Egypt. J. Chem. **Vol. 67**, No. 8 pp. 593 - 607 (2024)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

A Relationship Between Cytotoxicity, Antioxidant Activity and Seasonal Changes in Glucosinolates and Isothiocyanate in Kale Varieties Recently Cultivated in Egypt

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Abstract

The kale plant, an important member of the family Brassicaceae, was recently introduced to Egypt. Five varieties of kale were propagated in two growing seasons: winter and autumn. They have successfully grown and continue their growth to maturity. The first season propagated in sandy soil (SS) was taken in July 2022, while the second season in clay soil (CS) grew its cut took place in January 2023. The plant is consumed due to its high content of health-promoting glucosinolates (GS), and its hydrolysable product, isothiocyanate (ITC), is an important antitumor agent. These two substances were spectrophotometrically determined through different developmental growth stages. The yield of leaves differs between the varieties studied, which all increased gradually until they reached their maximum at the cutting stages. The prumier type produced a higher yield in the two cuttings and was higher in the second one due to the soil type propagated, which is clay in texture. Also, kale varieties differ in their content of GS and ITC. A multi-purpose chromatographic technique was used for analysis, where glucosinolates and isothiocyanates were separated simultaneously by heat and enzyme method, with the measure by spectrophotometer at 320 nm for isothiocyanate from K4: lacinato, giving 1.67 ± 0.10 ug/g in June by dichloromethane extract, and 425 nm for glucosinolates, giving 118.92 ± 0.34 ug/g in sandy soil compared to enzyme methods, giving 17.85 ± 1.53 ug/g isothiocyanates. In conclusion, the HPLC was giving the highest sulfuraphane concentration in K1: vates blue curled at January in the second season as 163.84 µg/g.

Keywords: Kale varieties, glucosinolates, isothiocyanates, antioxidant, enzymatic hydrolysis, sulfuraphane

1. Introduction

Brassicaceae is a large family with 350 genera and 3700 species[1].This family contains six agriculturally important vegetables, which are consumed in large quantities worldwide. The genus *Brassica*, and its species *olraceae* has evolved into a number of cultivars with edible parts. *Brassica* vegetables are considered healthy food due to their diverse biological activities which is attributed to their bioactive compounds such as carotenoids, glucosinolates and phenolics [2], with anti-inflammatory and antioxidant properties that contribute to chemo-preventive activity against cancer[3, 4].

Kale is the best representative variety of the species and is an important vegetable and forage crop. Two important species were found in this family, *B. sobillica* and *B. encephala* (the black cabbage kale) the latter variety has dark green wrinkled leaves that are usually eaten cooked because of their thickness leaves. The sobillica variety

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Received date 05 May 2024; revised date 03 June 2024; accepted date 09 June 2024
DOI: 10.21608/EJCHEM.2024.283173.9598

exhibits different morphological leaves with different colors. Detailed reviews on glucosinolates of *Brassica* have been published by several authors [2, 5-7]. kale is mainly cultivated in central and Northern Europe and Northern America as winter crops [8], but it can be also grown all the year in the tropical regions due to its tolerance to summer temperature. Its production is extended to Mexico, Chile and Argentina.

Glucosinolates are water soluble and sulphur rich natural products with varying side chains which are responsible for the unique physiological roles and specific olfactory functions. In addition glucosinolates also have great potential to function as health promoting compounds after myrosinase-mediated hydrolysis [9]. Hydrolysis of glucosinolates by the endogenous enzyme lead to the formation of isothiocyanates, nitriles, thiocyanates, epithionitrile and oxasolidine-9-thione products vary widely, and depend on different species, stages of development and micro-environment of reactions such as pH [10, 11]. Among these hydrolytic products isothiocyanates have been extensively studied for their health promoting functions including anticancer and antibacterial effects.

As a new plant introduced to Egypt an investigation was carried to study the effects of different factors on yield and active ingredients and following up the of kale through its growth stages from sprout to maturity, with special reference to its content of glucosinolates and hydrolysable byproducts[12, 13].

Isothiocyanates are known as a protective agent against the development of various types of cancer [14]. This suggests that a high intake of Brassicaceae plants can have a chemo-protective effect and reduce the risk of various types of disease. The anticancer mechanism of isothiocyanates is based on anti-inflammatory activity and inhibition of tumor invasion and cell proliferations. This mechanism has been observed in many types of cancer *in-vitro*[15]. Sulforaphane, a glucoraphanin derived isothiocyanate found in high concentration in kale has been shown to inhibit chemically induced breast cancer in mice [16]. Also contains abundant sulfur and is known to be a natural of phase II detoxification enzymes that act as triggers of cytostasis and apoptosis[17].

Significant glucosinolates varies from year to year have been reported due to differences in climatic conditions [18, 19], the level of glucosinolates is also influenced by the growing location[20]. Josefsson [21] evaluated the distribution of glucosinolates in two kale cultivars grown at three different locations. Rosa and Heaney [22] reported that differences in GC content between growing regions were greater than between cultivars.Factors that might contribute to these differences include soil type, application of sulfate and nitrate fertilizers, spacing and harvest dates[21, 22]. In a study in *Brassica napus*, it was reported that higher nitrogen intake increased progoythrin at the expense of sinigrin [23]. Several of plants isothiocyanates content can be associated with genetic environmental and breeding factors. The factors that regulate the levels of isothiocyanates in plant tissue are not well understood, but some studies suggest that environmental factors may play a role in determining the level of isothiocyante or their derivatives in plant tissues. The effect of season on the evolution of isothiocyante content in radish roots was reported by Blazevic and Mastelic [24]. Wang et al., [25] observed changes in isothiocyanates and nitriles during the development of radish, chinese cabbage, cauliflower and turnip greens. On the other hand Rosa et al., [26] observed differences in the isothiocyanate contents of *B. oleracea* var. capitate between years suggesting that weather is associated with the accumulation of the active compounds in *B. oleracea* plant.

The present study deals with the introduction of different kale varieties obtained from USA as new crop with different beneficial activity to human beings. This study was carried out over two years to examine the individual changes in isothiocyanate from seedlings to maturity in different kale cultivars. In parallel to dates of sowings were tried to confirm the ample date with higher yield components and the dynamic variation of the active biological isothiocyanates during the two growth seasons conducted.

2. Materials and methods

2.1. Plant material

Seeds of five varieties of kale were obtained from USA through Dr. Gamal Reid, vegetable crops Dept.

NRC to whom we appreciate. All varieties have the Latin name *Brassica olraceae* varieties *sabillica*. These varieties have the following names as (**K_1**: *Vates Blue Curled*, **K_2**: *Dwarf Siberian*, **K_3**: *Red Russian*, **K_4**: *Lacinato* and **K_5**: *Prumier*). The seeds were sown in foam tray in the green house and then transferred to the two types of soil as sandy and clay for the first growth season and the second one respectively on 2022 and 2023.Seedlings were transferred to the soil after being 15cm in length on rows of 60cm in between and 30cm between plants. After producing the third leaves, plants were thinned to 2 plant/whole and the thinned plants were considered as the first sample for analyzing the active glucosinolates and its byproducts. The plants were irrigated and fertilized as usual and samples were taken every month from the five varieties. Samples were oven dried at 45°C powdered and kept in paper bags in desiccator till extracted [27, 28].

2.2. Extraction of glucosinolates and isocyothianates

An accurate weight of leaves was extracted with 50% methanol as soon as collected from the field then put in refrigerator at -70 ° C till beginning the analysis [29].

The alcoholic extract was reduced to small volume by evaporation under temperature not exceeding 40ºC. The mixture was incubated in a water bath for 3 hours at 45ºC and left outside the water bath for 30 min to reach room temperature. 100 ml dichloromethane were added and the mixture was stirred for 15 min and filtered with Whatmann No.I paper [29].

The organic layers were combined into separating funnel for removal of water, and then dried with anhydrous sodium sulfate. Solvent was then evaporated under vacuum at 35ºC till reach a final volume of 10 ml then injected to HPLC for separation and identification of different Kale constituents [30].

2.3. HPLC analysis of the hydrolysable product of glucosinolates

The HPLC system was a HP 1100 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto-sampler (G1329B), quaternary pump and a diode array detector. The measurements were integrated by Chemostation chromatographic software interfaced to a personal computer [31]. The analytical column was ZORBAX Eclipse XDB C18 column (15 cm x 4.6 mm I.D., 5 µm, USA) according to Cai et al [32].

Sulforaphane the major constituent of the hydrolysable component was determined by HPLC through the samples taken during in the two growth seasons and reported in Tables (3-6).Acetonitrile and water at a flow rate of 1.0 ml/min were used for elution [33]. The procedure employed isocratic elution with 1.5% acetonitrile for the first 5 min; a linear gradient to 20% acetonitrile over the next 15 min, followed by isocratic elution with 20% acetonitrile for the final 10 min. Absorbance was detected at 226 nm. Ortho-nitrophenyl-β-D-galactopyranoside (Sigma) was used as an internal standard for HPLC analysis [34].

2.4. Enzymatic analysis

The enzymatic cleavage was carried out according to the described procedure [35] modified as follows: Freshly kale plant (1g) was extracted with 70% methanol (10 ml). The mixture was filtered and the extract was concentrated at 40°C. Water (0.5 ml) was added and the solution was again evaporated. Distilled water (4 ml) was added to the residue followed by the addition of 1 M citrate buffer (0.2 ml, pH 6.5), ascorbic acid (5 mg), a solution of the enzyme myrosinase (0.8 ml, corresponding to 0.15 mg of protein) and the solution kept at 37°C for 2 hours. Acetone (2 ml), borate buffer (pH 10, 3 ml), and thioglycolic acid (0.5 ml) was added, the mixture was shaken and kept at 40°C for 2 hours. Hydrochloric acid (4 N, 1 ml) was added; the solution was kept at 40°C for 2 hours and after having been cooled the solution was extracted with chloromethane (10 ml). The clear solution was evaporated under reduced pressure at 40°C and the residue was dissolved in acetic acid (1 ml). The solution thus obtained was used for qualitative determination by using spectrophotometer at absorption 296 nm.[36]

2.5. Determination of glucosinolates (TGS) and isothiocyanate (TITC) by spectrophotometer

The content of TGS in Kale varieties was determined according to the method described in Mawlong et al.[37]. In the meantime the TITC contents produced by hydrolysis performed according to the method of Drobnica and Knoppová [35].

2.6. Evaluation of antioxidant activity

DPPH and ABTS free radical-scavenging activity methods were adopted to measure the in vitro antioxidant activity of extracts of kale verities according to Mohammed et al. [38], dichloromethane extract; at different concentration (20,15,10, 5, µg/mL) ascorbic acid, and trolox were used as a positive control[39, 40].

2.7. Cytotoxic activity

Anticancer activity was assessed to investigate the five kale verities extracts against HepG2 cell line (human liver carcinoma)[41].

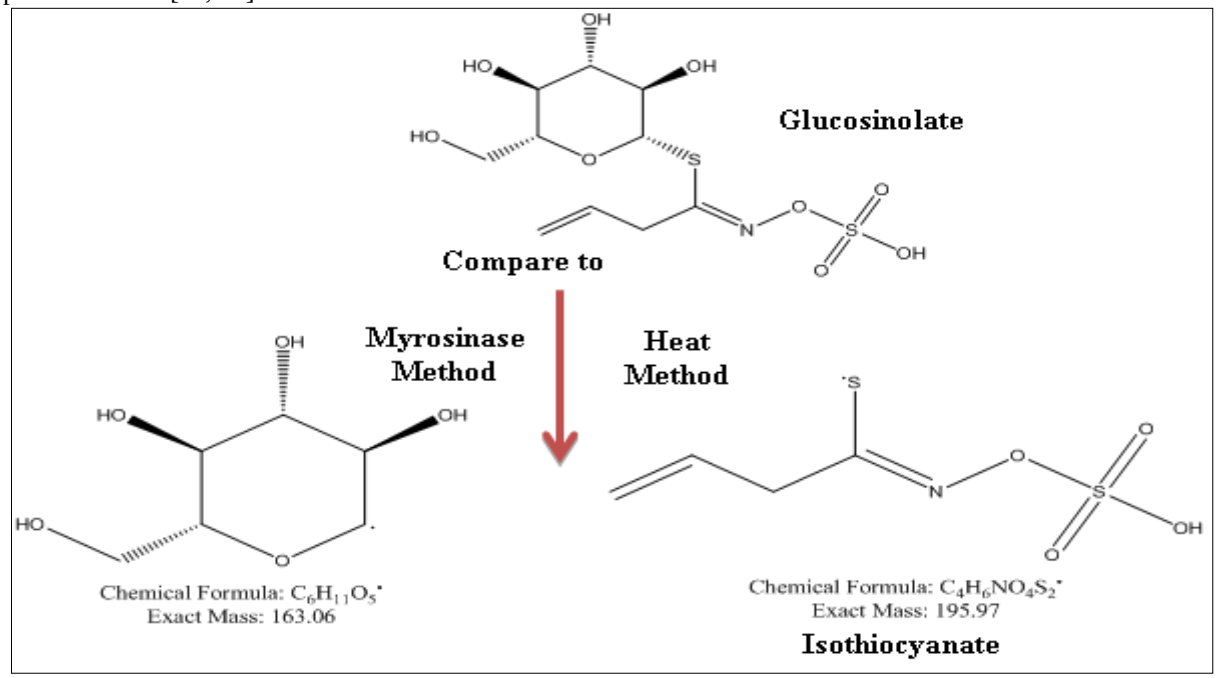

Fig. 1. Schematic illustrating the hydrolysis of sinigrin by myrosinase along with potential influencing factors

3. Results and Discussion

3.1. Different morphological character

Brassica oleracea is a vegetable crop with an amazing morphological diversity. Among the various crops derived from *B. oleracea*, kale has been in the spotlight globally due to its various health-benefiting compounds and many different varieties. Knowledge of the existing different morphological characters is essential for the differentiation of kale. Figure 2 illustrate the different photos of the five kale verities.

3.2. Yield components of kale plant

Yield components of kale plants are recorded through the developmental stages in the first growth season propagated in sandy soil in Figure (3) and second growth season propagated in clay soil in Figure (4).

3.2.1. Sprout stage

Seedlings were pulled from the trays when producing three leaves, their datum were recorded in Table (1). The table recorded the fresh weight of one seedling from the five kale varieties; whereas prumier exhibit the heaviest weight as 0.041g. The lowest weight was that of the variety K2 (dwarf sieberian) 0.16g. When considering the weight of plants, in samples taken through the developmental stages of the two growths season Figure (3&4) present the data obtained.

Table 1. Sprout vegetative characters of kale varieties

Variety	W_t/sample	Wt/one sprout
K1:Blue Curled	0.6602	0.026
K2:Dwarf Siberian	0.8730	0.016
K3:Red Russian	1.0555	0.033
K4:Lacinato	0.7150	0.029
K5:Prumier	1.8705	0.041

W_t=weight

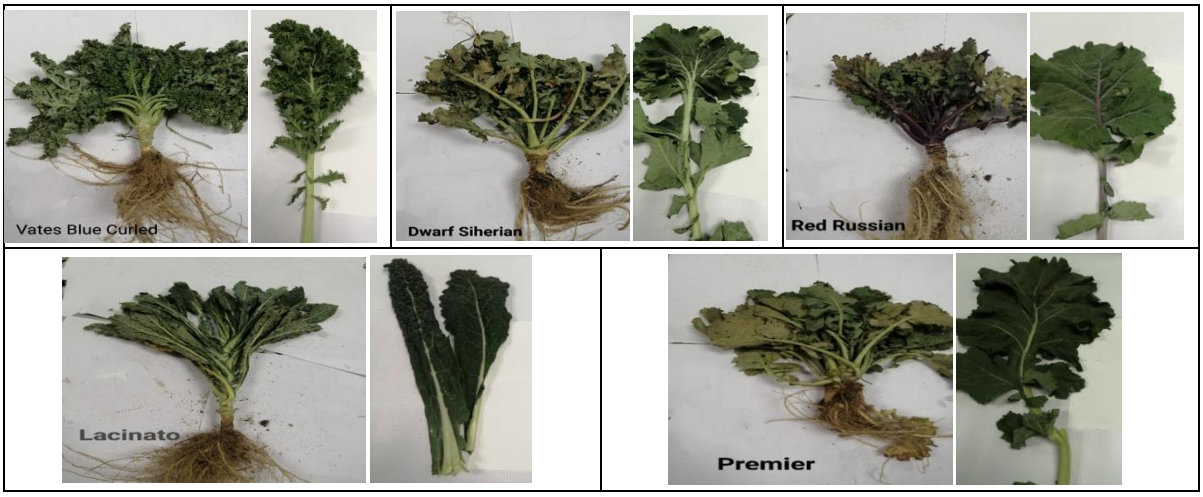

Fig. 2. Different morphological characters of the USA five Kale varieties

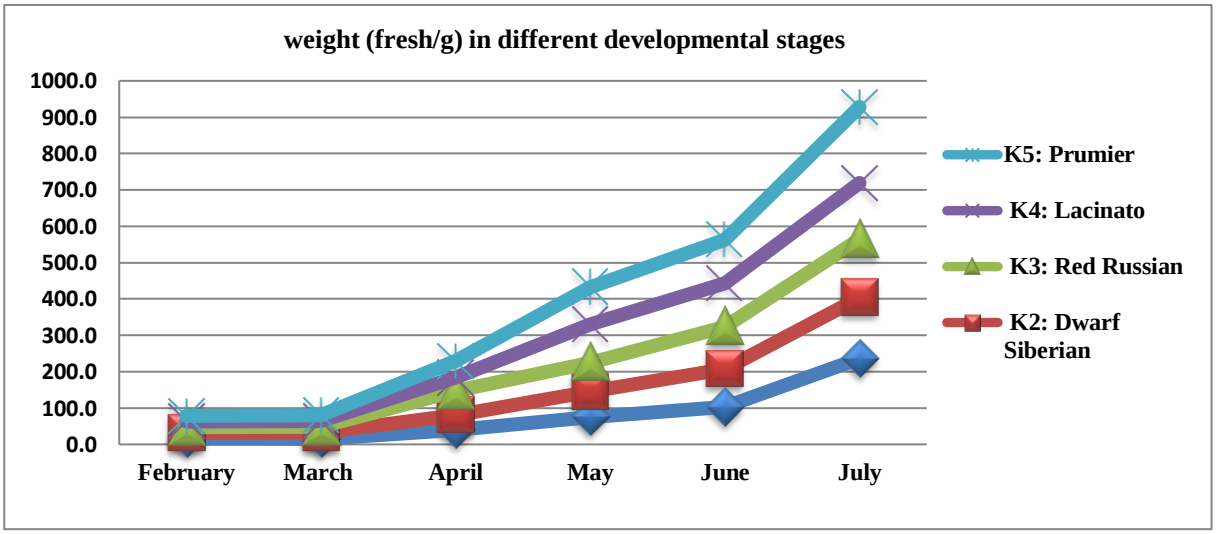

Fig.3. Fresh weight of kale varieties through the developmental stages in first season (sandy soil)

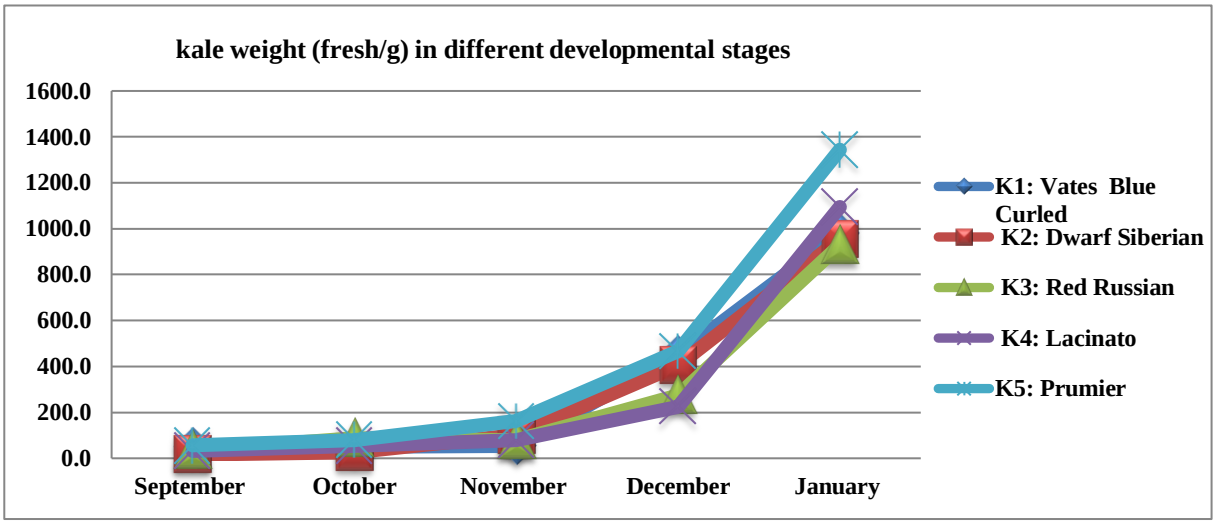

Fig.4. Fresh weight of kale varieties through developmental stages in second season (clay soil)

From the previously illustrated Figures (3&4) all kale varieties gradually increase in weight till reach maximum at cutting stages. However, when dealing with all the varieties studied, they also differ in their yield/plant, and in the two growth seasons.

3.2.2. Yield components through different developmental stages of growth

Figures (3&4) illustrate the changes occurred in the five kale varieties through the different developmental stages from February to July 2022 as fresh weight/g. the same figure shape was obtained for each of the individual. Kale weight increase gradually till June month then higher increase was noticed till July.

In the second growth season the same shapes of the figures were illustrated but with higher fresh weight per plant (Fig. 4), where *Lacinato* and *Prumier* show the heaviest weight, with top peak at January. Interestingly the plants of the second growth season were cultivated in clay soil and were propagated in February.

Comparing the yield obtained from the two growth seasons, the second one produces more yield then that of the first one. Table (2) predicted this comparison which may be attributed to climatic condition prevailing or to edaphic one especially soil type.

Table 2. Yield per unit area (20 m^2)

Varieties	Wt/unit area (Kg)	Wt/plant (g)
First season 2022 (sandy soil)		
Blue Curled	5.27±0.04	247.35±0.21
Dwarf Siberian	3.56±0.12	143.33±0.47
Red Russian	5.43±0.37	208.47±0.09
Lacinato	5.31±0.13	132.4±0.28
Prumier	6.61±1.08	297.27±0.12
Second season 2023(clay soil)		
Blue Curled	9.61±0.26	148.33±1.25
Dwarf Siberian	7.95±0.12	181±0.82
Red Russian	5.77±0.25	262.83±1.55
Lacinato	8.51±0.21	112.33±2.05
Blue Curled	9.24±2.11	185±4.08
Prumier	9.61±0.26	148.33±1.25

Brassica vegetables are family of six agriculture important species which are consumed in high quantities throughout the world, being very important in human nutrition. Within *Brassica* genus, the five *Brassica olreceae* species evolved into a number of varieties of different parts of the plant have become the edible constitutes. Kale plant is the most well represented of the species, being an important vegetable of forage crops[42].Pervious works have shown that nutrition quality as well as the yield of these crops can be affected by date of planting, environmental condition and length of growing seasons, [22]. Also *Brassica* species are reported to possess cancer preventive activity due to its glucosinolates which are responsible for the hot and pungent flavor of crucifer. Upon cellular disruption glucosinolates are hydrolyzes to various bioactive breakdown products isothiocyanate, nitriles and etc.

The results obtained in this study reveal the successful propagation of kale seeds in both the two growth seasons. Two dates of propagations were triable done, the first in winter in September and the second in January 2022. Two times of propagation produce high percentage of germination and consequently, the yield of leaves which is higher in autumn season as revealed from Table (2).

In this regard, the first growth season propagation of seeds were done in sandy soil of Nubaria farm, while the second growth season propagation was in heavy clay soil in Fac. Agric. Farm Cairo University. The higher yield obtained in the second season may be attributed to the nature of the soil; clay in its structure.

Glucosinolates species contents vary greatly with plant species and genotype [43], sinigrin, glucobrassicin and glucoiberin have been identified as the major glucosinolates in kales and cabbage [44]. While in broccoli, common glucosinolates like, Glucoraphanin (GR), progoitrin, glucoiberin and the indole glucosin are found [45].

Glucosinolates are rich in plant seeds and young sprouts which are similar to our obtained results, and decreased with plant growth which is attributed to the degradation of glucosinolates to support the formation of other sulfur containing compounds[46]. A total glucosinolates in young broccoli sprout are 20 fold that in mature plants. With plant organs roots have the most kinds and content of glucosinolates may be due to the complicated and stressful rhizosphere.

Sulfur fertilization will accelerate the biosynthesis of glucosinolates, specially the aliphatic and GR in particular, while a higher increase of indole GS are

detected when plants are supplied with nitrogen[46]. As a basic growth condition for growing, light can have a greater role in increasing GS content than dark[47]. In the present study clay soil produce higher yield of kale leaves than sandy and this is the first report in this regards.

So, the other factors affecting glucosinolate biosynthesis and content must study to ascertain or confirm their role and its accumulation. Also the specific condition for growth which affects the accumulation of glucosinolates is necessary to detect for individual glucosinolates and its hydrolysable byproducts that have a specific role as antitumor.

3.3. Antioxidant activity

Tables (S2-S4) and Figure (5) present the antioxidant activity of kale varieties using DPPH and ABTS radical scavenging activities.

In the first growth season plants were cultivated at sandy soil in Figure (5) present the antioxidant activity of the five kale varieties through all the developmental stages using DPPH and ABTS scavenging radical [48-50].

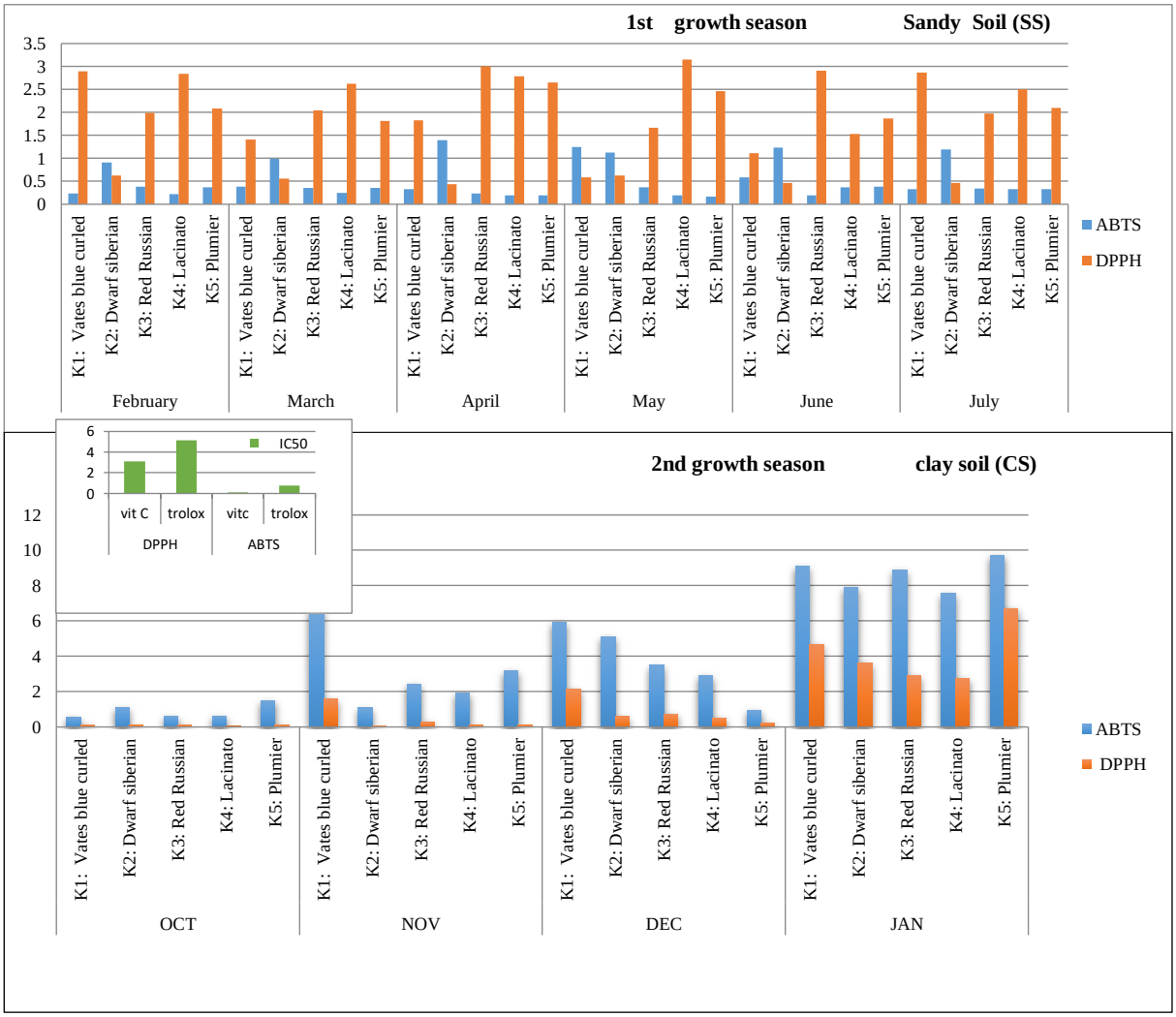

Fig.5. IC_{50} antioxidant activity of five kales varieties using DPPH and ABTS radical scavenging activities

Six samples were taken monthly through the first growth season (SS) begin from February till July 2022.,to determine the antioxidant activity of kale varieties using the DPPH and ABTS assay which aimed at measuring the capacity of the extracts to scavenges purple colored DPPH converting it to yellow colored via diphenylpicrylhydrazin. The result in Table (S3) and Figure (5) showed that CH_2CL_2 extract of Kale varieties at the 1st stage of growth on May 2022 has free radical scavenging activity with an IC_{50} value of 0.16ug/ml with prumier type similar to all the different varieties and in all stages of

growth against 1.39ug/ml at the smaller kale age in April 2022 with dwarf siberian variety but still had strong antioxidant activity in DPPH.

In general all the varieties exhibit higher free radical scavenging activity through the smaller growth stages from February to April then decreased when kale become old or at maturity. This higher activity may be due to the major role of polyphenolics, isothiocyanates in kale plants, which may be a consequence of higher level of active compounds (isothiocyanates, polyphenols and flavonoids). A moderate free radical (DPPH) scavenging was recorded whenever the plants proceed in growth to reach poor scavenge activity at maturity stages.

In the second growth season with plant cultivated in clay soil in October 2022 till January, 2023, samples collected in October produced the highest antioxidant activity in all the different concentrations prepared for the five kale varieties.

The activity was reduced as the plants grown in age towards maturity stages and then exhibited poor or less antioxidant activity Tables (4 and 5) Sand Figure (5) each kale variety exhibit higher activity, in the begging of its growth clearly noticed in October month with all concentration performed then decrease as plant proceed in age. The most active varieties through all concentration prepared and through stages of growth are lacina to and red Russian varieties.

3.4. Changes in the content of GS and ITC products by using heat methods

The beneficial and detrimental effects of GS are attributed to the ITC and not to glucosinolates. Therefore, determining glucosinolate levels can be misleading when evaluating the beneficial properties of kale plants.

Cruciferous vegetables such as kale, cabbage, and broccoli have been shown to produce not only isothiocyanate but also other bioactive breakdown products such as nitrils and epithionitrile. Isothiocyanates derived from glucosinolates have been associated with a reduced risk of certain types of cancer in humans, and therefore the most promising cultivars for future medicine purposes are those with the highest total isothiocyanate content.

HPLC is used to fractionate the isothiocyanates obtained through the two growth seasons (2022 and 2023). Samples are taken monthly through the two growth seasons and injected into HPLC with standard ITC to detect its components.

Sulforaphane is reported to be a major ITC and is clearly resolved in the HPLC chart; however, the other isothiocyanates are not clearly resolved, and HPLC is focused on sulforaphane in all the developmental stages of growth in the two growth seasons and in all kale varieties studied. Tables (S4 and S5) and figure 6 illustrate the dynamic variations of glucosinolates and their hydrolysable byproducts, the sulforaphane content, in the previously mentioned two growth seasons.

From the previous table, the total isothiocyanates determined spectrometrically in all five kale varieties increased with the age of the leaves from February to April in the first three kale varieties, commercially named K1: vates blue curled, K2: dwarf Siberian, and K3: red russian. However, the last two kale varieties (K4 and K5) named lacinato and plumier didn't show this decrement in isothiocyanates, meaning the increment of their isothiocyanates till April then decreased after that (Fig. 6).

In the second growth season in plant cultivated in clay soil, the glucosinolates, and the isothiocyanates were determined monthly from October 2022 tell Jan 2023recorded in Tables (5) and figure (6).

The pervious table represents the glucosinolates content and it's by product, the isothiocyanates show that, all the kale cultivars went the same trend increases gradually from October 2022 till Jan 2023 and the top peak of the two components occurred in December. Considering the amounts of the two chemical components, the kale variety lacinato (K4) accumulates the higher contents in May till July 2022 and in December and January 2023.

3.5. HPLC of the isothiocyanates (Heat method)

HPLC was the tool used to fractionate and determine the byproduct components of glucosinolates extracted from the leaves of kale cultivars during the two growth seasons. As already known, sulforaphane was the major isothiocyanate found in addition to nitrils. Some trials were made to detect the resolution of the other isothiocyanates found in the hydrolysable products, but they failed to produce a good separation. Sulphoraphane was successfully separated from other isothiocyanates, so sulforaphane was determined in all the samples

collected through the developmental stages of the two seasons; the following table presents this compound

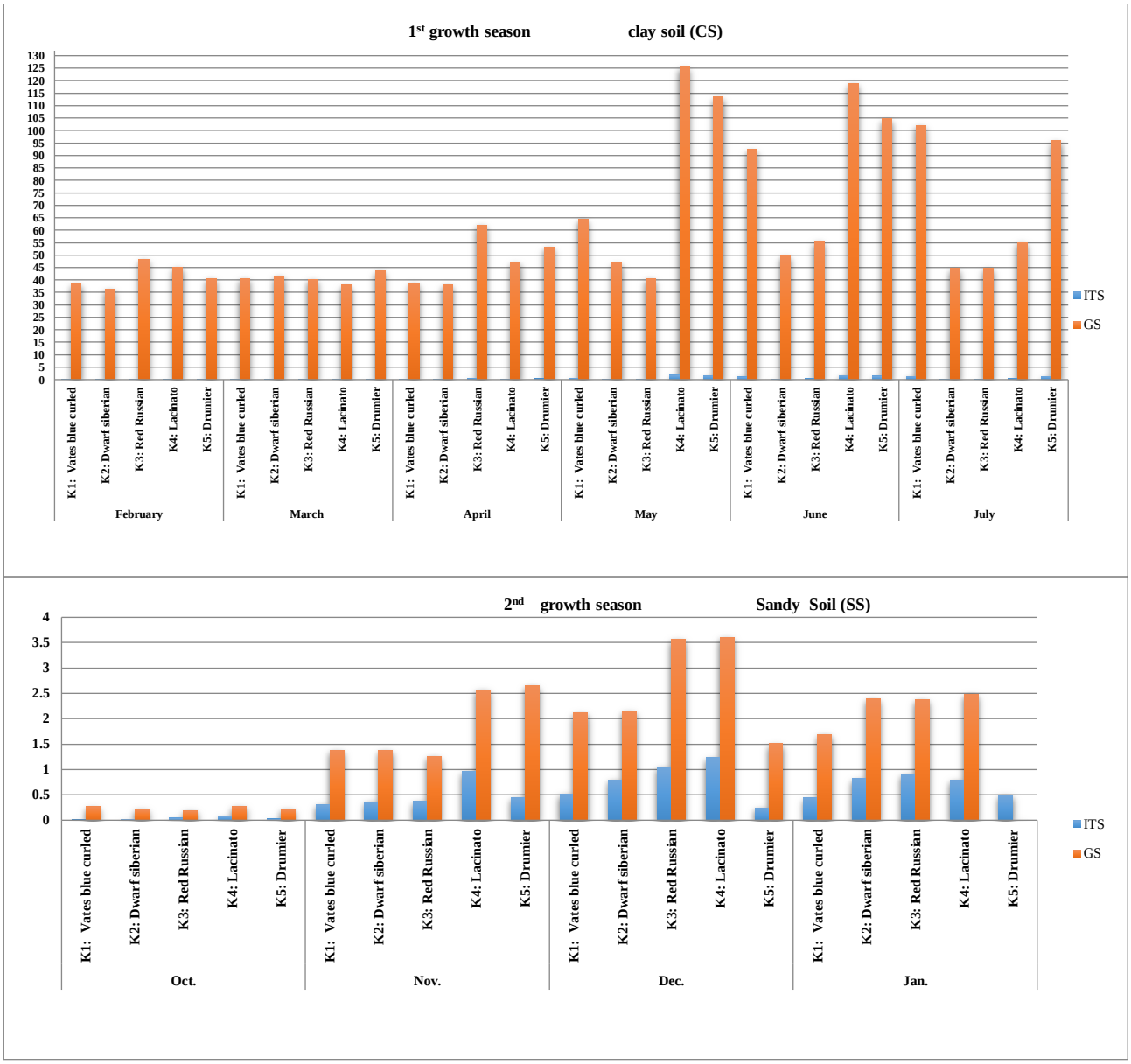

Fig.6. Glucosinolates and isothiocyanates in different developmental stages in plant cultivated in sandy soil first season and second season plants cultivated in clay soil using spectrophotometer determination

As already known, salforaphane was the major isothiocyanate found in addition to nitrils. Some trials were made to discover the resolution of the isothiocyanates; sulforaphane was successfully separated, but the others were compacted with each other. So, sulforaphane, the core compound, was determined in all the samples collected through the developmental stages of the two growth seasons. The following tables present the data obtained.

Two sources of kale seeds from USA and Poland were propagated in trays and the seedlings were collected and sulforaphane was determined, Table (3) represents the content which indicates great differences in the two sources.

From Tables (4&5) that recorded sulforaphane content during the two growth seasonsit is obviously seen that there was variations through varieties; Lacinato and Prumier produced the higher amount with top production through April and May in the first growth season.

In the second growth season, the results obtained confirmed the sulforaphane variation through cultivars. On the other hand in all cultivars sulforaphane increases from October till January with maximum production on January in all cultivars and (K1:vast Curled blue) and (K4: Lacinato) have the highest production Table (5).

These variations between cultivars in the two growth seasons could be attributed to climatic conditions, as the two seasons differed in propagation dates. These interactions between sulforaphane content and propagation time were also confirmed by its content at cutting stages for all varieties studied in Table (6).

Table 3. HPLC determination of sulforaphane content at sprout stage in different kale cultivars (µg/g)

Source of seeds	**kale varieties**				
USA	Vates blue curled (K1)	Dwarf Siberian (K2)	Red Russian (K3)	Lacinato (K4)	Prumier (K5)
	17.23	51.31	57.58	48.81	151.63
Poland	Kale kadet	Kale Kaproi	Kkale Jarmuz	Kale Curly	Fringed cabbage
	2.99	117.53	65.14	86.18	78.97

Table 4. HPLC determination of sulforaphane content during different developmental stages of the first season (µg/g)

Months 2022	**kale varieties ug/g**				
	vates blue curled (K1)	dwarf Siberian (K2)	Red Russian (K3)	Lacinato (K4)	Prumier (K5)
February	0.1	0.0975	0.1995	0.1445	0.1645
March	0.058	0.086	0.548	0.213	0.056
April	0.621	0.257	0.114	1.806	1.514
May	0.997	0.291	0.402	1.676	1.390
June	1.295	0.198	0.110	0.382	1.277
July	0.551	0.272	1.265	0.117	1.215

Table 5. HPLC determination of sulforaphane content during different developmental stages of the second season (µg/g)

Kale variety	kale varieties ug/g				
	Vates blue curled	Dwarf siberian	Red Russian	Lacinato	Prumier
October (2022)	0.810	0.022	0.045	0.075	0.025
November(2022)	107.8	79.61	60.84	56.54	23.93
December(2022)	142.01	83.02	22.40	11.27	19.82
January (2023)	163.84	85.04	36.37	91.22	36.341

Table 6. HPLC determination of sulforaphane content at the first and second seasons at the cutting stage (µg/g)

Soil type	kale varieties ug/g				
	Vates blue curled	Dwarf siberian	Red Russian	Lacinato	Prumier
1st season (Sandy soil)	72.3	85.18	40.83	52.83	64.22
2nd season (Clay soil)	163.84	87.4	36.87	21.22	36.04

3.6. Comparison between heats and enzymatic hydrolyses one on isothiocyanate production

An experiment was performed to compare the effects of enzymatic hydrolysis on isothiocyanate production. The sample from the second season, (December 2022) Figure 7 records the date obtained [35].

From the first view, the enzymatic hydrolysis method using the myrosinase enzyme produced higher isothiocyanates than the first one in (figure 6). The first method is a specific tool to break down the glucosidic compounds and, hence, increase byproduct compounds with a higher content as hydrolyzed products by heat.

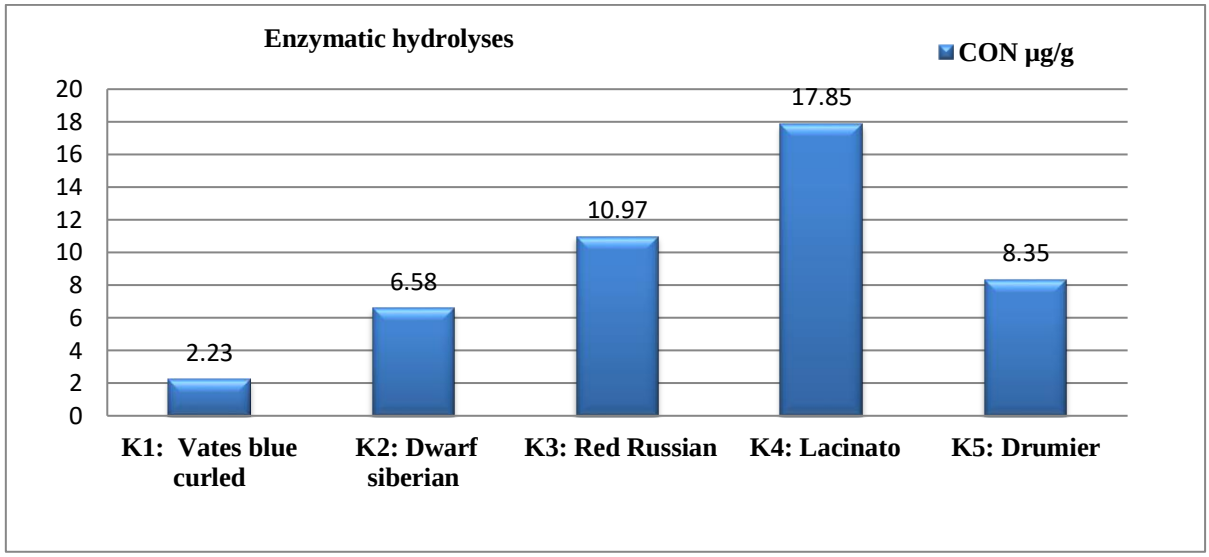

Fig.7. Isothiocyanate produced by Enzymatic hydrolyses

Kale plant a member of *Brassicaeae* family, an edible plant famous of its biological characters as reported by Šamec et al [51]. Any changes in its total and individual active ingredients; isothiocyanates through its growing season will clearly affect its active properties. For example such changes are expected to change its taste characters and consequently its biological activities.

So, as a new plant introduced to Egypt all these fluctuations must be investigated and confirmed during the different developmental growth.

The results obtained in this investigation recorded variation in the two chemical group's glucosinolates and its byproducts isothiocyanate the important compounds responsible for biological activity to human health.

Different cultivars from different sources contained higher concentration of glucosinolates at seedling stages than at later one. The product of the hydrolyses showed relatively rapid changes during the growing season, such changes properly may be due to climatic conditions reverted through the two propagation dates[26], Recently Leng et al [52]reported that ITC transformed each other and the affinity of myrosinase in broccoli sprouts was stronger than to other glucosinolates, and hence no accurate content can be fixed unless an optimized and controlled methodology was performed[53].

Isothiocyanates in the first growth season determined spectrophotometrically increased with plant age from February to May in nearly all the varieties studied however three of the five varieties K1 and K5 continue increment in their isothiocyanate contents in the first season.

In the second growth season, the glucosinolates and isothiocyantes increased gradually till cutting time with peak at December and with small decreasement in January in some varieties.

The results of dynamic variation of glucosinoltates and isothiocyantes are nearly the same in the two growth seasons, increase gradually till maturity in most of varieties and in the remainder decrease as the plant reaches the maturity probably due to the continuous formation of large number of typical shoots and young leaves which may led to cell and vacules disruption and activation of glucosinolates.

The endogenous enzyme myrosinase, which catalyses the hydrolysis of GSLs, is usually responsible for their separation. Substituted isothiocyanates, thiocyanates, nitriles, epithionitrils, and oxazolidines are among the breakdown byproducts of GSls. Depending on the specific substrate and reaction circumstances, these breakdown products' composition changes [54].

Isothiocyantes are physiologically active substances having a variety of therapeutic benefits, including anticancer, anticoagulant, anti-inflammatory, anti-asthmatic, antibiotic, and antifungal properties. ITC concentrations varied depending on the type of plant tissue. Its concentration in seeds is typically high and can reach 10% of the dry weight. The level is roughly ten times lower in the leaf, stem, and root. Numerous fresh or precooked *Brassica* vegetables have undergone

extensive research on the composition of volatile compounds and the discovery of GSL breakdown products; nevertheless, virtually little is known about the profiles of these chemicals. Therefore, the focus of the current study is on the volatile components of several kale cultivars. The main ingredient, sulforaphane, is extracted and quantified in all the types examined in this study. The biological activity of each particular isothiocyanate will be determined by further research, which will also identify the additional volatile isothiocyantes present in all kale cultivars.

3.7. Determination of Antiproliferative activity

Anticancer activity of kale varieties extracts was assessed against HepG2 cell line (human liver carcinoma). All exhibited pronounced low cytotoxic effects Table (7).

Table 7. Antiproliferative activity of kale variety extracts

Plant	100µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml
K1	78.67±4.21	68.10±8.43	55.87±4.81	54.1±1.51
K2	ND	ND	ND	ND
K3	70.07±7.26	70.17±8.21	67.00±5.56	66.4±7.12
K4	56.85±4.81	54.00±6.86	52.87±6.47	33.5±2.72
K5	64.73±4.21	63.37±7.17	61.37±5.95	56.3±4.10

In numerous animal models, kale has been shown to be useful as a chemopreventive. The isothiocyanates exhibit some organ- and carcinogen-specificity and have a wide range of structural variations. In some tissues, isothiocyanates prevent carcinogenesis caused by several carcinogens. In protocols including the injection of the isothiocyanate before or during carcinogen exposure, the majority of isothiocyanates have demonstrated chemoprotective action.

4. Conclusions

Five kale varieties were successfully propagated in two seasons: winter and autumn. Leaves the edible part bears higher amounted of glucosinolates and isothiocyantes. The high levels of isothiocyantes, especially sulforaphane, in kale varieties cultivated in Egypt at the end of this study, which deals with five cultivars, all exhibit biological activities. It's important dichloromethane (isothiocyanate extract) has a different structure with different biological activities: antitumor, antiinflamatory, antidiabetic, antibiotic, and antifungal. This plant has these multipurpose activities, which make it a plant of national wealth like the cotton plant in Egypt. However, the studied cultivars need further investigation of their potential use to increase the levels of their potential phytochemicals in these edible plants. The varieties K1: Vates blue curled, K2: Dwarf Siberian, K3: Red Russian, K4: Lacinato, and K5: Plumier can be used as good candidates for future anticancer programs due to their high total isothiocyanate content, as sulforaphane, its major isothiocyanate, is reported to have anticancer prevention activity.

Conflict of interest

There is no conflict of interest to declare

Acknowledgements

The authors acknowledge National Research Centre for funding this study Project No. 12060118.

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