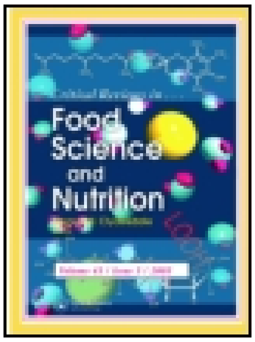

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Kale (*Brassica oleracea* var. *acephala*) as a superfood: review of the scientific evidence behind the statement

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Abstract

Kale (*Brassica oleracea* var. *acephala*) is a cruciferous vegetable, characterized by leaves along the stem, which, in recent years, have gained a great popularity as a ´superfood´. Consequently, in a popular culture it is listed in many ´lists of the healthiest vegetables´. Without the doubt, a scientific evidence support the fact that cruciferous vegetables included in human diet can positively affect health and well-being, but remains unclear why kale is declared superior in comparison with other cruciferous. It is questionable if this statement about kale is triggered by scientific evidence or by some other factors. Our review aims to bring an overview of kale's botanical characteristics, agronomic requirements, contemporary and traditional use, macronutrient and phytochemical content and biological activity, in order to point out the reasons for tremendous kale popularity.

Keywords: *Brassica oleracea* var. *acephala*, cruciferous vegetables, superfood, phytochemistry, biological activity

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1. Introduction

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In the last couple of decade, in parallel with the growing number of evidence that food and food components significantly influence human health and well-being, consumer perception about food has considerably changed. More and more consumers believe that food, in addition to fulfill the primary task by satisfying hunger and providing nutrients, could directly contribute to their health through prevention of nutrition-related diseases and improvement of physical and mental well-being. This concept follows well known and long-time ago justified Hippocrates statement 'Let food be thy medicine and medicine be thy food', and in modern days launches new trends. Foods based on the scientific evidence that may improve the general conditions of the body, decrease the risk of some diseases or/and be used for curing some illnesses are known under the name 'functional food'. This term was first used thirty years ago in Japan and has been accepted across the world. Functional food, besides being able to lower the cost of healthcare of the aging population, might also give a commercial potential for the food industry (Siro et al. 2008). In general population, the commonly used term for functional food is 'superfood'. A vegetables that is often on the lists of 'the most healthy foods' or 'superfoods' are kales- the vegetables belonging to the genus *Brassica,* species *Brassica oleracea,* group *acephala*, characterized by the leaves that do not form a head. Although kale has been cultivated for several centuries and it has been included in many traditional meals, especially in Mediterranean area, it has become very popular in the United States after 2010. Due to good tolerance for the extreme temperature fluctuations caused by climate change in recent decades, kale is very popular crop among farmers. Kale production significantly increased from 3,994 to 6,256 harvested acres in in US, in the time period from 2007 to 2012, respectively (USDA 2012).

Vegetables from genus *Brassica,* known under the common name cruciferous vegetables, are under the scientific attention for several decades because numerous epidemiological studies provide evidence that diets rich in cruciferous vegetables are associated with lower risk of several types of cancer and other chronical diseases (reviewed by Šamec and Salopek-Sondi 2018.). Mainly, health benefits of cruciferous vegetables are associated with the presence of various phytonutrients from the glucosinolate, polyphenol, carotenoid or terpenoid group (reviewed by Murillo and Mehta, 2001; Podsedek 2007; Cartea et al. 2008; Jahangir, Kim, Choi and Verpoorte 2009; Tse and Eslick 2014).

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Till few years ago 'the most popular' cruciferous vegetables, due to the health benefits, was broccoli whose phytochemistry and biological activity were extensively studied and reviewed (Latté, Appel and Lampen 2011). However, in the last couple of years, kale is in the center of popularity, and there is even an event such as the *day of the kale* celebrated in US. The question is whether the kale popularity was trigged by scientific evidence, which prove the kale superiority in comparison with other cruciferous vegetables, or by public relations experts who promote the kale in collaboration with The American Kale Association. This review will try to shed light on that question through a comprehensive overview of botanical characteristics, agronomical requirements, phytochemistry and biological activity of kale (*B. oleracea* var. *acephala)*.

2. Botanical characteristic

Brassicaceae family has very complex taxonomy and systematics. The Brassicaceae family belongs to order Brassicales and by recent findings have 341 genera and 3977 species (Franzke et al. 2011). These numbers change every few years due to findings of new species or use of new modern techniques that can distinguish family genetic diversity in a more details (Al-Shehbaz, Beilstein and Kellogg 2006; Huang et al. 2016). These abundant resources influence proper Brassicaceae classification, and respectable data on the species name are hard to find. In the last years few valuable databases have been developed to overcome these limitations, such as BrassiBase (http://brassibase.cos.uni-heidelberg.de) which covers all taxonomic, systematic and phylo- and cytogenetic literature of Brassicaceae family, and Brassicaceae Genera of the World (http://flora.huh.harvard.edu/Brassicaceae/intkey/WWW/Genera.htm) that includes more morphological and geographical identification of family genera.

Brassica oleracea L. (2n = 18) is a member of the Brassicaceae family which wild species have been found as more or less isolated populations in maritime habitats on the Atlantic coast of Spain, France and British islands. It is suggested that modern Brassica crops derived from these species and that early selection of crop varieties have occurred in Mediterranean area (Christensen et al. 2011). It was indicated that early cultivated forms of *B. oleracea* were not originally from Mediterranean area and

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that they were brought from Atlantic coast. This issues is still under reconsideration, as other opinions implicate that the origin of *Brassica* crops is in the Mediterranean Basin (Swarup and Brahmi 2005; Izzah et al. 2013).

The *B. oleracea* primitive ancestors were under cultivation for several millennia, and written evidence are available since ancient Greek and Roman times. These long-lasting human-mediated selections resulted in significant morphological diversity of plant organs that are specific for particular Brassicas. Simplified, we can recognize leaves along the stem in kales, leaves surrounding the terminal bud in cabbages, enlarged axillary buds in Brussels sprouts, inflorescences in cauliflower and broccoli, and swollen stem in kohlrabi and marrow stem kale All of these types were classified in botanical varieties and co-varieties, but under modern cultonomic terms they would be cultivar-groups (Spooner, Hetterscheid, Van den Berg and Brandenburg, 2003). Among these groups, *acephala* group includes leafy, non-heading cabbages with common names: kale and collards. These names occur in different ways in many languages. Kale is often called 'borecole'; a term developed from Scottish word 'coles' or 'caulis', originally used by the Greeks and Romans in referring to all cabbages and cabbage like plants. The German word 'kohl' has the same origin. In English 'cole' also refers to all cultivated *B. olearacea* varieties. Collards are a derivative of the words coleworts or colewyrts, Anglo-Saxon terms that literally mean 'cabbage plants', and in America collards are sometimes called 'sprouts'. As already noted, in everyday use and scientific literature, word kale (kales) includes different varieties, such as: kale (*B. oleracea* L. var. *acephala* DC.), scotch kale (B. *oleracea* L. convar. *acephala* (DC.) Alef. var. *sabellica* L.), collard (B. *oleracea* L. var. *viridis* L.), palm kale (*B. oleracea* L. convar. *acephala* (DC.) Alef. var. *palmifolia* L.), marrow stem kale (B. *oleracea* L. convar. *acephala* (DC.) Alef. var. *medullosa* L.), thousand-head kale (*B. oleracea* L. var. *ramosa* DC.) and Portuguese Tronchuda cabbage (*B. oleracea* L. var. *costata* DC.) (Diederichsen, 2001). Classification presented in specific database for Brassica genus made by the European Cooperative Program for Crop Genetic Resources (http://ecpgr.cgn.wur.nl/Brasedb) is in agreement with above referred terminology/taxonomy.

A full range of morphotypes can be found among *acephala* group. Collard has smooth, broad leaves without blistering; kale has dark green and curled leaves, while Scotch kale types have gray-green and

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very curled and crumpled leaves. Marrow stem kale or fodder kale, used mostly for livestock, has soft stem and different leaves types. Palm tree kale (Lacinato or Tuscan kale) has dark blue-green and elongated leaves. Thousand-head kale is perennial bush, while trochunda cabbage is dwarf rosette plant with a variable loose pseudo-head (Dias and Monteiro 1994; Dias 2012). It is important to mention Siberian kale (*Brassica napus* L. var. *pabularia*), that is used as other kales, but new evidence showed this species is hybridization product of *Brassica oleracea* L. var. *acephala* and *Brassica rapa* L. (Körber et al. 2012), and can be distinguished from others by bluish-green foliage, less curled and with lacerate leaf incision.

As already mentioned, different kale types have a long history of extensive horticultural use that enables a great genetic variability, and resulted in a large number of kale populations/landraces across world. This variability can be result of intra-population variability generated by cross-pollination of plants, and as inter-population variability resulted from farmer's selection and adaptations to local ecological conditions (Cartea, Picoaga, Soengas, and Ordas 2002). In the last decades, morphological, agronomical, genetically and phytochemical characteristics of local kale varieties have been considered and investigated in many countries, as Spain (Cartea, Picoaga, Soengas, and Ordas 2002; Padilla, Cartea and Ordás 2007), Portugal (Dias and Monteiro 1994), Turkey (Balkaya and Yanmaz 2005), Croatia (Urlić, Dumičić, Ban and Romić 2016) and others (Izzah et al., 2013; Hahn, Müller, Kuhnert and Albach 2016).

3. Agronomic requirements

Crop management for kales are similar as for all *Brassica* crops. Cultivation field of *B. oleracea* var. *acephala* can be seen on Figure 1. It is recommended to make rotation with crops other than Brassicaceae, and to plant them again on the same soil every 3-4 years. Although kales can be cultivated in wide range of soils, the best production can be achieved on low acid or neutral (pH 6-6.5) soils, deep, with loamy texture and proper water and air capacity. *Brassica* plants have a high demand for nutrient uptake. Thus, basic soil preparation includes deep plugging with incorporation of high amounts of organic manure. Kale crops respond positively to application of nitrogen fertilizer by

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improving a vegetative growth and by delay of premature bolting, although concern about nitrate accumulation in plant tissues and environmental pollution should be highlighted (D'Antuono and Neri 1997). Kale type plants are mostly produced from seed. Seedlings develop strong taproot and several strong lateral roots. In warmer climate, seedlings are normally planted on the end of summer or early autumn. Cultivars with higher frost hardiness and resistance to premature bolting after induced vernalization by low temperatures produce edible leaves all winter. In addition, some cultivars could be planted in spring and harvest occurs during summer and autumn (Farnham and Garrett, 1996). Therefore, a fresh kale can be available on the market all year round. Extensive consumption of kale across Europe, Asia, and the United States and its recent huge popularity could be triggered with the fact that kale is easy and cheap for cultivation, and tolerant to unfavorable climate conditions (increased salinity, drought, high and low temperature etc.). Leaves can be picked 4-6 weeks after planting depending on desired leaf size and tenderness. Kales are harvested by removal of the older leaves on the stem what promotes the growth of the new ones and accordingly higher yield. Another commercial practice is to cut off completely the young plant or rosette.

4. Contemporary and traditional use

Kale (*B. oleracea* L var. *acephala*), has an important place in the culinary and diet of the population in Europe, Asia and America (Balkaya and Yanmaz 2005, Velasco et al. 2007, Lemos et al. 2011, Batelja et al., 2009). It has been traditionally cultivated by farmers on small plots mostly for family consumption, either human or animal food. Recent trend and promotion of kale as a 'superfood' bring kale to the menus of many restaurants across USA, especially those focused on the *healthy food*. Since it is tolerant to low temperatures, plants may survive the winter time and serve as fresh vegetable from late autumn to early spring next season. Younger and tenderer leaves of kale are used for human consumption while older leaves are more appropriate as fodder (Cartea, Picoaga, Soengas, and Ordas 2002). Kale leaves are usually consumed fresh in salads and as kale leaf juice, and cooked as diverse soup dishes, omelets, and stir-fry. In a Europe, kale is often served with the smoked pork. Some plant parts are occasionally prepared as pickles. Recently, dried kale or so-called ´kale chips´ became very popular, although draying significantly decreases its nutritive and phytochemical content (Oliveira,

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Ramos, Brandao and Silva 2015). Besides leaf, the kale seed may be used as crude oil supplement to breads and cakes (Ayaz et al. 2006). In development of novel functional food, Biegańska-Marecik, Radziejewska-Kubzdela and Marecik (2017) reported beverages based on apple juice with addition of frozen and freeze-dried kale leaves which are rich source of minerals and healthy phytochemicals. Although headed *Brassica* crops, such as white cabbage are very often used as fermented dish (sauerkraut), kale is not reported to be traditionally prepared and consumed as fermented vegetable. However there was reported an attempt to produce kale juice fermented with *Lactobacillus* strains which possessed a good nutritional composition (Kim 2017). Author suggested the fermented kale juice as healthy beverage with essential nutrients, although the acceptability of the fermented kale juice for the consumers' taste needs to be investigated.

As we mention above, in a many languages, especially in a records about traditional medicine, the same word has been used for all leafy *Brassica* vegetables, so it is hard to distinguish when in traditional medicine was used cabbage (*capitata* group) and when kale (*acephala* group). Therefore, use of kale in traditional medicine is very similar such as other *Brassica* crops that have been used in traditional medicine for centuries, mostly to cure gastritis and gastric ulcer (Leonti and Casu 2013). In addition to relief of symptoms of gastric ulcers, *B. oleracea* var. *acephala* has been reported to use for treating diabetes mellitus, rheumatism, bone weakness, ophthalmologic problems, hepatic diseases, anemia, obesity etc. (Lemos et al., 2011; Gonçalves et al. 2012, Kuerban et al. 2017).

5. Macronutrients, vitamins and minerals

According to the eight edition of Dietary guidelines for Americans from 2015-2010 realized by USDA, an adult woman needs about 2-2.5 and a man about 2.5-3 cups of vegetables every day, (USDA, 2015). In Guides, some cruciferous vegetables such as kale, collards and broccoli are counted in 'dark green vegetables' group whereas cabbage and cauliflowers are in 'other vegetables' group. Table 1. shows comparable macronutrients content in different Brassica vegetables according to the USDA Food Composition Databases (USDA, 2015). Cruciferous vegetables from *acephala* group (kale and collards) have higher Ca, folate, riboflavin,and vitamin K content while just vitamin C

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amount is much higher in kale than in other vegetables listed in Table 1. According to the Kamchan et al., (2004) calcium from kale is highly bioavailable, therefore kale could be considered as a good calcium source. According to the Becerra-Moreno et al. (2013) one serving size of kale provides more than 100% of the recommended daily intake (RDI) of vitamin A and more than 40% of the RDI of vitamin C. Other authors also recognized kale, among cabbages, as the best source of vitamins (A, B1, B2, B6, C and E), folic acid and niacin, fatty acids, and essentials minerals (especially K, Ca, Mg, Fe and Cu) (Ayaz et al. 2006; Jahangir, Kim, Choi and Verpoorte 2009; Eryilmaz Acikgoz and Deveci, 2011; Thavarajah et al. 2016) but their level may depend on the the environmental and growing factors (Fadigas et al. 2010; Björkman et al., 2011; Westwood, Cutts, Russell and O'Brien 2014). For example, Fadigas et al. (2010) reported that kale samples collected during winter have a higher concentration of micronutrients (Fe, Zn and Mn) and macronutrients (Ca and Mg) than those collected during summer. Maturity stage is also important factor which influence macronutrients, vitamin and mineral content in cruciferous vegetables (Šamec et al., 2011) including kale (Eryilmaz Acikgoz 2011). In last couple of years, kale, as well as other cruciferous, have been used in culinary in juvenile stage, as a sprouts, and in addition to the good sensory properties, sprouts are considered as a good source of proteins and dietary fibers (Vale et al., 2015). Eryilmaz Acikgoz (2011) who analysed vitamin C, mineral and protein content in kale during three harvesting stage found that with maturity vitamin C content increased, mineral content remained stable, while protein content was higher in younger plants. As we can noticed from the Table 1. all listed vegetables contain macronutrients which are crucial for human well-being and can support our health. It is hard to conclude that kale is 'superior' vegetables in comparison with other cruciferous vegetables, especially due to the fact that is very similar to collards. This is quite expected since both vegetables belong to the acephala group of non-headed Brassica vegetables and are genetically pretty similar.

6. Phytochemistry

In the recent years, new approaches in metabolomic profiling accelerated determination of phytochemicals in different plant-based food. In general, metabolomic may consider targeted and untargeted analysis. Targeted analysis include identification of selected (targeted) metabolites while

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untargeted metabolomics describes the process by which hundreds of molecules are globally profiled. Unfortunately, to the best of our knowledge, papers on untargeted metabolomic profiling of kale are not available. Targeted analysis showed that positive effect of the cruciferous vegetables in cancers prevention might be associated with the presence of health-promoting phytochemicals such as glucosinolates, polyphenols and carotenoids (Ferreres et al. 2009, Björkman et al. 2011, Becerra-Moreno et al. 2013; Šamec and Salopek-Sondi 2018). Jeon et al. (2018) reported transcriptome analysis and metabolite profile of kale where he annotated 26 glucosinolate biosynthetic genes, 23 phenylpropanoid biosynthetic genes, and 22 carotenoid biosynthetic genes. In the same paper, HPLC analysis revealed 14 glucosinolates, 20 anthocyanins, 3 phenylpropanoids, and 6 carotenoids in the kale. This founding supports the fact that also in the kale, the main phytochemicals with health benefites belong to the glucosinolates, polyphenols and carotenoids groups.

6.1. Glucosinolates

Glucosinolates are sulfur-containing compounds, found in *Brassica* plants, primary associated with health-benefits of vegetables belonging to this group. They are characterized by the core structure consisting of sulfated isothiocyanate group linked to thioglucose. Further modification of the core structure by side chain groups results in a great diversification of glucosinolates. Glucosinolates are not bioactive until they have been enzymatically hydrolyzed by the endogenous plant enzyme myrosinase to various bioactive breakdown products which include isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidinethiones (Vaughn and Berhow 2005; Cartea et al. 2008). Today is known around 200 different aliphatic, aromatic and indolic glucosinolates, but their presence in different Brassica species is most likely genetically predetermined. Each type of cruciferous shows a characteristic glucosinolates profile that includes more than ten different glucosinolates in each species/varieties, although only 3–4 are predominate (Fahey Zalcmann and Talalay 2001). Fresh kale contains glucosinolates in concentration 2.25-93.90 µmol/g dw (Table 2.), but ratio of indolic and aliphatic glucosinolates differs in samples from different location. For example, Turkey kale varieties were reported to contain significantly higher amount of indolic glucosinolates (Sarıkamış, Balkaya and Yanmaz 2008), while Polish ones were higher in aliphatic (Korus, Słupski, Gebczynski and Bana

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2014). Glucobrassicin (Figure 2.) was reported as predominant indole glucosinolate in American (Charron, Saxton and Sams 2005.), Spanish (Velasco et al. 2007), Polish (Korus, Słupski, Gebczynski and Bana 2014; Kapusta-Duch, Kusznirewicz, Leszczynska and Borczak 2016), Norwegian (Steindal, Rødven, Hansen and Mølmann 2015) and Korean (Park et al. 2017) kale varieties. Hydrolysis product of glucosinolates glucobrassicin- indole-3-carbinol and its further derivative 3,3′-diindolylmethane received considerable interest as cancer chemoprotective agents (reviewed by Fujioka et al., 2016). Predominant aliphatic glucosinolates in kale are reported to be sinigrin, glucoiberin and glucoraphanin (Charron, Saxton and Sams 2005; Velasco et al., 2007; Sasaki et al., 2012; Korus, Słupski, Gebczynski and Bana 2014) (Figure 2.). Sinigrin and its hydrolysis product allyl isothiocyanate are associated with numerous therapeutic benefits (Mazumder, Dwivedi and Plessis 2016), while hydrolysis product of glucoraphanin, sulforaphane, according to the review article by Elbarbry and Elrody (2011) has the potential to reduce risk of various types of cancers, diabetes, atherosclerosis, respiratory diseases, neurodegenerative disorders, ocular disorders, and cardiovascular diseases.

In recent years, kale sprouts have become also popular in culinary. Recent report by Jeon et al. (2018) showed that in 10 days old kale seedlings predominant glucosinolates are glucobrassicin and sinigrin, similar as in mature vegetables. Therefore, kale contains glucobrassicin and sinigrin, two glucosinolates well studied and associated with health benefits. However, when glucobrassicin content was comparatively analyzed in different Brassica vegetables, kale contained significantly lower amount than broccoli and Brussels sprouts (Charron, Saxton and Sams 2005). According to available literature, in comparison with other Brassica vegetables, kale does not contain significantly higher levels of glucosinolates, and therefore probably does not possess a genetic background that would result with the increased glucosinolates content. Rather, glucosinolates content of kale, as well as in other Brassica species, depend on conditions of cultivation, locations and developmental stage, what is demonstrated in several studies (Charron, Saxton and Sams 2005; Velasco et al., 2007; Cartea et al., 2008; Sarıkamış, Balkaya and Yanmaz et al. 2008; Steindal, Rødven, Hansen and Mølmann 2015).

6.2. Phenolic compounds

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Phenolic compounds are wide group of specialized metabolites associated with the health benefits. They are the best studied and extensively reviewed group of specialized metabolites attributed to the management of obesity (Farhat Drummond and Al-Dujaili 2017), type 2 diabetes (Guasch-Ferré et al., 2017), metabolic syndrome (Chiva-Blanch and Badimo, 2017), neurodegenerative diseases (Hossen et al., 2017), atherosclerosis (Bahramsoltani et al., 2017) and cancer (Russo, Tedesco, Spagnuolo and Russo 2017). It was reported that polyphenols, in synergy with other compounds, significantly contribute to the biological activity of *Brassica* plants (Šamec et al., 2011; Šamec, Pavlović and Salopek-Sondi 2016). Their level in kale varies depend on the variety, maturity stage, growing location and environmental condition, so it is hard directly compare results of the different authors. The most commonly total phenolic content is measured using Folin–Ciocalteu method which is very unspecific (reagent can react with any reducing substance), thus these data can not be well correlated with the health benefits potential of certain vegetables. More relevant data are those collected using modern hyphenated methods (HPLC-MS/MS, HPLC-DAD, GC-MS) which provide the data of specific polyphenols levels. The best studied groups of polyphenols are flavonoids. Flavonoids quercetin and kaempferol (Figure 3.) are reported to be predominant flavonoids in kale (Hagen, Borge, Solhaug and Bengtsson 2009; Akdaş and Bakkalbaşı 2017), more exactly their mono- to tetraglycosides (Lin and Harnly 2009; Ferreres et al. 2009; Olsen, Aaby and Borge 2009; Olsen, Aaby and Borge 2010). Red variety of curly kale also contains anthocyanins among which cyanidin-glycosides (Figure 3.) are predominant (Olsen, Aaby and Borge 2009; Olsen, Aaby and Borge 2010; Jeon et al., 2018). Furthermore, phenolic acids present a large group of phenolic compounds, widespread in plants. They are derived from benzoic and cinnamic acids and are associated with health benefits, but also can affect the organoleptic properties of the plant-based food (Gruz, Novak and Strnad 2008). Dominant phenolic acids in kale varieties are those from hydroxycinnamic group (up to 92.8% of identified phenolic acids) (Ayaz et al., 2008; Lin et al., 2009; Olsen, Aaby and Borge 2009; Olsen, Aaby and Borge 2010). In the kale leaves derivatives of caffeic, ferulic and sinapinic acid (Figure 3.) were the most commonly identified phenolic acids (Ayaz et al., 2008; Lin and Harnly 2009; Olsen, Aaby and Borge 2009; Olsen, Aaby and Borge 2010). Comparative studies of phenolic profiles of different *B. oleracea* vegetables showed very similar patterns, and there was not any

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particular variety underlined as significantly rich in these compounds (Lin and Harnly 2009; Lin and Harnly 2010; Cartea, Francisco, Soengas and Velasco 2011).

6.3. Carotenoids

Although the color of the carotenoids is masked by chlorophyll, cruciferous vegetables are good sources of β-carotene (provitamin A) and lutein, which, together with zeaxanthin, due to the strong antioxidant activity, are considered to play a role in ocular health (Manikandan et al. 2016). Both β- and α-carotenes are precursors to vitamin A, which is important for healthy skin, bone, gastrointestinal, and respiratory systems. Principal carotenoids in kale are lutein, β-carotene, violaxanthin and neoxanthin (and Rodriguez-Amaya 2005), but also the presence of 13-cis-β-carotene, α-carotene, 9-cis-β-carotene and lycopene were reported (Jeon et al., 2018). Carotenoids in vegetables may contribute to health benefits, but it is important to understand absorption from plant foods. In order to study bioavailability of lutein and β-carotene from kale, Novotny, Kurilich, Britz and Clevidence (2005) feed seven adult volunteers with 13C labeled kale, and analyzed serial plasma samples for labeled lutein, β-carotene, and its metabolite retinol. Concentrations of labeled β-carotene, lutein, and retinol in plasma increased markedly after ingestion of the isotopically labeled kale. Lutein was first detected between 3 and 6 h after the dose, β-carotene was first detected at 4 or 5 h after the dose, and retinol was first detected between 4 and 6 h after the dose. This showed that kale could be considered as a good source of carotenoids. Their quantity in kale depends on environmental factors during growing and maturity stage (Azevedo and Rodriguez-Amaya, 2005; Lefsrud, Kopsell, Wenzel and Sheehan 2007). For example, according to the Lefsrud, Kopsell, Wenzel and Sheehan (2007) the β-carotene and lutein content during growth period followed a quadratic trend, with maximums occurring between the 1st and 3rd week of leaf age. In order to compare carotenoid content of different *B. oleracea* vegetables, Kurilich et al., (1999) exanimated 50 broccoli, 13 cabbage, kale, cauliflower, and Brussels sprouts accessions, and found that kale contained comparatively the highest amount of β -carotene. In the same study they found that kale contain higher amount of α-tocopherol, a form of vitamin E important for maintaining stable cell membranes and preventing oxidative damage to tissue.

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7. Biological activity

As can be seen above, kales contain phytochemicals, which are associated with the biological activity in human body after consumption. In general, biological activity of certain food/food component can be measure *in vitro* and *in vivo*. However, the biological activity *in vitro* does not mean necessarily the activity *in vivo*, although *in vitro* results can direct future research and help in a selection of a potentially interesting food/food component. Unfortunately, *in vivo* experiments on kale are very limited. The main biological activities associated with kale are antioxidant activity, anticancerogenic activity and protection of cardiovascular and gastrointestinal tract (Figure 4.)

7.1. Antioxidant activity

Kale contains compounds such as polyphenols, carotenoids, glucosinolates' hydrolysis products, vitamin C and E that show antioxidant activity. It is widely accepted that food high in phytochemicals with antioxidant activity can help with the protection against free radicals and reactive oxygen species (ROS), and therefore in prevention from chronical diseases. Several *in vitro* methods are used for determination of antioxidant activity among which ORAC (Oxygen Radical Absorbance Capacity) attracts a lot of attention and was considered to become standard, however correlation with the *in vivo* efficacy has not still been fully clarified. For example, in 2010, U.S. Department of Agriculture realized USDA Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods but later they removed it from the website due to mounting evidence that the values indicating antioxidant capacity have no relevance to the effects of specific bioactive compounds. Cruciferous vegetables from *acephala* group were not listed in those database, however antioxidant activity of kale was evaluated *in vitro* in several studies (Zhou and Yu 2006; Ayes et al. 2008; Hagen, Borges, Solvang and Bentsen 2009; Korus and Lisiewska 2011; Korus 2011; Becerra-Moreno et al. 2013). Zhou and Yu (2006) measured antioxidant capacity of 38 commonly consumed vegetable using several methods, and reported kale, together with the spinach, broccoli, and rhubarb, as a vegetable with the highest antioxidant activity. Furthermore, Sikora et al. (2008) measured antioxidant activity in kale, broccoli, Brussels sprouts and green and white cauliflower and found that kale had much higher antioxidant

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activity than other analyzed vegetables. Kale extracts showed also *in vitro* protective effect on the oxidation of very low density (VLDL) and low density (LDL) lipoproteins which may indicate protective role from cardiovascular diseases (Kural, Küçük, Yücesan and Örem 2011).

7.2. Anticancerogenic activity

Several epidemiological studies over the last decades supported the fact that Brassica vegetables are promising in cancer prevention. Meta-analysis that combined results of different authors showed an inverse relationship between cruciferous vegetable consumption and the risk of cancers of the reproductive system (Liu, Mao, Cao and Xie 2012; Liu and Live, 2013; Han, Li and Yu 2014), gastrointestinal system (Wu et al., 2013a; Wu et al., 2013c), urinary system (Liu et al., 2013a; Liu et al., 2013b) and lung (Wu et al., 2013b). Mechanisms underlying anticancer activity have been attributed to the decomposition products of glucosinolates, which are also present in kale. So far, indole-3-carbinol (Figure 1), the hydrolysis product of glucobrassicin, has been the best studied component, which has received considerable interest as cancer chemoprotective agent. Indole-3-carbinol and its dimeric product 3,3'-diindolylmethane target multiple aspects of cancer cell-cycle regulation and survival including Akt-NFjB signaling, caspase activation, cyclin-dependent kinase activities, estrogen metabolism, estrogen receptor signaling, endoplasmic reticulum stress, and BRCA gene expression (Weng, Tsai, Kulp and Chen 2008; Caruso et al. 2014). However, little is known about bioavailability of indole-3-carbinol from foods including kale. The most important factor for conversion of glucobrassicin into indole-3-carbinol is myrosinase (Martinez-Ballesta and Carvajal 2015) whose endogenous activity in kale is comparative as those in broccoli (Dosz, Ku,Juvik and Jeffery 2014.).

Anticancerogenic activity of plant extracts can be measured by several *in vitro* tests, which may or may not include tumor cells. Such as test can detect potential activity of certain plant extract or particular fraction, but it does not necessarily mean the same activity *in vivo*. One of the parameters important for cancer prevention is antigenotoxicity that describes the extracts properties that prevent damages of the genetic information within a cell, which can cause mutations and may lead to cancer.

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Antigenotoxic potential was confirmed in a kale extracts in the study on Brazilian kale variety ‘’couve’ (Goncalves et al. 2012). In addition to antigenotoxicity, easy and often used method for determination of anticancer potential of some plant extract/compound is cancer cell proliferation assay, which is based on the measurement of extract impact on the cell proliferation. Traditional cell proliferation assays involve incubating cancer cells for a few hours to overnight with tested plant extract or compounds of interest. Then, using specific dyes could be monitored metabolic activity of cells, and if tested extract influence proliferation we can attribute it with the potent cytotoxic activity. Radošević et al. (2017) studied cytotoxicity of *B. oleracea* from *italica* and *acephala* groups on a breast adenocarcinoma (MCF-7) and cervical cancer (HeLa) cells line. Tested extracts showed inhibitory effect on cell viability with concentration 50 and 100 mg/ mL. In another, comparative study, which include testing of antiproliferative activity of 34 common vegetables on 8 different tumor cell lines (stomach adenocarcinoma, CRL-1739; mammary gland adenocarcinoma, HTB-22; pancreatic carcinoma, CRL-1469; prostatic adenocarcinoma, CRL-1435; lung carcinoma, CCL-185; medulloblastoma, HTB-186; glioblastoma, HTB-14; renal carcinoma, HTB-47), Boivin et al. (2009) classified kale as a vegetable with very high chemopreventive potential together with other cruciferous vegetables such as Brussel sprouts, cabbage and curly cabbage and as well the plants from Allium family. In that study, all extracts had strong inhibitory activities against a glioblastoma cell line, but had negligible effects on the growth of normal cells, strongly suggesting that the antiproliferative properties of these vegetables are specific to cells of tumor origin.

7.3. Effects on gastrointestinal tract

Brassica oleracea plants including *acephala* group have been used in traditional medicine of different culture to treat gastrointestinal problems (Šamec, Pavlović and Salopek-Sondi 2016; Šamec and Salopek-Sondi, 2018). In Brasil, plants from acephala group are known under the common name ‘couve’ and are most commonly used for the treatment of gastritis and especially gastric ulcer (Lemos et al., 2011). Antiulcer activity was demonstrated experimentally in rats and mice, and protection mechanisms may be explained with the stimulation of the mucus synthesis, increase pH and decrease H+ ions in the stomach after kale ingestion (Lemos et al., 2011). Gastritis and peptic ulcers are

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commonly caused by infection with the *Helicobacter pylori*, which dramatically enhance the risk of gastric cancer (Fahey, Stephenson, Wade and Talalay 2013). Fahey, Stephenson, Wade and Talalay (2013) found that glucoiberin hydrolysis product sulforaphane (Figure 1) inhibits extracellular, intracellular, and antibiotic-resistant strains of *Helicobacter pylori* and prevents some types of stomach tumors. As we mention above, kale contains glucoraphanin, which is sulforaphane precursor, therefore, antiulcer activity of kale may be related with the sulforaphane anti- *Helicobacter pylori* activity. Kale also possesses antimicrobial activity against *Staphylococcus aureus, Enterococcus faecalis*, *Bacillus subtilis* and *Moraxella catarrhalis* (Ayaz et al., 2008).

Different Brassica vegetables in traditional medicine have been used for relaxing of the symptoms of the inflammatory bowel disease (IBD) and kale effect was studied *in vitro* using animal models (Albuquerque et al., 2010). Rats received, orally, 500 mg/kg of rat weight three treatments of dried vegetables: papaya, kale and the mixture of both vegetables (60% of kale plus 40% of papaya) but only the administration of the mixture was able to modulate the bacterial flora in healthy rats, as well as in rats with the induced colitis (Albuquerque et al., 2010). IBD, according to some hypothesis, may be caused by an imbalance in the intestinal microflora, the relative predominance of aggressive bacteria, and an insufficient amount of protective species. Therefore, therapeutic approach may be to modify the intestinal micro flora by administration of probiotics or prebiotics which may promote the growth or activity of beneficial microorganisms. Prebiotic-rich diets promote intestinal microbial diversity, stimulate the immune system, promote mineral micronutrient absorption, decrease the risk of developing colon cancer, reduce excessive blood glucose and cholesterol levels, and improve insulin sensitivity (Dey, 2017). Thavarajah et al., (2016) analyzed 25 different kale genotypes grown in Pelion, South Carolina, USA, and found that kale in a diet can provide adequate quantities of prebiotic carbohydrates, therefore, presence of the prebiotics in kale may contribute to the their association as an functional food or superfood.

7.3. Effects on cardiovascular system

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Presence of compounds such as polyohenols, glucosinolates, carotenoids, Vitamins E and C in food are associated with the cardiovascular protection (reviewed by Dinkova-Kostova and Kostov 2012). According to the review article by Manchali, Murthy and Patil (2012) bioactive compounds of cruciferous vegetables help in heart health mainly through their ability to reduce LDL, to combat free radicals and up-regulate GST activity (reduction of oxidative stress). *In vitro* study by Kural, Küçük, Yücesan and Örem (2011) showed that kale methanolic extract possess protective effect on the oxidation of very low density (VLDL) and low density (LDL) lipoproteins. Cholesterol-lowering (atherosclerosis amelioration) or detoxification of harmful metabolites (cancer prevention) potential of food fractions could be predicted by evaluating their *in vitro* bile acid binding, based on positive correlations found between *in vitro* and *in vivo* studies showing that cholestyramine (bile acid–binding, cholesterol-lowering drug) binds bile acids and does not bind cellulose (Kahlon, Chiu and Chapman 2008). In two studies by the same group (Kahlon Chapman and Smith 2007; Kahlon, Chiu and Chapman 2008) kale had the highest bile acid binding in comparison with other vegetables and capacity increased after steam cooking what suggest that kale would lower the risk of cardiovascular disease and cancer also consumed after cooking (Kahlon, Chiu and Chapman 2008). Kim et al. (2008) confirmed *in vivo* kale effect on reducing coronary artery disease risk. In their study thirty-two men with hypercholesterolemia consumed 150 mL of kale juice per day for a 12-week intervention period. Kale juice supplementation resulted in substantial improvements in serum lipid profiles, especially with respect to HDL and LDL cholesterol levels, the ratio of HDL- to LDL-cholesterol, and in antioxidant status. It was established that cardiovascular disease is a disease of inflammation, and consequently is amenable to intervention *via* molecules that have anti-inflammatory effects (Kris-Etherton et al., 2004).Therefore, presence of the compounds with anti-inflammatory activity such as carotenoids and flavonoids (Ciccone et al., 2013) may contribute to cardiovascular protection of the compounds which contain them, such as kale.

7.4. Other activity

Brazilian study showed that the use of *B. oleracea* var. *acephala* leaf juice once a day for 24 months resulted in bone mass stabilization in women after menopause (Pereira et al., 2006): however, authors

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did not suggested possible mechanisms and study was done on relatively small number of women, only 13. Furthermore, kale seeds extract was shown as an effective acetylcholinesterase inhibitor, which implicates that may be used in therapeutic applications in neurological diseases such as in Alzheimer's disease, senile dementia, ataxia, myasthenia gravis, and Parkinson's disease (Ferreres et al. 2009). This effect may be explained due to the presence of sinapine, an analogue of acetylcholine, which is suggested to be acetylcholinesterase inhibitor (Dohi, Terasaki and Makino 2009).

8. Conclusions

Considering great popularity of *Brassica oleracea* var. *acephala* plants in recent years, relatively small number of research are available on those plants, especially those which include *in vivo* biological studies. Kale showed antioxidant and anticancerogenic potential in several *in vitro* studies, very similar as other cruciferous vegetables. Unfortunately, there are no aveilable studies, which would explain *in vivo* biological activity of kale. Kale and collards are higher in Ca, folate, riboflavin, vitamine C, K and A content than other cruciferous while their phytochemical content is comparable with other *Brassica* vegetables. The levels of specific phytochemicals are more under the influence of environmental growth factors. Without doubt, cruciferous vegetables can provide variety of compounds with health benefits, but it is hard to conclude at that point that one cruciferous is healthier than another. Kale is easy and cheap for cultivation, and tolerant to unfavorable climate conditions so these are likely reasons for kale promotions, especially in recent years where climate changes cause extreme temperature fluctuations. Kale can be considered as a superfood, the same as another cruciferous. Currently there is lack of literature, which will support the fact that kale provides more health benefits than other cruciferous, but maybe in the future this statement can change. At this point, according to the available literature, the choice of cruciferous in the diet can be based on availability and consumer preferences.

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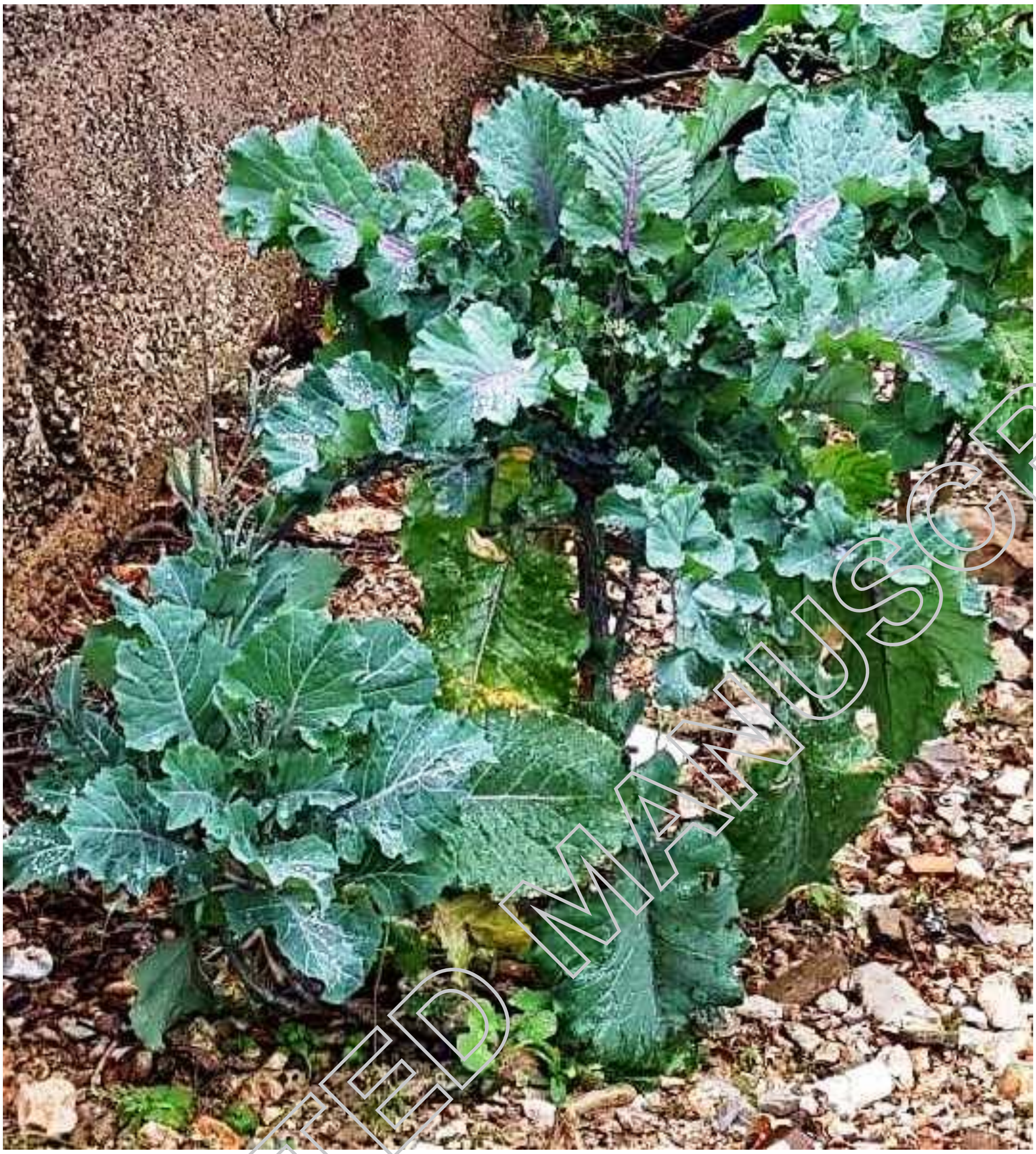

Figure 1. *Brassica oleracea* var. *acephala* growing in a southern Croatia (Dubrovnik-Neretva county).

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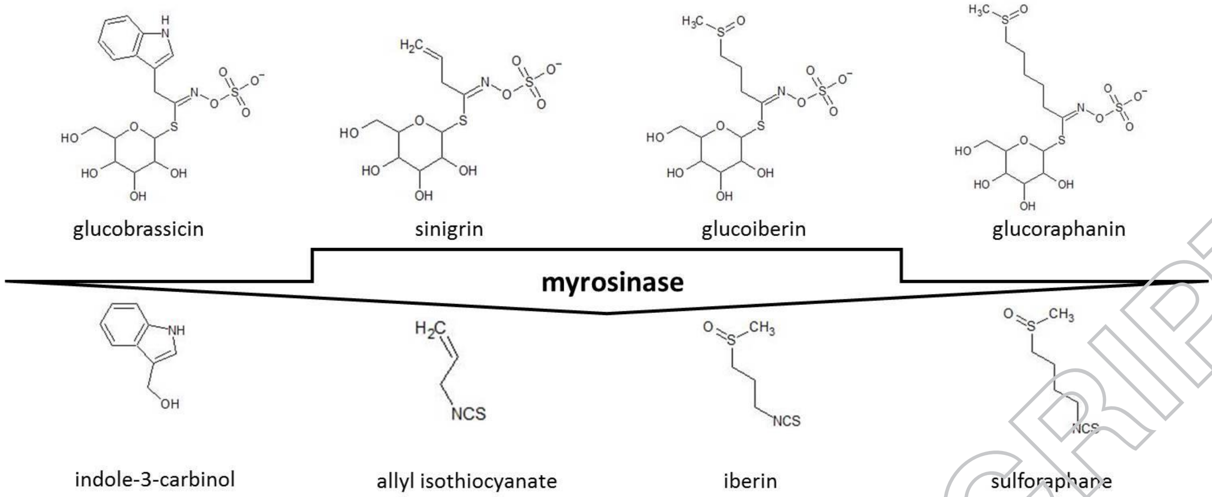

Figure 2. The most abundant glucosinolates in *Brassica oleracea* var. *acephala* and their hydrolysis products.

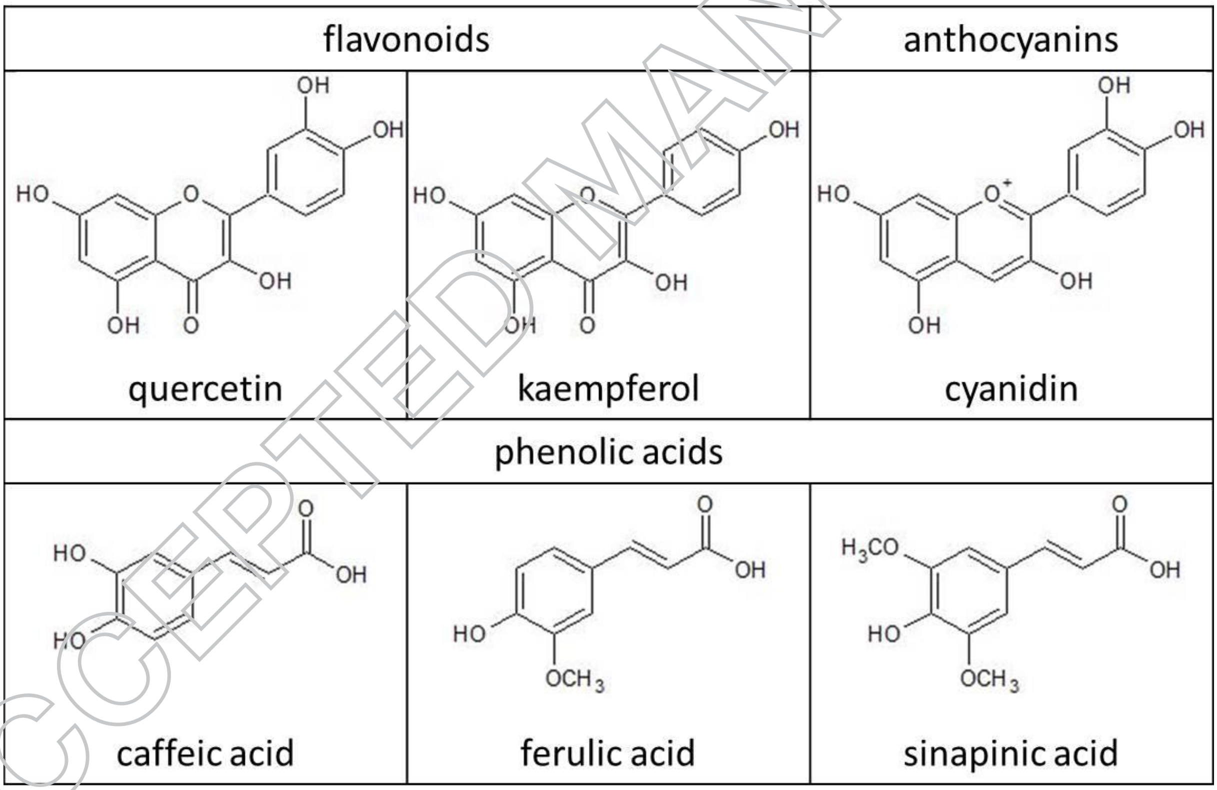

Figure 3. Main polyphenolic compounds identified in *Brassica oleracea* var. *acephala*.

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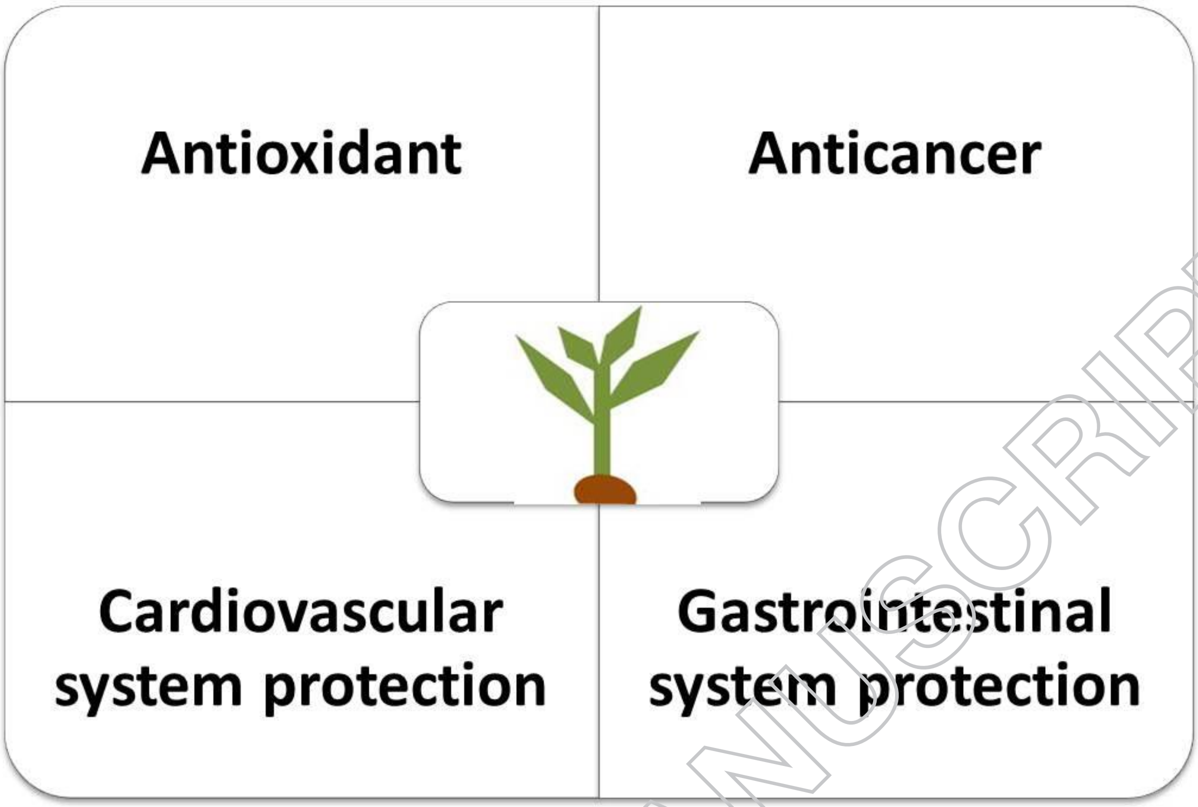

Figure 4. *Brassica oleracea* var. *acephala* biological activities.

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Table 1. Content of proximates, minerals, vitamins and fatty acids according to the USDA Food Composition Databases (available at https://ndb.nal.usda.gov/ndb/)

		kale	collards	brocolli	cauliflower	cabbage	Brussels sprouts
Proximates	water (g)	84.04	89.62	89.30	92.07	92.18	86.00
	energy (kcal)	49	32	34	25	25	43
	protein (g)	4.28	3.02	2.82	1.92	1.28	3.38
	total lipids (g)	0.93	0.61	0.37	0.28	0.10	0.30
	Carbohydrate (g)	8.75	5.42	6.64	4.97	5.80	8.95
	Fibres (g)	3.6	4.0	2.6	2.0	2.5	3.8
	Sugars (g)	2.26	0.46	1.70	1.91	3.20	2.20
Minerals	Ca (mg)	150	232	47	22	40	42
	Fe (mg)	1.47	0.47	0.73	0.42	0.47	1.40
	Mg (mg)	47	27	21	15	12	23
	P (mg)	92	25	66	44	26	69
	K (mg)	491	213	316	299	170	389
	Na (mg)	38	17	33	30	18	25
	Zn (mg)	0.56	0.21	0.41	0.27	0.18	0.42
Vitamines	vitamine C (mg)	120	35.3	89.2	48.2	36.6	85
	thiamin (mg)	0.110	0.054	0.071	0.050	0.061	0.139
	riboflavin (mg)	0.130	0.130	0.117	0.060	0.040	0.090
	niacin (mg)	1.000	0.742	0.639	0.507	0.234	0.745
	B-6 (mg)	0.271	0.165	0.175	0.184	0.124	0.219

	folate (µg)	141	129	63	57	43	61
	vitamin A (µg)	500	251	31	0	5	38
	vitamin E (mg)	1.54	2.26	0.780	0.08	0.15	0.88
	vitamin K (µg)	704.8	437.1	101.6	15.5	76.0	177.0
fatty acids	saturated (g)	0.091	0.055	0.039	0.130	0.034	0.062
	monounsaturated (g)	0.052	0.030	0.011	0.034	0.017	0.023
	polysaturated (g)	0.338	0.201	0.038	0.031	0.017	0.153

Table2. Total glucosinolates content in kale from different growing locations

location	content (µmol/g dW)	reference
Poland	2.25±0.09	Kapusta-Duch et al., 2016
Poland	26.87 ± 0.23	Korus et al., 2014
Spain	23.04- 93.90	Velasco et al., 2007
USA	4.2-10.6	Charron et al., 2005
Turky	34.52-60.94	Sarıkamış et al., 2008
Spain	11.00–52.79	Cartea et al., 2008

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