption. The effects of sulforaphane are shown in detail, with Figure 12.

Fig 12. Biological effects of sulforaphane [29]



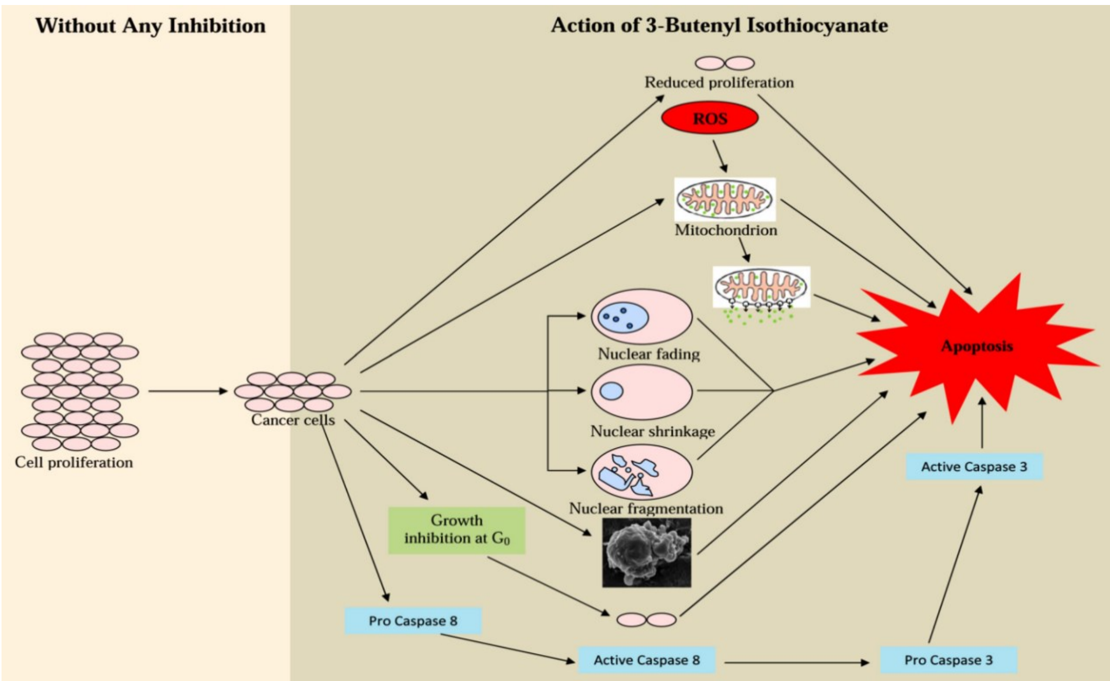
Although predominantly sulforaphane has been mentioned in the prevention of colon, rectal, and thyroid cancers, there are other tumors where this substance will be able to exert its anticancer effects. One such tumor is the hepatic tumor. Interestingly, sulforaphane will not reveal a direct effect on liver tumor cells but is required to undergo hydrolysis to form a compound that possesses these effects. Enzymatic hydrolysis of sulforaphane results in the formation of 4-methylthiobutyl. This sulforaphane metabolism product selectively induces cytotoxicity of liver tumor cells. Selectivity for tumor cells was demonstrated by the absence of apoptosis and necrosis when the drug was administered to healthy liver cells [36]. The exact mechanism by which 4-methylthiobutyl induces cytotoxicity is not known, but it is known that this cytotoxicity is not due to the activation of the p-53 dependent pathway.

Plants of the family Brassicaceae also contain allyl isothiocyanates. As expected, due to its volatile nature, evaporation occurs during heat treatment and a significant loss of concentration of this substance. Allyl isothiocyanate is subject to hydrolysis at physiological temperature. This process is accelerated as the temperature increases, and a significant loss is achieved by increasing the temperature to 88 ° C [30]. With increasing temperature, the rate at which isothiocyanate is lost from the body increases [37].

Allyl isothiocyanate shows antibacterial and antifungal activity. Bacteria such as *E. coli, S. aureus, C. jejuni, and P. aeruginosa* are known to be sensitive to allyl isothiocyanate [30]. In addition to the antimicrobial effects of allyl isothiocyanate, this VSC has been attributed to anticancer effects. Since the concentration of this VSC is ten times higher in the urinary tract than in the plasma, the most important site of the anticancer activity of allyl isothiocyanate may well be in the urinary tract [39].

3-butenyl isothiocyanate has shown significant activity against different tumor lines in preclinical studies. The highest sensitivity of 3-butenyl isothiocyanate was expressed by PC-3 and DU145 prostate cancer cell lines [40, 41]. 3-butenyl isothiocyanate induces cancer cell apoptosis, and a detailed mechanism of action is shown in Figure 13 [32]. It should be noted that 3-butenyl isothiocyanate has not yet been studied on humans.

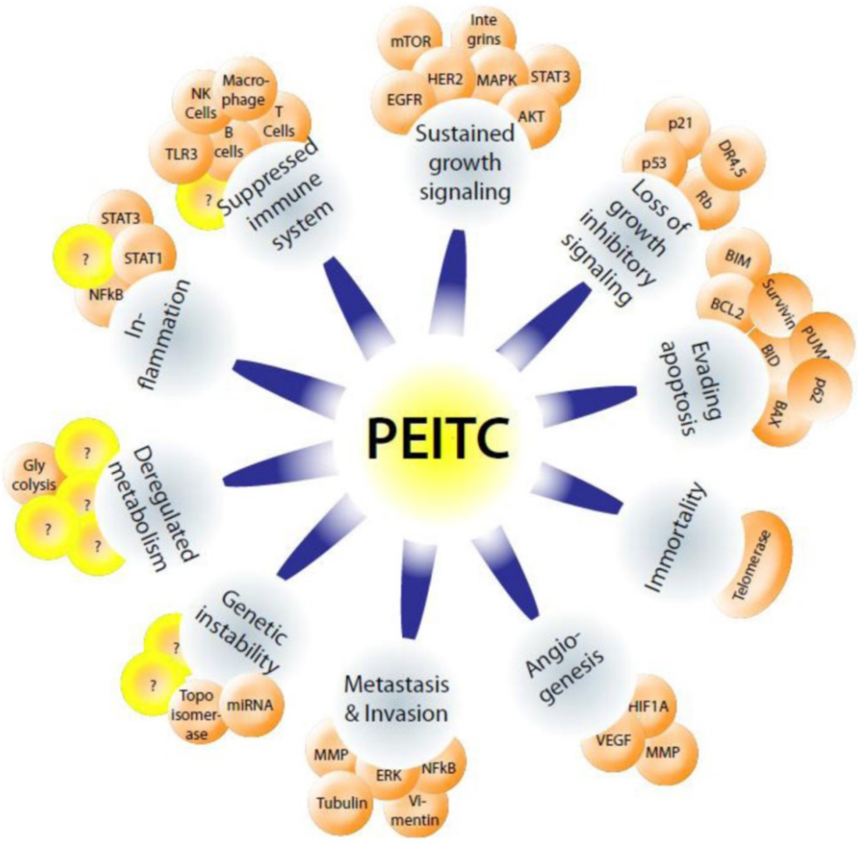
Fig 13. Mechanism of action [40]



Although the most significant interest is given to the anticancer and antimicrobial action of VSC originating from plants of the family Brassicaceae, there are other interesting biological effects. One of these has been attributed to phenethyl isothiocyanate, which can activate leptin in the hypothalamus and thus reduce hunger [42]. So far, the effects of phenethyl isothiocyanate on food intake have only been investigated in animal studies. Further research in this direction should answer the question of whether phenethyl isothiocyanate can also affect hunger in humans.

In addition to this effect, anticancer effects have been attributed to phenethyl isothiocyanate - among others, in melanoma, prostate cancer, and colon cancer in experimental studies on cell lines or animal studies [43-46]. The exact mechanism of action has not yet been determined, but there are different indications of the mechanism of action of this compound in Figure 14.

Fig 14. Phenethyl isothiocyanate anti-cancer mechanisms [46]



PEITC - Phenethyl isothiocyanate

2.2 Amaryllidaceae family

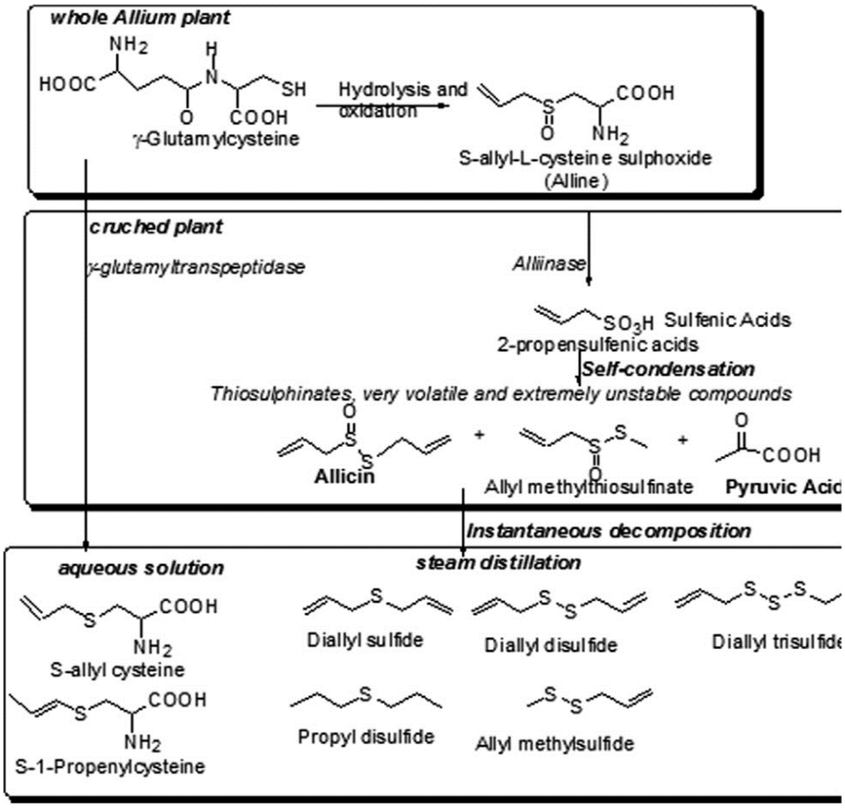
There are several different plant species in *Allium spp.*:

1. *Allium cepa*
2. *Allium ampeloprasum*
3. *Allium chinense*
4. *Allium fistulosum*
5. *Allium sativum*
6. *Allium schoenoprasum*
7. *Allium tuberosum*

Allium spp. represent the genus of plants from the Amaryllidaceae family. Plants of the genus *Allium* are the most widespread and most numerous plants of the Amaryllidaceae. From the economic point of view, this is also the most significant genus in this family since vast quantities of plants of this genus are used daily in nutrition worldwide [47]. The main reason for their daily use in the diet is the significant nutritional components in plants of this family. They can also be used in the treatment of various diseases as they have significant phytonutrients. Thus, edible parts of these plants are known to be useful in the prevention of diseases such as cancer, obesity, diabetes, coronary disease, hypercholesterolemia, and various inflammatory diseases [48-53]. The positive effects of these plants on human health are also explained by the existence of VSC in these plants that are introduced into the organism [47]. VSCs are also responsible for the appearance of specific odor and taste [47].

Organic sulfur compounds of alliin and g-glutamyl cysteines (both S-alk (en) yl-L-cysteine sulfoxides) are responsible for the taste and smell of onions and garlic. During the breach of the structural integrity of these plants, damage to the cell membrane occurs, and substances from the cell cytoplasm and vacuole become free and able to interact with the environment. This leads to the hydrolysis of various sulfur compounds resulting in VSC such as allicin, diallyl sulfide, and diallyl disulfide [47]. A schematic representation of the formation of different VSC during hydrolysis is shown in Figure 15.

Fig 15. Enzymatic reactions of various VSC from *Allium spp.* [47]



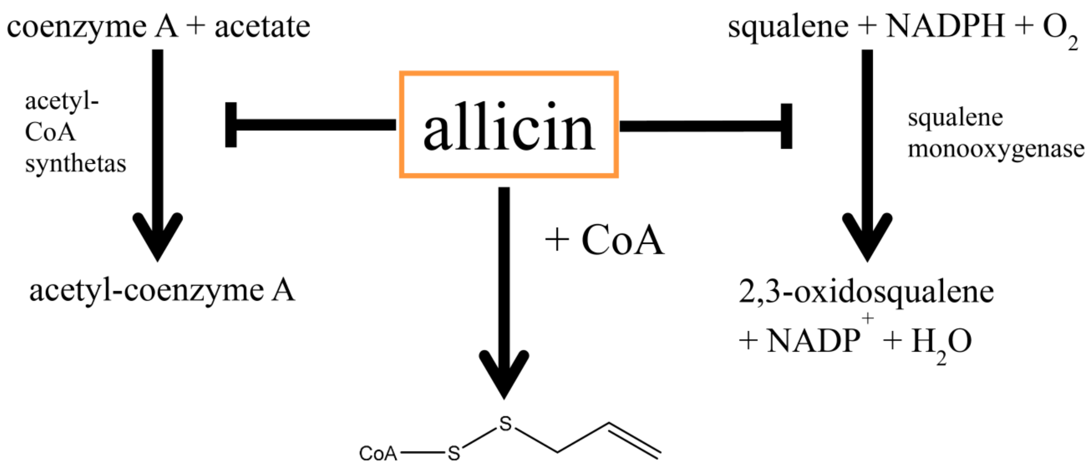
In addition to the aforementioned VSC in plants of the genus *Allium*, methyl propyl disulfide, dipropyl disulfide, dimethyl trisulfide, dimethyl disulfide, methyl allyl disulfide, methyl propyl disulfide, methyl propyl trisulfide, and many other compounds can be found.

Allicin is a biologically active VSC formed from the amino acid alliin in a reaction that is mediated by an enzyme called alliinase [54]. Its biological activity is very significant, and it has numerous beneficial effects on human health.

Allicin has antibacterial and antifungal activity [54]. Dose dependence was determined when it comes to effects against bacteria and fungi [54]. Allicin can interrupt the proliferation of bacteria and fungi, and may additionally kill these microorganisms directly [54]. Interestingly, allicin may also affect the methicillin-resistant *Staphylococcus aureus* strain (MRSA) [54].

In addition to its antimicrobial activity, allicin has been shown to play an important role in the disruption of cholesterol biosynthesis (Figure 16). This effect of allicin can be explained by the direct inhibition of squalene-monooxygenase and acetyl-CoA synthetase [54]. Additionally, since coenzyme A contains a thiol group, there is a direct reaction between coenzyme A and allicin, which further prevents coenzyme A from being used in cholesterol biosynthesis.

Fig 16. Effect of allicin on biosynthesis of cholesterol. [54]

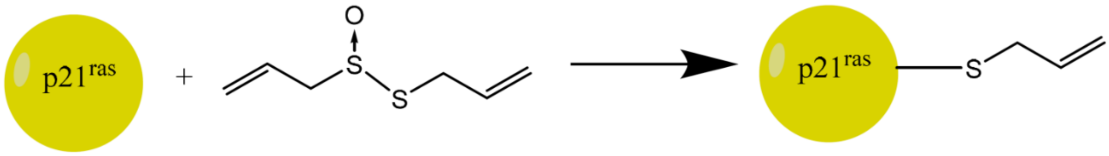


Allicin and other thiosulfates are capable of blocking platelet aggregation. A 0.4 mM concentration of allicin inhibited 90% of platelet aggregation, while inhibition of about 35% was demonstrated for a similar concentration of Aspirin® (0.36mM). [54].

In addition to the cardiovascular effects and effects mentioned before, allicin also has an antihypertensive effect. The anti-hypertensive effect of allicin is due to the hydrogen sulfide produced during the breakdown of allicin. Hydrogen sulfide is a signaling molecule that influences smooth muscle cell relaxation leading to vasodilation and a reduction of blood pressure [54].

Allicin also affects the functioning of the immune system. It stimulates the activity of immune cells, has been observed to prevent neutrophils from migrating to the epithelium, which is of great importance for the inflammation process. Additionally, allicin acts on T cells by inhibiting SDF 1α-hominin-induced chemotaxis. Allicin activates T cells. A key thing in lymphocyte activation is the p21ras molecule that inactivates RAS GTPs. Allicin directly acts on p21ras and activates this molecule (Figure 17).

Fig 17. Interaction, allicin and $p21^{ras}$ [54]



Allicin has also been shown to have anticancer effects [54]. Its impact on the occurrence of apoptosis in tumor cells has been demonstrated. In tumor cell apoptosis triggered by allicin, the participation of Nrf2 has been observed, which makes it an exciting and somewhat controversial finding as it was known by then that Nrf2 exerted only anti-apoptotic effects.

Although all of the positive effects of allicin on human health are clearly outlined above, some problems preclude its clinical use. The most important of these is the chemical instability of this compound. Due to the chemical instability of allicin, upon its entry into the systemic circulation, this compound interacts with other potential reactants resulting in the inactivation of allicin [54].

Diallyl sulfide is a VSC isolated mainly from garlic [55]. So far, it has been shown that this compound exhibits a large number of biological effects that, like the VSCs above, are related mainly to antimicrobial, anticancer, anti-inflammatory, and antioxidant activity [55].

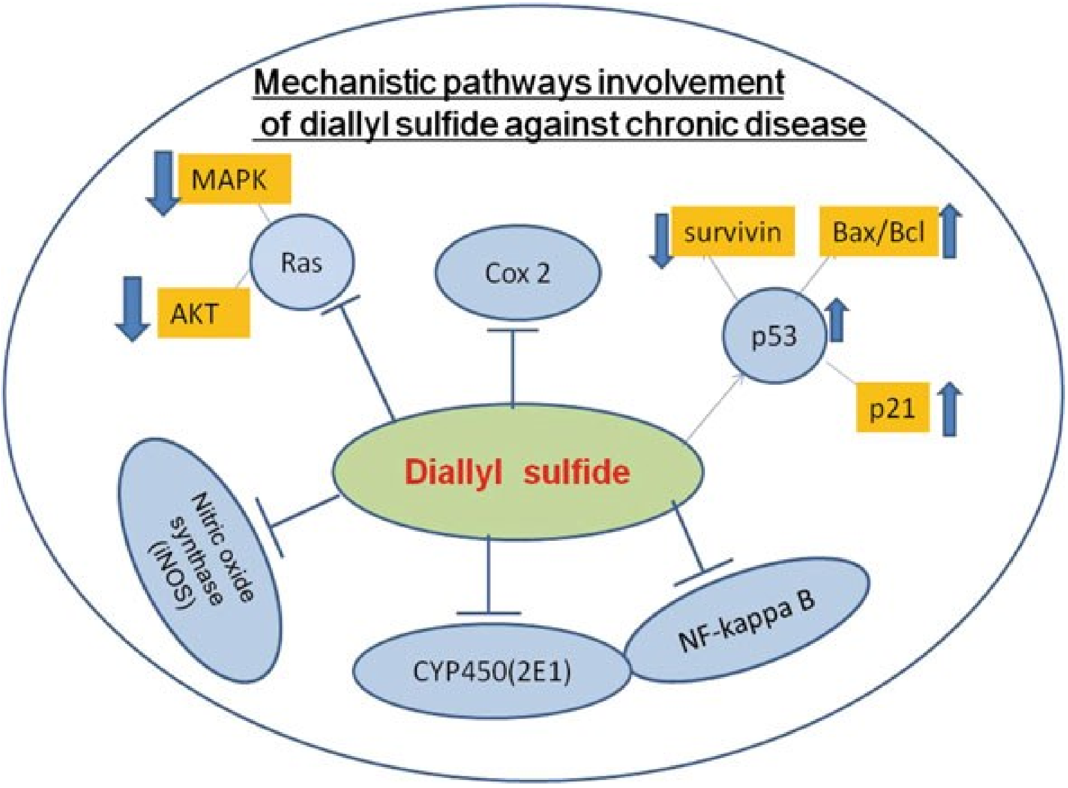
The antimicrobial effects of diallyl sulfide are reflected in its effect on various Gram-positive and Gram-negative bacteria. Multiple studies have shown that diallyl sulfide is capable of acting on *Helicobacter pylori* and *Pseudomonas aeruginosa* [55].

Diallyl sulfide also affects the modulation of the immune system. Studies have shown that diallyl sulfides can modulate the immune system by means of the modulation of various interleukins such as IL1β, IL-6, and IL-10 [55].

The compound reveals antioxidant effects that support its direct anticancer activity [55]. Diallyl sulfide has also shown an antimutagenic effect, further evidence in support of the possibility that it exerts anticancer effects.

There are various assumptions about the mechanism of action by which it exhibits its biological effects. The possible mechanisms by which diallyl sulfide shows its biological effects are summarized in Figure 18.

Fig 18. Mechanism of action - diallyl sulfide [55]



Diallyl disulfide, as well as the diallyl sulfide mentioned above, possesses biological effects of great importance. In contrast to diallyl sulfide, the biological effects of diallyl disulfide have been less extensively investigated and reported in the scientific literature. Particular attention has been paid to the effects of this volatile compound on the prevention and treatment of various tumors. Studies conducted with diallyl disulfide have shown that this compound exerts positive effects in suppressing multiple cancers such as squamous cell carcinoma, prostate cancer, gastric, lung, cervical, and other [56-60].

Diallyl disulfide affects said tumors by inducing apoptosis of tumor cells. It is able, *in vitro*, to block signaling pathways responsible for metastases in esophageal-gastric junction adenocarcinoma [61].

In addition to these, other VSC can be extracted from plants of the genus *Allium*. However, these compounds either have no significant biological effects, or their positive biological effects have not yet been demonstrated.

2.3 Moringaceae family

Within the Moringaceae family are three genera - *Anom*, *Hyperanthera,* and *Moringa* [62]. There are 13 different plant species within the *Moringa* genus:

1. *M. arborea*
2. *M. borziana*
3. *M. concanensis*
4. *M. drouhardii*
5. *M. hildebrandtii*
6. *M. longituba*
7. *M. oleifera*
8. *M. ovalifolia*
9. *M. peregrine*
10. *M. pygmaea*
11. *M. rivae*
12. *M. ruspoliana*
13. *M. tenopetala*

Currently, studies are focused only on *Moringa oleifera, Moringa stenopetala, Moringa concanensis,* and *Moringa peregrina* [62].

This genus of plants is traditionally used to promote health. The royal families used these plants to keep their skin healthy and nourished. The Indians used these plants to prepare for battle and to reduce the fatigue and pain that occurs during combat. In addition to the historically relevant applications listed above, plants of this genus can also be used in anxiety, asthma, fever, diarrhea, for wound healing, as well as in many other indications [62].

These plants reveal significant and practical use in different fields, especially in various branches of industrial production. For example, the seeds of these plants can be used to purify water. However, despite all other effects, the highest importance of these plants is reflected in the promotion of human health. Among the plants of this genus *M. oleifera* is the one that stands out, it has been called "Miracle Tree" and "Mother's Best Friend" because of the nutritional value it possesses. In addition to vitamins A and C, potassium, calcium, essential amino acids are found [63].

One part of the aforementioned positive effects caused by plants of this family is due to the VSC present. Isothiocyanates found predominantly in plants of the Brassicaceae Burnett family are also sporadically found in the Moringaceae family [4]. Several different VSC were isolated from the *Moringa oleifera* plant, which is known to be the most effective of the plants belonging to the Moringaceae family; benzyl isothiocyanate, isopropyl isothiocyanate, isobutyl isothiocyanate, 1-methyl propyl isothiocyanate, 4-hydroxy benzyl isothiocyanate, and 5,5-dimethyl oxazolidine-2-thione were isolated [4]. VSCs were also found in another plant species from the Moringaceae family, namely *Moringa peregrine*. The compounds isothiocyanate isobutyl, isothiocyanate, and 1-methyl propyl isothiocyanate were isolated from this plant [4].

Some of the representative isocyanates have been described above, most of them in the section on plants from the Brassicaceae Burnett family. Benzyl isothiocyanate, not addressed before, has a great ability to kill Gram-negative periodontal pathogens (*Actinobacillus actinomycetemcomitans* and *Phorphyromonas gingivalis*) [64]. In addition to the pathogens mentioned, benzyl isothiocyanate is capable of killing other medically significant Gram-negative bacteria such as *Salmonella enterica*, *Haemophilus influenza*, and what is perhaps the most critical *Pseudomonas aeruginosa* [64]. The antimicrobial effect of isopropyl isothiocyanate on *E. coli* was tested; however, no positive results were obtained [38].

2.4 Rosaceas family

This family is also referred to as the rose family and is one of the most abundant plant families (3000 to 4000 plant species). Four subfamilies (Rosoideae, Spiraeoideae, Amygdaloideae, and Maloideae) with a large number of species are included.

Plants from this family with isolated VSCs are *Caylusea abyssinica*, *Reseda alba*, *Reseda lutea*, *Reseda luteola*, *Reseda media*, *Reseda odorata*, *Reseda phyteuma*, *Reseda stricta*, *Sesamoides canescens var. canenscens* and *Sesamoides pygmaea* [4]. The most commonly isolated VSC are isothiocyanates, and among the isolated compounds from these plants, the most frequent is 2-phenylethyl isothiocyanate and 4-hydroxy benzyl isothiocyanate. In addition to the compounds mentioned above, benzyl isothiocyanate, 5,5-dimethyl oxazolidine-2-thione, 3-hydroxybenzyl isothiocyanate, 2-hydroxy benzyl isothiocyanate, 5-phenyl oxazolidine 2-thione can be isolated [4].

2-phenylethyl isothiocyanate exerts a proven antibacterial activity. Studies have shown that this compound is more active against Gram-positive than Gram-negative bacteria [81]. This compound shows a considerable level of activity against bacteria such as *Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, and *Listeria monocytogenes*. On the other hand, 2-phenylethyl isothiocyanate has no activity against *Serratia marcescens* and *Shigella sonnei* [65].

4-hydroxy benzyl isothiocyanate is considered a donor of H_2S, which is why this compound is attributed to antiproliferative events [82]. In preliminary studies, this VSC had been shown to inhibit the proliferation of neuroblastoma (SH-SY5Y) and glioblastoma (U87MG) cell lines [66]. The inhibition of proliferation was accompanied by an increase in thiosulfate levels [66].

2.5 Caricaceae

This family includes multicellular green algae that live in clean and sometimes saltwater. The plant species from this family from which the VSC were isolated are *Empress cauliflora, E. chilensis, E. papaya, E. pennata, E. quercifolia, Cycliomorpha solmsii* and *Jarilla chocola*. Only one VSC, benzyl isothiocyanate, was found in all four [4]. The biological effects of this VSC are described in the preceding pages, under Moringaceae family.

2.6 Capparaceae family

This is one large family of grass-like plants. The family includes a large number of different genera with over 5500 known plant species. The plant species from which the VSC have been isolated are *Boscia senegalensis, Capparis spp*. (*angulata, baducca, cartilagena, ferruginea, flexuosa, galeata, hastate, inermis, linearis, masaikai, mitchellii, nobilis, odoratissima, ovalifolia, ovata var. canescens, ovata var. palaestina, quiniflora, rupestris, spinosa, spinicololia aegyptia, spinosa var. desertii, Teureckheimii, Tweediana*), *Cleome spp*. (*anomala, arabica, arborea, chelidonii, chrysantha, diandra, gigantean, graveolens, integrifolia, isomeris, machycarpa, monophylla,, ornithopodioides, papillosa, pilosa, pungens, rosea, serrutala, sonorae, speciosysima, spinosa, spinach, spinach, spinach , Courbonia virgata, Crataeva roxburghii, Crataeva tapia, Dhofaria macleishii, Dithyrea californica, Gynandropsis spp.* (*gynandra, pentaphylla* and *speciosa*), *Isomeris arborea, Maerua spp*. (*aethiopica, hoehnellii* and *ovalifolia*), *Polanisia spp.* (*dodecandra, viscose* and *dodecandra*), *Puccionia macradenia, Ritchiea albersii, Thylachium spp.* (*africanum* and *thomasii*) and *Wislizenia refracta* [4]. As one can see, many plants in this family contain some of the VSC. Most of the plants in this family comprise methyl isothiocyanate. Other VSC isolated from these plants are: dimethyl trisulfide, dipropyl disulfide, isopropyl isothiocyanate, butyl isothiocyanate, 3-butenyl isothiocyanate, 4-hydroxy benzyl isothiocyanate, 5-vinyl oxazolidine-2-thione, 4-hydroxybutyrate isothiocyanate, 3-hydroxybutyl isothiocyanate, 4-ethyl oxazolidine-2-thione, 5,5-dimethyl oxazolidine-2-thione, 5,5-methyl ethyl oxazolidine-2-thione, 3- (methyl sulfinyl) propyl isothiocyanate, 4-oxoheptyl isothiocyanate, 5-oxoheptyl isothiocyanate, 3-methyl-3-butenyl, oxazolidine-2-thione, 2-ethyl thiophene, ethyl thiocyanate, 2-propyl thiophene. [4].

The most commonly isolated VSC from these plants is methyl isothiocyanate. However, this compound is toxic to humans [67] and is used in agriculture [67]. Symptoms that occur during exposure to this compound may vary depending on the duration of contact, from mild when short-term, and if more extended contact and significant resorption occur, more severe symptoms develop [67]. Transient contact with this compound may cause enhanced lacrimal gland action [67]. Further, if prolonged contact, irritation in the nose and throat, headache, nausea, cough, and difficulty breathing occur [8].

Other VSCs isolated from plants belonging to the Capparaceae family have either been described previously or no biological activity has been shown so far. Among other VSC, 3-butenyl isothiocyanate (described previously) should be mentioned, and if further studies go well, it will be a candidate substance for the treatment of prostate cancer.

2.7 Rutaceae family

This family is also called the citrus family. Among others, plants from this family containing VSC are *Agathosma spp*. (*apiculata, clavisepal*, and *puberol*), *Citrus spp*. (*junos, paradisi, reticulata*, and *tangerine*), *Melicope obscura*, *Poncirus rifoliate, Ruta spp*. (*chalepensis* and *graveolens*) [4].

The isolated VSCs of this family are: S- (3-methyl-2-butenyl) -2-methyl propanthionate, S- (3-methyl-2-butenyl) ethanthioate, S- (3-methyl-2-butenyl) 3-methyl butanethioate, 3-mercaptohexanol, 3-mercaptohexanol acetate, methional, β-mintsulfide, diethyl disulfide, 1-p-menthene-8-thiol, 4-mercapto-4-methyl pentane-2-ol, 1-methoxyhexane- 3-thiol, 4-methoxy-2-methyl butane-2-thiol, 4-methyl-4-sulfanylpentane-2-ol, 3-sulfanylhexyl butanoate, 3-sulfanylhexyl hexanoate, 3-sulfanylhexyl octanoate, 3-sulfanylhexyl decanoate, 3- sulfanylhexyl dodecanoate, 3-sulfanylhexyl tetradecanoate, 3-sulfanylhexyl hexadecanoate, 3-sulfanylhexyl octadecenoate [4]. So far, no significant biological effects of these volatile sulfur compounds have been demonstrated.

2.8 Resedaceae family

The Resedaceae family is a small family of plants that, according to different classifications, includes between 3 and 7 genera with about 68 species of plants. Resedaceae includes the following plants, which have a VSC: *Caylusea abyssinica,* *Reseda spp*. (*alba, lutea, luteola, media, odorata, phyteum* and *stricta*) and *Sesamoides spp*. (*grandis* and *canescens*) [4]. The following VSC can be isolated from the plants of this family:

2-phenylethyl isothiocyanate, benzyl isothiocyanate, 4-hydroxybenzyl isothiocyanate, 5-phenyl oxazolidine 2-thione, 5,5-dimethyl oxazolidine-2-thione, 3-hydroxybenzyl -hydroxy benzyl isothiocyanate [4]. The biological effects of relevant VSC have been described in previous chapters.

2.9 Solanaceae family

The Solanaceae family is a family of plants that are very important from an economic point of view. This family includes about 98 genera of plants with about 2700 plant species. Among the plant species from which volatile sulfur compounds can be isolated are *Lycopersicon esculentum, Mandragora autumnalis, Solanum lycopersicum,* and *Capsicum annuum* [4]. Methional, dimethyl sulfide, 2-ethyl thiophene, 2-Isobutyl thiazole, 3-(methylthio) propyl acetate, 2-heptanethiol, 1-(2-thienyl)-2-pentanethiol, 4-mercapto-2-nonanol, 1,3-oxathiane, ethyl methyl propanethioate, 1-methoxyhexane-3-thiol [4]. The biological effects of isolated VSC from these plants have either been previously described, are not in the widely available scientific literature, or no positive biological effects have been reported so far.

2.10 Other families

In the remaining families, there are generally few species (up to three) of plants from which VSC can be isolated. Also, only a few VSCs can be isolated from these plants. These families include Bignoniaceae, Phytolaccaceae, Meliaceae, Chenopodiaceae, Fabaceae, Pittosporaceae, Buxaceae, Theaceae, Gyrostemonaceae, Cucurbitaceae, Poaceae, Cannabaceae, Limnanthaceae, Myrtaceae, Lamiaceae, Moringaceae, Oleaceae, Poaceae, Fabaceae, Passifloraceae, Pentadiplandraceae and Phytolaccaeae [4].

3. Conclusion

VSC, from a biological point of view, are an exciting group of compounds that originate from different sources in nature. Of great interest from the pharmaceutical and medical point of view are the VSCs that originate from plants. In part, the positive effects of various medicinal plants are explained by the effects of VSC. So far, a large number of VSC from plants are known and found in a large number of plant families and species. However, not all VSC possess significant biological effects. One can say with great certainty that the most productive plants when considering the concentration and number of different VSC are plants from the Brassicaceae Burnett and Amaryllidaceae families. In addition to these two families, biologically active sulfur compounds are also isolated from other families, and a significant number of assays have been conducted to detect all possible biological effects of these compounds. Mostly the positive biological effects are linked to the impact on cancer. More specifically, these substances have been shown to have antioxidant, antiproliferative, proapoptotic, as well as direct anticancer effects. To date, the influence of these substances has been investigated in cell line studies as well as in animal models of tumors.

Further investigations are necessary to identify all the potential tumor types that these substances may affect, as well as to find candidates for future drugs, to identify stable substances applicable in humans. In addition to the anticancer activity, the antibacterial and antifungal effects of these substances were investigated. The effect of these compounds on various strains of bacteria and fungi is noticeable. Some of these compounds have shown effects against resistant bacteria such as MRSA. To a lesser extent, with the noticeable increase in recent years, tests have also been directed towards the metabolic effects of VSC. All these facts direct to the potentially significant effect that the VSC originating from plants show on human health, as well as the need to further search for and clarify their biological effect.

.

References

[1] A. Sekowska, H. F. Kung, and A. Danchin, "Sulfur metabolism in Escherichia coli and related bacteria: facts and fiction," *J Mol Microbiol Biotechnol,* **2**(2), 145-77, 2000.

[2] N. U. Frigaard and C. Dahl, "Sulfur metabolism in phototrophic sulfur bacteria," (in eng), *Adv Microb Physiol,* **54**, 103-200, 2009.

[3] T. Linder, "Assimilation of alternative sulfur sources in fungi," *World J Microbiol Biotechnol,* **34**(4), 51, 2018.

[4] M. Iranshahi, "A review of volatile sulfur-containing compounds from terrestrial plants: biosynthesis, distribution and analytical methods," **24**(4), 393-434, 2012.

[5] J. Reiter, A. M. Hubbers, F. Albrecht, L. I. O. Leichert, and A. J. Slusarenko, "Allicin, a natural antimicrobial defence substance from garlic, inhibits DNA gyrase activity in bacteria," *Int J Med Microbiol,* 151359, 2019.

[6] R. Tocmo and K. Parkin, "Data on chromatographic isolation of cysteine mixed-disulfide conjugates of Allium thiosulfinates and their role in cellular thiol redox modulation," *Data Brief,* **21**, 1445-1450, 2018.

[7] Yi L, Shan J, Chen X, Li G, Li L, Tan H, Su Q, "Involvement of calreticulin in cell proliferation, invasion and differentiation in diallyl disulfide-treated HL-60 cells," *Oncol Lett,* **12**(3), 1861-1867, 2016.

[8] C. Grundemann and R. Huber, "Chemoprevention with isothiocyanates - From bench to bedside," *Cancer Lett,* vol. 414, pp. 26-33, Feb 1 2018.

[9] J. W. Fahey, A. T. Zalcmann, and P. Talalay, "The chemical diversity and distribution of glucosinolates and isothiocyanates among plants," *Phytochemistry,* **56**(1), 5-51, 2001.

[10] L. Bell and C. Wagstaff, "Rocket science: A review of phytochemical & health-related research in Eruca & Diplotaxis species," *Food Chem X,* **1**, 100002, 2019.

[11] D. Prakash and C. Gupta, "Glucosinolates: the phytochemicals of nutraceutical importance," *J Complement Integr Med,* **9**(13), 2012.

[12] P. Soundararajan and J. S. Kim, "Anti-Carcinogenic Glucosinolates in Cruciferous Vegetables and Their Antagonistic Effects on Prevention of Cancers," *Molecules,* **23**(11), 2018.

[13] J. W. Fahey, Mark E. Olson, Katherine K. Stephenson, Kristina L. Wade, Gwen M. Chodur, David Odee, Wasif Nouman, Michael Massiah, Jesse Alt, Patricia A. Egner & Walter C. Hubbard, "The Diversity of Chemoprotective Glucosinolates in Moringaceae (Moringa spp.)," *Sci Rep,* **8**(1), 7994, 2018.

[14] O. A. Ismaiel, E. Abdelghani, H. Mousa, S. I. Eldahmy, and B. E. Bayoumy, "Determination of Estragole in Pharmaceutical Products, Herbal Teas and Herbal Extracts Using GC-FID," **6**(12), 144-150, 2016.

[15] I.-Y. Kim, S.-H. Suh, I.-K. Lee, R. R. Wolfe, "Applications of stable, nonradioactive isotope tracers in in vivo human metabolic research," *Exp Mol Med,* **48**, 203, 2016.

[16] A. T. Nielsen and S. Jonsson, "Quantification of volatile sulfur compounds in complex gaseous matrices by solid-phase microextraction," *J Chromatogr A,* **963**(1-2), 57-64, 2002.

[17] M.-H. Yang, N.-H. Kim, J.-D. Heo, J.-D. Rho, K. J. Ock, E.-C. Shin, E. J. Jeong, Eun, "Comparative Evaluation of Sulfur Compounds Contents and Antiobesity Properties of Allium hookeri Prepared by Different Drying Methods," *Evid Based Complement Alternat Med,* **2017**, 2436927–2436936, 2017

[18] A. F. Al-Rubaye, I. H. Hameed, M. J. Kadhim, "A Review: Uses of Gas Chromatography-Mass Spectrometry (GC-MS) Technique for Analysis of Bioactive Natural Compounds of Some Plants," **9**(1), 81-85, 2017.

[19] N. Rao and K. D. Gawde, "Review on gas chromatography & its hyphenation techniques," *World Journal of Pharmaceutical Research*, **7**(2), 444-455, 2017.

[20] S. Soltani, G.-R. Amin, M. H. Salehi-Sourmaghi, B. Schneider, S. Lorenz, and M. J. F. Iranshahi, "Sulfur-containing compounds from the roots of Ferula latisecta and their cytotoxic activities," **124**, 108-112, 2018.

[21] N. S. Radulović, A. B. Miltojević, M. B. Stojković, and P. D. Blagojević, "New volatile sulfur-containing compounds from wild garlic (Allium ursinum L., Liliaceae)," **78**, 1-10, 2015.

[22] M. E. Cartea and P. Velasco, "Glucosinolates in Brassica foods: bioavailability in food and significance for human health," **7**(2), 213-229, 2008.

[23] I. T. Johnson, "Anticarcinogenic effects of diet-related apoptosis in the colorectal mucosa," **40**(8), 1171-1178, 2002.

[24] I. T. Johnson, "Glucosinolates in the human diet. Bioavailability and implications for health," **1**(2), 183-188, 2002.

[25] A. F. Brown, G. G. Yousef, E. H. Jeffery , B. P. Klein, M. A. Wallig, M. M. Kushad, J. A. Juvik "Glucosinolate profiles in broccoli: Variation in levels and implications in breeding for cancer chemoprotection," **127**(5), 807-813, 2002.

[26] C. Fimognari and P. Hrelia, "Sulforaphane as a promising molecule for fighting cancer," **635**(2-3), 90-104, 2007.

[27] I. Herr and M. W. Büchler, "Dietary constituents of broccoli and other cruciferous vegetables: implications for prevention and therapy of cancer," **36**(5), 377-383, 2010.

[28] K. P. Latté, K.-E. Appel, A. Lampen, "Health benefits and possible risks of broccoli–an overview," **49**(12), 3287-3309, 2011.

[29] N. Sanlier and S. Guler, "The benefits of Brassica vegetables on human health," **1**, 1-13, 2018.

[30] T. Oliviero, R. Verkerk, M. Dekker, "Isothiocyanates from Brassica Vegetables—Effects of Processing, Cooking, Mastication, and Digestion," *Mol Nutr Food Res,* **62**(18), 1701069, 2018.

[31] S. F. Vaughn, M. A. Berhow "Glucosinolate hydrolysis products from various plant sources: pH effects, isolation, and purification," **21**(2), 193-202, 2005.

[32] I. E. Sønderby, F. Geu-Flores, and B. A. Halkier, "Biosynthesis of glucosinolates–gene discovery and beyond," **15**(5), 283-290, 2010.

[33] X.-L. Tan, M. Shi, H. Tang, W. Han, and S. D. Spivack, "Candidate dietary phytochemicals modulate expression of phase II enzymes GSTP1 and NQO1 in human lung cells," **140**(8), 1404-1410, 2010.

[34] A. Pawlik, M. A. Szczepanski, A. Klimaszewska, L. Gackowska, A. Zuryn, and A. Grzanka, "Phenethyl isothiocyanate-induced cytoskeletal changes and cell death in lung cancer cells," *Food Chem Toxicol,* **50**(10), 3577-3594, 2012.

[35] D. Kalpana Deepa Priya, R. Gayathri, G. R. Gunassekaran, S. Murugan, and D. Sakthisekaran, "Apoptotic role of natural isothiocyanate from broccoli (Brassica oleracea italica) in experimental chemical lung carcinogenesis," *Pharm Biol,* **51**(5), 621-628, 2013.

[36] E. Lamy, A. Hertrampf, C. Herz, J. Schüler, M. Erlacher, D. Bertele, A. Bakare, M. Wagner, T. Weiland, U. Lauer, O. Drognitz, R. Huber, S. Rohn, T. Giesemann, and V. Mersch-Sundermann, "Preclinical evaluation of 4-methylthiobutyl isothiocyanate on liver cancer and cancer stem cells with different p53 status," *PLoS One,* **8**(8), e70846, 2013.

[37] M. N. Wieczorek and H. H. Jeleń, "Volatile Compounds of Selected Raw and Cooked Brassica Vegetables," **24**(3), 391, 2019.

[38] L. Romeo, R. Iori, P. Rollin, P. Bramanti, and E. J. M. Mazzon, "Isothiocyanates: An overview of their antimicrobial activity against human infections," **23**(3), 624, 2018.

[39] Y. Zhang, "Allyl isothiocyanate as a cancer chemopreventive phytochemical," **54**(1), 127-135, 2010.

[40] R. Arora, R. Kumar, J. Mahajan, A.P. Vig, B. Singh, B. Singh, S. Arora "3-Butenyl isothiocyanate: a hydrolytic product of glucosinolate as a potential cytotoxic agent against human cancer cell lines," **53**(9), 3437-3445, 2016.

[41] M. Núñez-Iglesias, S. Novio, S. García-Santiago, M.E. Cartea, P. Soengas, P. Velasco, M. Freire-Garaba, "Effects of 3-butenyl isothiocyanate on phenotypically different prostate cancer cells," **53**(5), 2213-2223, 2018.

[42] M. Yagi, Y. Nakatsuji, A. Maeda, H. Ota, R. Kamikubo, N. Miyoshi, Y. Nakamura, M. Akagawa, "Phenethyl isothiocyanate activates leptin signaling and decreases food intake, " **13**(11), e0206748, 2018.

[43] K.-C. Lai, Y.-T. Hsiao, J.-L. Lang, Y.-S. Ma, Y.-P. Huang, T.-A. Chiang, J.-G. Chung "Benzyl isothiocyanate and phenethyl isothiocyanate inhibit murine melanoma B16F10 cell migration and invasion in vitro," **51**(3), 832-840, 2017.

[44] Y. Liu and M. Dey, "Dietary phenethyl isothiocyanate protects mice from colitis associated colon cancer," **18**(9), 1908, 2017.

[45] H. Huang, Y. He, L. Zhang, H. Xiang, D. Li, W. Liu, X.-T. Xu, S. Goodin, K. Zhang, X. Zheng, "Phenethyl isothiocyanate in combination with dibenzoylmethane inhibits the androgen-independent growth of prostate cancer cells," **9**(4), 2398-2408, 2018.

[46] P. Gupta, S. E. Wright, S.-H. Kim, and S. K. Srivastava, "Phenethyl isothiocyanate: a comprehensive review of anti-cancer mechanisms," **1846**(2), 405-424, 2014.

[47] L. Gitin, R. Dinica, C. Neagu, and L. Dumitrascu, "Sulfur compounds identification and quantification from Allium spp. fresh leaves," *J Food Drug Anal,* **22**(4), 425-430, 2014.

[48] S. Gorinstein, Z. Jastrzebski, J. Namiesnik, H. Leontowicz, M. Leontowicz, S. Trakhtenberg, "The atherosclerotic heart disease and protecting properties of garlic: contemporary data," **51**(11), 1365-1381, 2007.

[49] G. Siegel, M. Malmsten, J. Pietzsch, A. Schmidt, E. Buddecke, F. Michel, M. Ploch, W. Schneider, "The effect of garlic on arteriosclerotic nanoplaque formation and size," **11**(1), 24-35, 2004.

[50] Y. Shukla and N. Kalra, "Cancer chemoprevention with garlic and its constituents," **247**(2), 167-181, 2007.

[51] G. Hodge, S. Hodge, and P. Han, "Allium sativum (garlic) suppresses leukocyte inflammatory cytokine production in vitro: potential therapeutic use in the treatment of inflammatory bowel disease," **48**(4), 209-215, 2002.

[52] S. Kalayarasan, P. N. Prabhu, N. Sriram, R. Manikandan, M. Arumugam, and G. Sudhandiran, "Diallyl sulfide enhances antioxidants and inhibits inflammation through the activation of Nrf2 against gentamicin-induced nephrotoxicity in Wistar rats," **606**(1-3), 162-171, 2009.

[53] M. Takahashi, T. Shibamoto, "Chemical compositions and antioxidant/anti-inflammatory activities of steam distillate from freeze-dried onion (Allium cepa L.) sprout," **56**(22), 10462-10467, 2008.

[54] J. Borlinghaus, F. Albrecht, M. C. Gruhlke, I. D. Nwachukwu, and A. J. Slusarenko, "Allicin: chemistry and biological properties," *Molecules,* **19**(8), 12591-12618, 2014.

[55] S. Suman and Y. Shukla, "Diallyl Sulfide and Its Role in Chronic Diseases Prevention," *Adv Exp Med Biol,* **929**, 127-144, 2016.

[56] X. Yin., R. Zhang, C. Feng, J. Zhang, D. Liu, K. Xu, X. Wang, S. Zhang, Z. Li, X. Liu, H. Ma "Diallyl disulfide induces G2/M arrest and promotes apoptosis through the p53/p21 and MEK-ERK pathways in human esophageal squamous cell carcinoma," *Oncol Rep,* **32**(4), 1748-1756, 2014.

[57] D. Y. Shin, G. Y. Kim, J. H. Lee, B. T. Choi, Y. H. Yoo, and Y. H. Choi, "Apoptosis induction of human prostate carcinoma DU145 cells by diallyl disulfide via modulation of JNK and PI3K/AKT signaling pathways," *Int J Mol Sci,* **13**(11), 14158-14171, 2012.

[58] H. Tang, Y. Kong, J. Guo, Y. Tang, X. Xie, L. Yang, Q. Su, X. Xie, "Diallyl disulfide suppresses proliferation and induces apoptosis in human gastric cancer through Wnt-1 signaling pathway by up-regulation of miR-200b and miR-22," *Cancer Lett,* **340**(1), 72-81, 2013.

[59] C. Hui, W. Jun, L. N. Ya, and X. Ming, "Effect of Allium sativum (garlic) diallyl disulfide (DADS) on human non-small cell lung carcinoma H1299 cells," *Trop Biomed,* **25**(1), 37-45, 2008.

[60] C. Di, C. Sun, H. Li, J. Si, H. Zhang, L. Han, Q. Zhao, Y. Liu, B. Liu, G. Miao, L. Gan, Y. Liu "Diallyl disulfide enhances carbon ion beams-induced apoptotic cell death in cervical cancer cells through regulating Tap73 /DeltaNp73," *Cell Cycle,* **14**(23), 3725-3733, 2015.

[61] X.Yin, C. Feng, L. Han, Y. Ma, Y. Jiao, J. Wang, L. Jia, F. Jing, X "Diallyl disulfide inhibits the metastasis of type esophagealgastric junction adenocarcinoma cells via NF-kappaB and PI3K/AKT signaling pathways in vitro," *Oncol Rep,* **39**(2), 784-794, 2018.

[62] N. Z. Abd Rani, K. Husain, and E. Kumolosasi, "Moringa Genus: A Review of Phytochemistry and Pharmacology," *Front Pharmacol,* **9**, 108, 2018.

[63] K. T. Mahmood, T. Mugal, I. U. Haq, "Moringa oleifera: a natural gift-A review," **2**(11), 775, 2010.

[64] A. Sofrata, E. M. Santangelo, M. Azeem, A. K. Borg-Karlson, A. Gustafsson, and K. Putsep, "Benzyl isothiocyanate, a major component from the roots of Salvadora persica is highly active against Gram-negative bacteria," *PLoS One,* **6**(8), e23045, 2011.

[65] M. Jang, E. Hong, and G. H. Kim, "Evaluation of antibacterial activity of 3-butenyl, 4-pentenyl, 2-phenylethyl, and benzyl isothiocyanate in Brassica vegetables," (in eng), *J Food Sci,* **75**(7), M412-M416, 2010.

[66] H. Jurkowska, M. Wrobel, D. Szlezak, and E. Jasek-Gajda, "New aspects of antiproliferative activity of 4-hydroxybenzyl isothiocyanate, a natural H2S-donor," *Amino Acids,* **50**(6), 699-709, 2018.

[67] M. L. Dourson, M. J. Kohrman-Vincent, and B. C. Allen, "Dose response assessment for effects of acute exposure to methyl isothiocyanate (MITC)," *Regul Toxicol Pharmacol,* **58**(2), 181-188, 2010.