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ORIGINAL PAPER

Variation in biochemical profile and health beneficial compounds and biological activities of *Brassica oleracea* var *gongylodes* L. morphological parts

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Abstract

Brassica oleracea var. *gongylodes*, commonly used as food, is loaded with biochemicals that fend diabetes, cancer, high cholesterol and it improves the general human health. The present study aimed at evaluating the antioxidant and antibacterial activities of leaves, flesh and peel extracts of *B. oleracea* var. *gongylodes* cultivated in Tunisia. Besides, biochemical, phenolic and nutritional composition was investigated. Phenolics and ascorbic acid were detected and quantified by high performance liquid chromatography (HPLC) method, while fatty acids and sugars were identified by gas chromatography flame ionization detector (GC-FID). Nutritional composition was determined for each plant part. Antioxidant activities were estimated by DPPH, ABTS and reducing power assays. Agar disk diffusion and micro-dilution methods were adopted to determine antibacterial activity. Leaves showed significantly high antiradical (EC_{50} = 0.26–0.32 mg/mL), antioxidant (EC_{50} = 0.46 mg/mL) and antibacterial (MIC = 0.625 mg/mL) activities. Chlorogenic acid, catechol, epigallocatechin and epicatechin 3-*O*-gallate were present in leaves. Isorhamnetin-3-*O*-glucoside was detected in peel and flesh. Gallic acid was present only in flesh. Peel showed higher ascorbic acid content (223.2 mg/100 g DW) than flesh and leaves. Hemicellulose, chlorophylls, proteins, Na, P and microelements amounts were significantly elevated in leaves. Sucrose is the main sugar in stem tissues. Linoleic, palmitic and oleic acids were the major fatty acids in this species. The present study provides important data on phytochemical, nutritional composition, bioactivities and emphasizes *B. oleracea* var. *gongylodes* as a potential nutraceutical and functional food. Moreover, this species can be employed as an alternative and complementary therapy for the running of related diseases of oxidative stress.

Keywords *B. oleracea* var. *gongylodes* · Antioxidant · Antibacterial · Biochemicals · Phenolics

Introduction

Brassica oleracea var. *gongylodes* L. (Kohlrabi) belongs to the family of Brassicacea. It is a biennial vegetable, with a bulbous stem at the plant basis [1]. Stems of kohlrabi may be green or purple red with flesh that is always white and it is harvested in northwestern areas of Europe and is consumed in North America, India, China, Vietnam, Korea, Europe, Mediterranean, and some zones of Asia. Kohlrabi is popular as a diet food thanks to its high fiber, cellulose and pectin amounts; it is also popular for its nutritional values with high levels of potassium and vitamin C and low amounts of fat [2]. The stem part of this plant is commonly utilized as a cooked vegetable, but the raw grated stem forms a component of winter salads [3]. In Tunisia, the green kohlrabi stem is consumed as a cooked or pickled vegetable. This species

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contains a considerable amount of phenolic compounds, which support its antioxidant, anticarcinogenic and anti-hyperglycemic potentials [4–6]. Park et al. [7] demonstrated that the total glucosinolates and phenylpropanoids levels in the flesh of green Kohlrabi were higher than those in the skin. Consumption of kohlrabi aids people to be healthy by reducing free radicals as it provides high concentrations of vitamin C and protects against prostate and colon cancer by providing the body with important phytochemicals such as isothiocyanates and indole-3-carbinol [8].

However, researches focusing on the bioactivities, metabolic profile and nutritional value of *B. oleracea* var. *gongylodes* are limited, and only a few have been published. To our knowledge, no previous report has established phytochemical profiling and antioxidant activity of leaves, flesh and peel of *B. oleracea* var. *gongylodes* growing in Tunisia. Therefore, this comparative study focused on profiling and quantifying phenolic compounds, dietary fibers, chlorophylls, minerals, proteins, soluble sugars and fatty acids of leaves, flesh and peel of *B. oleracea* var. *gongylodes*. Furthermore, in vitro antioxidant and antibacterial properties of aqueous extracts of *B. oleracea* var. *gongylodes* morphological parts were evaluated and compared to the corresponding phytochemical profile.

Materials and methods

Plant material and extraction

Brassica oleracea var. *gongylodes* was collected in December 2017 (in a vegetative stage) from Menzel Bourguiba area in northern of Tunisia and was botanically identified by the botanist Fethia Skhiri (High Institute of Biotechnology, University of Monastir, Tunisia). The Plant was separated into leaves, flesh and peel and air-dried in the shadow for 15 days. Voucher specimen (number 12/2017/BOG) was deposited at the Herbarium of the Laboratory of Botany, Faculty of Pharmacy, University of Monastir, Tunisia.

An aqueous extract from leaves, flesh and peel was prepared by putting dried material (10 g) in boiling distilled water (100 mL) [9]. The mixture was boiled for 30 min and then filtered. The obtained extract was lyophilized and the different extract yields were determined.

Phenolic content determination

Total phenolic content was measured by a Folin-Ciocalteu method [10] and results are reported as mg of gallic acid equivalent per 100 g of dry weight (mg GAE/100 g DW). Total flavonoid and flavonol contents of extracts were evaluated by the aluminum chloride method [11] and were presented as mg catechin equivalent per 100 g of dry weight (mg CE/100 g DW). Total tannins were evaluated by the method of Julkunen-Titto [12] and tannin content is reported as mg catechin equivalent per 100 g of dry weight (mg CE/100 g DW). Analysis was done in three repetitions.

Phenolic compounds identification and ascorbic acid content determination by high performance liquid chromatography (HPLC)

The identification of phenolic compounds in aqueous extracts from leaves, flesh and peel was carried out using HPLC system as described by Bettaieb Rebey et al. [13]. Phenols were identified according to their retention times and the spectral characteristics of their peaks against those of standards and by spiking the sample with standards.

Ascorbic acid content was determined using HPLC system, according to the method described by Lo Scalzo et al. [14]. Thus, ascorbic acid concentration was calculated from the experimental peak area by analytical interpolation in a standard calibration curve, and was expressed as mg per 100 g of sample dry weight (mg/100 g DW).

Proximate composition and mineral analysis

Samples were analyzed for content of water (WC), crude protein (CP), nitrogen (N), neutral detergent fibers (NDF), acid detergent fibers (ADF) and acid detergent lignin (ADL). WC was determined after drying the sample at 45 °C to a constant weight and results were expressed as percentage (%) of water content. The Kjeldahl method [15] was used to determine nitrogen content and CP was calculated as N × 6.25. The contents of NDF, ADF and ADL were determined according to the methods of Van Soest et al. [16] using an ANKOM 220 Fiber Analyzer (ANKOM Technology Corporation, NY, USA). Hemicellulose was calculated as NDF–ADF and cellulose as ADF–ADL [17]. Mineral content was determined using the official analytical methods [18]. Phosphorus (P) was determined by molybdovanadophosphoric acid method described by Kitson and Mellon [19].

Chlorophyll content determination

Chlorophylls were extracted with 80% acetone (v/v) [20]. Chlorophyll a, chlorophyll b and total chlorophyll concentrations were determined according to the equations of Arnon [20].

Soluble sugar profile determination

Soluble carbohydrates of *B. oleracea* var *gongylodes* leaves, flesh and peel were extracted according to the method described by Bartolozzi et al. [21]. Identification

of individual soluble sugars was carried out using a gas chromatograph equipped with a flame ionization detector (FID) and a HP-5MS column (30 m × 0.25 mm), and was achieved by mean of relative retention times, in comparison to that of standards [21]. The contents of soluble sugars were expressed as g per 100 g of fresh weight (FW) (g/100 g FW).

Fatty acid analysis by gas chromatography

Fatty acids were transformed into their methyl esters as described by Dhibi et al. [22]. Fatty acid methyl esters (FAMEs) were separated and quantified by gas chromatography, according to their percentage area, obtained by integration of the peaks and were identified by comparing their retention times with respect to pure standard FAMEs and analyzed under the same conditions [22]. Results were expressed as a percentage of individual fatty acid in the lipid fraction [22].

Antioxidant activity assays

Three antioxidant activity assays were performed for different extracts: ABTS and DPPH radical scavenging and reducing power assays. In ABTS assay [23], an aqueous solution of ABTS (7 mM) was mixed with a potassium persulfate solution (2.45 mM). Then, 990 µL of the ABTS radical solution (at absorbance of ≈ 0.70 at 734 nm) was added to 10 µL of extract. The absorbance was determined at 734 nm. In DPPH assay [24], DPPH (950 µL, 10^{-4} M) was mixed with diluted extract (50 µL). After 30 min, the mixture absorbance was read at 515 nm. The anti-radical activity was determined by the formula: % inhibition of radical ABTS or DPPH = $[(A_{control} - A_{sample})/A_{control}] \times 100$; $A_{control}$ is control reaction absorbance, and A_{sample} is sample test absorbance. Reducing power was evaluated by the capacity to convert Fe^{3+} into Fe^{2+} [25], measuring the absorbance at 690 nm. In all tests, the results were presented as EC_{50} values (sample concentration providing 50% of antiradical activity or 0.5 of absorbance in the reducing power assay) in mg/mL of extract. Trolox was used as a positive control and absorbance was measured in three replications.

Antibacterial activity

The antibacterial activity of aqueous extracts from leaves, flesh and peel of green kohlrabi, was determined using the agar disk diffusion and micro-dilution methods adopted by Hlila et al. [26], to determine inhibition zone diameters (IZD in mm), Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Activity was tested against Gram positive: *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Bacillus anthracis* (clinical strain) and Gram negative: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella enterica* CIP 8039 bacteria. These strains were provided by the Laboratory of Transmissible Diseases and Biological Active Substances (Faculty of Pharmacy of Monastir, Tunisia). Gentamicin was used as a positive reference standard. All tests were performed in triplicate.

Statistical analysis

The means and standard deviation of data were calculated from independent experiments. Data analysis was carried out using one-way ANOVA analyses followed by multi-range post hoc of Duncan's test and ($p < 0.05$) or less was considered significant.

Results and discussion

Yields and phenolic contents

The yield percentages of aqueous extractions from *Brassica oleracea* var. *gongylodes* leaves, flesh and peel are shown in Table 1. Aqueous extract from leaves (33.4%; $p < 0.05$) presented the significantly highest yield, followed by peel (27.52%) and flesh (27.48%) extracts. As shown in Table 1,

Table 1 Yields, total phenol, flavonoid, flavonol and tannin contents and antioxidant activity (EC_{50} mg/mL) of aqueous extracts of *B. oleraceae* var *gongylodes* leaves, flesh and peel

Plant part	Yield (%)	Phenol (mgGAE/100 g DW)	Flavonoïd (mgCE/100 g DW)	Flavonl (mgCE/100 g DW)	Tannin (mgCE/100 g DW)	EC_{50} (mg/mL) (DPPH assay)	EC_{50} (mg/mL) (ABTS assay)	EC_{50} (mg/mL) (reducing power assay)
Leaves	33.4^{b}	5290.56 ± 8.9^{b}	718.73 ± 6.7^{b}	479.32 ± 7.2^{b}	38.24 ± 0.35^{a}	0.26^{b}	0.32^{b}	0.46^{b}
Flesh	27.48^{a}	3143.71 ± 9.2^{a}	643.52 ± 9.1^{a}	419.54 ± 7.6^{a}	48.36 ± 0.44^{b}	0.88^{d}	1.20^{d}	0.94^{c}
Peel	27.52^{a}	6736.89 ± 12.35^{c}	1288.91 ± 9.3^{c}	504.18 ± 9.55^{c}	363.99 ± 10.17^{c}	0.52^{c}	1.05^{c}	0.92^{c}
Trolox						0.096^{a}	0.153^{a}	0.072^{a}

Means in the same column (a–d) bearing different superscripts in sample are significantly different by Duncan's multiple range test ($p < 0.05$)

EC_{50} effective concentration at 50%, *mg GAE/100 g DW* mg gallic acid equivalents per 100 g of dry weight of the plant material, *mgCE/100 g DW* mg catechin equivalents per 100 g of dry weight of the plant material

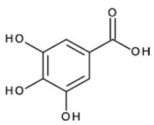
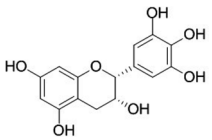
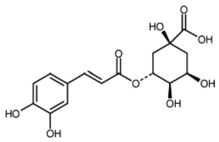
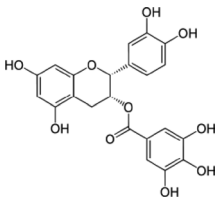
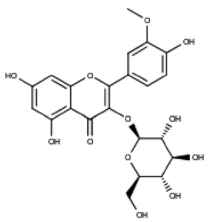
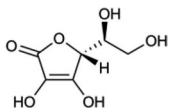
phenol, flavonoid, flavonol and tannin contents varied significantly with the organ type. Among all samples, peel aqueous extract had significantly the highest levels of total phenols (6736.89 mg GAE/100 g DW; $p < 0.05$), flavonoids (1288,91 mg CE/100 g DW; $p > 0.05$), flavonols (504,18 mg CE/100 g DW; $p > 0.05$) and tannins (363,99 mg CE/100 g DW; $p > 0.05$).

These results that demonstrated that the peel and leaves of *B. oleracea* var. *gongylodes* were richer in phenolic compounds than flesh are in accordance with these reported by Pak et al. [27]. According to Chauhan et al. [28], phytochemicals such as flavonoids and tannins are present in distilled water and methanol extracts of *B. oleracea* var. *gongylodes* leaves. Consequently, these results have demonstrated that water is a good solvent in extracting polyphenols and that peel is significantly richer on phenols, flavonoids, flavonols and tannins than flesh and leaves. Secondary metabolites are accumulated in all plant cells but their concentration varies according to the plant parts, particular growth phases, climates and seasons.

Phenolic profile and ascorbic acid content

The aqueous extracts of *B. oleracea* var. *gongylodes* parts were analyzed using HPLC method and results are presented in Table 2. To the best of our knowledge this is the first study about the phenolic compounds in aqueous extracts of the studied parts of green kohlrabi grown in Tunisia,

Table 2 Phenolic and ascorbic acid composition (mg/100 g DW) of aqueous extracts of *B. oleracea* var. *gongylodes* leaves, flesh and peel, using the high performance liquid chromatography

	Formula	Leaves	Flesh	Peel
Gallic acid		nd	31.74 ± 0.41	nd
Catechol		89.86 ± 1.2^{c}	52.53 ± 0.72^{a}	59.99 ± 0.71^{b}
Epigallocatechin		82.19 ± 1.08	nd	nd
Chlorogenic acid		106.36 ± 2.1^{b}	nd	55.62 ± 0.68^{a}
Epicatechine-3-*O*-gallate		113.51 ± 1.65	nd	nd
Isorhamnetin-3-*O*-glucoside		nd	44.42 ± 0.58^{b}	18.45 ± 0.27^{a}
Ascorbic acid		212.741 ± 2.45^{b}	118.493 ± 1.3^{a}	233.15 ± 1.97^{c}

Means in the same row (a–c) bearing different superscripts in sample are significantly different by Duncan's multiple range test ($p < 0.05$)

nd not detected

performed by HPLC technique. In leaves aqueous extract, one hydroxycinnamic acid (chlorogenic acid) and three flavonoids (catechol, epigallocatechin and epicatechin 3-*O*-gallate) were identified. Also, catechol and isorhamnetin-3-*O*-glucoside were detected in flesh and peel, but, gallic acid was present only in flesh with a level of 31.74 mg/100 g DW. Catechol was significantly more concentrated in leaves (89.86 mg/100 g DW; $p < 0.05$) compared to peel (59.99 mg/100 g DW) and flesh (52.53 mg/100 g DW) (Table 2).

The most diverse and widespread polyphenols group in *Brassica* species are hydroxycinnamic acids and flavonoids [3]. The major common hydroxycinnamic acids in *Brassica* vegetables are ferulic, *p*-coumaric and sinapic acids, frequently found in conjugation with other hydroxycinnamic acids or sugar [29]. A study reported the presence of 4-hydroxybenzoic, caffeic, *p*-coumaric, benzoic and trans-cinnamic acids, quercetin, and kaempferol in the skin and flesh of *B. oleracea* var. *gongylodes* [7]. These reports are inconsistent with our study results, where we noted the absence of *p*-coumaric acid in aqueous extracts from different parts of kohlrabi. Leaves and peel contained chlorogenic acid with the highest amount in leaves aqueous extract (106.362 mg/100 g DW; $p < 0.05$). Significant amounts of chlorogenic acids were reported in leafy *Brassica* species, like cabbage, kale and Brussels sprouts [30].

Only aqueous extracts from flesh and peel contained flavonol, isorhamnetin-3-*O*-glucoside with the greatest amount in the flesh. Isorhamnetin-3-*O*-glucoside is identified here for the first time in *B. oleracea* var. *gongylodes* flesh and peel, in disagreement with previous reports in *Brassica* species [31]. These reports mentioned that isorhamnetin derivatives were absent in *B. oleracea* and present in *B. rapa* group (Romani et al. 2006). Nevertheless, isorhamnetin, quercetin and kaempferol are the main flavonols in *Brassica* crops and are frequently found as *O*-glycosides [32, 33]. The quantification of isorhamnetin is recommended for the differentiation of varieties [34], as well as for investigations on cooking conditions [35].

The peel ascorbic acid concentration (233.15 mg/100 g DW; $p < 0.05$) was significantly higher than that of leaves (212.741 mg/100 g DW) and flesh (118.5 mg/100 g DW) extracts (Table 2). These results are consistent with data published by Cha et al. [36], who reported that peel was richer in vitamin C (402.74 mg/100 g) than flesh (231.36 mg/100 g). Thus, vitamin C can be found reduced (ascorbic acid) or oxidized (dehydroascorbic acid); the sum of these is total vitamin C and the variation of acid ascorbic and vitamin C probably arises from different factors such as variations in post-harvest handling conditions and seasonal cultivar selection. Vitamin C is essential for collagen and carnitine biosynthesis, and has antioxidant and anticarcinogenic activities [37].

Proximate, dietary fiber, chlorophyll and mineral composition

The proximate, dietary fiber, chlorophyll and mineral composition values are shown in Table 3. Water content (87.28%; $p < 0.05$), crude protein (18.69%; $p < 0.05$) and nitrogen (2.99%; $p < 0.05$) were significantly higher in leaves than in flesh and peel. In this context, Cha et al. [36] demonstrated that the crude protein content of flesh and peel of *B. oleracea* var. *gongylodes* were 16.63 and 20.45% of dry weight, respectively and that moisture varied from 5.58% (peel) to 16.52% (flesh).

All plant parts contained similar content of NDF (36.86–38.35%; $p < 0.05$), but ADF was more concentrated in flesh (31.53%) and peel (30.92%). Flesh was richer in ADL (14.88%; $p < 0.05$) than peel and leaves. A significantly high amount of hemicellulose (11.49%; $p < 0.05$) was

Table 3 Proximate, dietary fiber, chlorophyll and mineral composition of leaves, flesh and peel of *B. oleracea* var. *gongylodes*

	Leaves	Flesh	Peel
Water content (%)	87.28 ± 4.27^{c}	81.43 ± 5.42^{a}	85.83 ± 5.20^{b}
Crude protein (%)	18.69 ± 1.2^{c}	16.88 ± 1.20^{b}	15.30 ± 0.95^{a}
N (%)	2.99 ± 0.36^{c}	2.7 ± 0.30^{b}	2.45 ± 0.25^{a}
NDF (%)	36.86 ± 3.78^{a}	38.35 ± 1.20^{a}	36.90 ± 4.82^{a}
ADF (%)	25.37 ± 2.32^{a}	31.53 ± 1.77^{b}	30.92 ± 5.16^{b}
ADL (%)	8.76 ± 0.25^{a}	14.88 ± 2.36^{b}	8.91 ± 4.84^{a}
Hemicellulose (%)	11.49 ± 2.42^{b}	6.82 ± 0.58^{a}	5.98 ± 0.66^{a}
Cellulose (%)	16.61 ± 0.31^{a}	16.65 ± 0.75^{a}	22.02 ± 1.59^{b}
Chlorophyll (mg/100 g FW)			
Chlorophyll a	89.1 ± 2.1^{c}	1.00 ± 0.30^{a}	2.00 ± 0.60^{b}
Chlorophyll b	30.23 ± 1.2^{c}	0.60 ± 0.0^{a}	1.70 ± 0.35^{b}
Total chlorophyll	113.14 ± 2.8^{c}	2.10 ± 0.40^{a}	4.00 ± 0.83^{b}
Mineral constituents (mg/100 g DW)			
K	1600.0 ± 21.0^{b}	1660.21 ± 11.26^{c}	1410.34 ± 12.13^{a}
Na	100.0 ± 10.8^{c}	37.00 ± 5.80^{a}	45.00 ± 6.10^{b}
P	80.0 ± 1.3^{b}	10.00 ± 1.50^{a}	9.00 ± 0.98^{a}
Fe	8.425 ± 0.2^{b}	3.18 ± 0.40^{a}	3.87 ± 0.46^{a}
Zn	4.89 ± 0.1^{b}	2.38 ± 0.32^{a}	2.68 ± 0.24^{a}
Mn	3.19 ± 0.07^{c}	1.32 ± 0.12^{a}	2.60 ± 0.38^{b}
Ni	0.888 ± 0.16^{b}	0.46 ± 0.10^{a}	0.54 ± 0.11^{a}
Cu	0.490 ± 0.15^{a}	0.46 ± 0.10^{a}	0.30 ± 0.06^{a}
Cr	0.303 ± 0.01^{a}	0.18 ± 0.01^{a}	0.22 ± 0.01^{a}
Co	0.083 ± 0.03^{b}	0.02 ± 0.001^{a}	0.038 ± 0.002^{a}

Means in each line followed by different letters are significantly different by Duncan's multiple range test ($p < 0.05$)

N nitrogen, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *ADL* acid detergent lignin, *DW* dry weight, *FW* fresh weight

present in leaves. Cellulose was elevated in peel (22.02%; $p < 0.05$).

No reports on the dietary fiber composition of leaves of *B. oleracea* var. *gongylodes* have been presented. Nevertheless, Cha et al. [36] reported that peel of kohlrabi is richer in total dietary fiber (19.58%) than flesh (1.44%). NDF had the highest share in dietary fiber, followed by ADF, which includes lignin and cellulose. Cellulose fibers are virtually undigested in the gastrointestinal tract; however, they aid intestine peristalsis, as similar as ADL [38]. Thus, dietary fibers have advantageous effects on body function and human health and can only be provided by plants. Indeed, the consumption of dietary fibers is related to a reduced incidence of frequent disorders such as obesity, diabetes, cancer and cardiovascular diseases [39].

The mineral elements are expressed in mg/100 g DW and their contents are given in Table 3. The leaves, flesh and peel proved to be a source of potassium (1410.34–1660.21 mg/100 g DW; respectively) and sodium (37.0–100.0 mg/100 g DW). Comparing potassium contents, the flesh showed the highest amount (1660.21 mg/100 g DW; $p < 0.05$). On the contrary, leaves exhibited greater levels of sodium (100.0 mg/100 g DW; $p < 0.05$) and phosphorus (80.0 mg/100 g DW) than flesh and peel. In terms of microelements, Fe, Mn, Zn, Ni, Cu, Cr and Co were detected in the leaves, flesh and peel of Kohlrabi (Table 3). Fe is the most abundant microelement and cobalt is the least (Table 3).

No work on the mineral composition of green kohlrabi leaves has been published. However, Cha et al. [36] mentioned that peel contained an elevated amount of sodium (115.6 mg/100 g DW) and that flesh and peel of Kohlrabi were richer in potassium and contained 366.0 and 4440.0 mg/100 g of K, respectively. Also, Cha et al. [36] reported the presence of Cu and Mn in Kohlrabi peel, but its absence in flesh sample. Briefly, the same authors detected eight minerals (K, Na, Ca, Mg, Fe, Cu, Mn and Zn) in peel and six minerals (K, Na, Ca, Mg, Fe and Zn) in the flesh of *B. oleracea* var. *gongylodes* cultivated in Korea [36]. Our present study identified four minerals (P, Ni, Cr and Co) which have not yet been reported in the literature for this plant material.

The results of the present investigation of chlorophyll a, chlorophyll b and total chlorophyll contents are shown in Table 3. Chlorophyll a (89.1 mg/100 g), chlorophyll b (30.23 mg/100 g) and total chlorophyll (113.14 mg/100 g) concentrations of leaves were significantly higher ($p < 0.05$) than those of the peel and flesh of *B. oleracea* var. *gongylodes*.

Kohlrabi leaves contained a significantly elevated level of chlorophylls such as a majority of leafy vegetables. Chlorophylls are green pigment photoreceptors present in all photosynthetic organisms and absorb solar light energy and provide mechanisms for its utilization in photosynthetic reactions. Chlorophyll pigments are classified among the least stable natural pigments. Some researchers suggest that these pigments contribute to antioxidant, antiviral and antimutagenic activities [40].

Soluble sugars composition

The sugar composition of different parts of *B. oleracea* var. *gongylodes* was presented in Table 4. Leaves were poor in sugar (0.828 g/100 g DW; $p < 0.05$) compared with flesh (1.934 g/100 g DW) and peel (1.983 g/100 g DW). Sucrose (1.001–1.189 g/100 g DW) was the main sugar in flesh and peel, but arabinose (0.001–0.003 g/100 g DW) was the least in all raw materials.

The total sugar content (0.828–1.983 g/100 g DW) reported here was lower than that quantified by Cha et al. [36] in flesh and peel of Kohlrabi from Korea (314.94–419.15 g/100 g). These authors demonstrated that the main sugars presented in the flesh and peel were fructose (152.23 g/100 g) and glucose (182.23 g/100 g), respectively [36]. In the present study, six sugars (sucrose, inositol, mannitol, raffinose, rhamnose and arabinose) were identified for the first time in *B. oleracea* var. *gongylodes* cultivated in Tunisia. Sugars are important chemical compounds with nutritional value for humans and constitute the main energy source in vegetarian diets.

Fatty acids composition

The fatty acids analysis of the studied parts of green kohlrabi indicated the presence of 17 components (Table 5). Linoleic

Table 4 Soluble sugars composition (g/100 g DW) of leaves, flesh and peel of *B. oleracea* var. *gongylodes*

	Leaves	Flesh	Peel
Monosaccharides			
Fructose	0.023 ± 0.01^{b}	0.027 ± 0.01^{b}	0.015 ± 0.01^{a}
Glucose	0.015 ± 0.00^{a}	0.022 ± 0.02^{b}	0.025 ± 0.002^{b}
Galactose	0.025 ± 0.01^{a}	0.031 ± 0.01^{b}	0.035 ± 0.001^{b}
Arabinose	0.001 ± 0.001^{a}	0.003 ± 0.01^{a}	0.002 ± 0.001^{a}
Rhamnose	0.016 ± 0.004^{a}	0.019 ± 0.04^{a}	0.022 ± 0.004^{a}
Mannose	0.506 ± 0.09^{b}	0.616 ± 0.03^{c}	0.453 ± 0.001^{a}
Polysaccharides			
Sucrose	0.105 ± 0.05^{a}	1.001 ± 0.02^{b}	1.189 ± 0.03^{c}
Raffinose	0.003 ± 0.001^{a}	0.041 ± 0.01^{b}	0.060 ± 0.001^{c}
Polyols			
Inositol	0.102 ± 0.02^{a}	0.112 ± 0.002^{b}	0.109 ± 0.01^{b}
Mannitol	0.032 ± 0.02^{a}	0.062 ± 0.01^{b}	0.073 ± 0.01^{c}
Total	0.828^{a}	1.934^{b}	1.983^{b}

Means in each line followed by different letters are significantly different by Duncan's multiple range test ($p < 0.05$)

Table 5 Fatty acids composition (relative percentage) of *B. oleracea* var. *gongylodes* leaves, flesh and peel

Fatty acids	Nomenclature	Leaves	Flesh	Peel
		Content (%)		
C6:0	Caproic acid	0.20 ± 0.02^{a}	0.22 ± 0.03^{a}	0.31 ± 0.03^{b}
C12:0	Lauric acid	3.15 ± 0.05^{b}	4.12 ± 0.35^{c}	2.89 ± 0.02^{a}
C14:0	Myristic acid	2.36 ± 0.03^{b}	3.15 ± 0.31^{c}	1.97 ± 0.08^{a}
C15:0	Pentadecylic acid	0.10 ± 0.005^{a}	0.18 ± 0.04^{b}	0.08 ± 0.001^{a}
C16:0	Palmitic acid	23.25 ± 1.53^{b}	20.89 ± 1.21^{a}	24.49 ± 1.22^{c}
C16:1	Palmitoleic acid	8.36 ± 1.09^{c}	7.51 ± 0.91^{b}	2.8 ± 0.3^{a}
C17:0	Margiric acid	0.98 ± 0.23^{a}	1.98 ± 0.08^{b}	1.99 ± 0.026^{b}
C18:0	Stearic acid	6.87 ± 1.04^{a}	9.65 ± 0.56^{b}	10.68 ± 1.05^{c}
C18:1	Oleic acid	20.98 ± 1.52^{c}	18.68 ± 1.05^{a}	19.88 ± 1.01^{b}
C18:2	Linoleic acid	23.07 ± 1.48^{b}	22.00 ± 1.21^{a}	24.66 ± 1.1^{c}
C18:3	α-Linolenic acid	4.58 ± 0.05^{b}	2.98 ± 0.12^{a}	5.32 ± 0.72^{c}
C20:0	Arachidic acid	4.03 ± 0.04^{b}	6.43 ± 0.05^{c}	3.08 ± 0.85^{a}
C20:1	Eicosenoic acid	0.54 ± 0.08^{b}	0.4 ± 0.02^{a}	$0,88 \pm 0.05^{c}$
C20:2	Eicosadienoic acid	0.43 ± 0.04^{b}	0.1 ± 0.01^{a}	0.55 ± 0.03^{c}
C22:0	Behenic acid	0.22 ± 0.03^{b}	0.66 ± 0.05^{c}	0.08 ± 0.002^{a}
C23:0	Tricosylic acid	0.78 ± 0.08^{b}	1.03 ± 0.04^{c}	0.23 ± 0.02^{a}
C24:0	Lignoceric acid	0.10 ± 0.01^{b}	0.02 ± 0.002^{a}	0.11 ± 0.01^{b}
Total identified		100.00	100.00	100.00
Saturated fatty acids		42.04 ± 1.59^{a}	48.33 ± 1.51^{c}	45.91 ± 1.52^{b}
Unsaturated fatty acids		57.96 ± 1.43^{c}	51.67 ± 1.68^{a}	54.09 ± 1.48^{b}
Monounsaturated fatty acids		29.88 ± 1.08^{c}	26.59 ± 1.1^{b}	23.56 ± 1.1^{a}
Polyunsaturated fatty acids		28.08 ± 1.07^{b}	25.08 ± 1.07^{a}	30.53 ± 1.2^{c}

Means in each line followed by different letters are significantly different by Duncan's multiple range test ($p < 0.05$)

acid was the major fatty acid with a concentration of 24.66% in peel, 23.07% in leaves and 22.00% in flesh, followed by palmitic (20.89–24.49%), and oleic (18.68–20.98%) acids. Nevertheless, the relative percentages of each quantified fatty acid always showed statistically significant differences between both plant parts. The unsaturated fatty acids were predominant (51.67–57.96%) compared to saturated fatty acids (42.04–48.33%). On the contrary, it was reported that the fatty acid profile of kohlrabi was dominated by saturated fatty acids in peel (82.2%) and flesh (72.99%) [36]. Furthermore, the same authors reported that palmitic acid was the dominant fatty acid (36.04%) in peel, followed by heneicosanoic acid (11.85%). However, the most abundant fatty acid in flesh was heneicosanoic acid (21.71%), followed by arachidic acid (18.65%) and linoleic acid (13.56%) [36]. Nevertheless, caproic, lauric, pentadecylic, margiric, eicosenoic and tricosylic acids were identified here for the first time in green kohlrabi grown in Tunisia.

Antioxidant activity

The antioxidant activity of the tested samples was determined using the DPPH, ABTS and reducing power assays and was compared to that of Trolox. The aqueous extracts of different plant parts exhibited remarkable antioxidant activities (Table 1). The best effect was observed in the aqueous extract of leaves with EC_{50} of 0.26, 0.32 and 0.46 mg/mL, by DPPH, ABTS and reducing power assays, respectively. This might be explained by the high level of hydrogen-donating constituents in the leaves extract, especially phenolic compounds. Besides, these activities are slightly lower than a Trolox with EC_{50} ranging from 0.072 to 0.153 mg/mL.

Aqueous extracts from leaves were more active than those from flesh and peel. Our results are in agreement with those reported by Pak et al. [27], who demonstrated that water and ethanol extracts of leaves were more active than those of flesh and peel, by DPPH and reducing power tests. Hassan et al. [6] proved the existence of a considerable amount of phenolic compounds in *B. caulorapa* var. *gongylodes* that is supporting its potential role as antioxidant and anticarcinogenic agent. *B. oleracea* var. *gongylodes* juices have been reported to exhibit potent antioxidant activity conferred by high total phenolic content [5]. Chun et al. [41] demonstrated the high correlation between antioxidant potential and phenol and flavonoid contents for different varieties of *B. oleracea* (green cabbage, red cabbage, Savoy cabbage and Napa cabbage) using an ABTS radical scavenging assay.

In fact, our results indicate that aqueous extracts of *B. oleracea* var. *gongylodes* leaves, flesh and peel, which are rich in phenolics and flavonoids, have an antioxidant activity. Generally, high antioxidant activity is probably due to the combined action of the polyphenol compounds present in variable levels and their high hydrogen atom donating abilities. Moreover, from the present study results, it is evident that the antioxidant activities of *B. oleracea* var. *gongylodes*, are associated to various phenolic compounds such as chlorogenic, gallic and ascorbic acids and catechol, epigallocatechin, epicatechin 3-*O*-gallate and isorhamnetin-3-*O*-glucoside present in different plant parts.

Antibacterial activity

Antibacterial activity of aqueous extracts of *B. oleracea* var. *gongylodes* parts was assessed in vitro against a panel of $Gram^+$ and $Gram^-$ bacteria. According to the results presented in Table 6, the samples exhibited an antibacterial activity against some tested bacteria. *Staphylococcus aureus* appeared the most sensitive towards extracts (IZD range from 6.16 to 11.33 mm, and MIC ranged from 0.625 to 5 mg/mL). Additionally, the leaves extract was the most active against *S. aureus* (MIC = 0.625 mg/mL and MBC = 2.5 mg/mL), but this antibacterial activity was lower than that of Gentamicin (Table 6).

According to literature investigation, no reports have been found out on the antimicrobial activity of *B. oleracea* var *gongylodes*. Nevertheless, it is well known that *Brassica* vegetables have been shown to possess antimicrobial properties [42]. It has been reported that cauliflower juice inhibits the growth of *E. coli*, *Salmonella enteritidis*, *Listeria monocytogenes* and all foodborne pathogens found in a great variety of foods [42]. Most phenolic compounds are biologically active constituents of plant origin having antibacterial, antiviral and anticancerous properties. In our work, we have found interesting results regarding the antibacterial potential of the aqueous extracts of *B. oleracea* var *gongylodes* and its phenolic analysis has demonstrated the phenols and flavonoids richness. Thus, phenolic compounds present in the different plant parts of *B. oleracea* var *gongylodes* can contribute to its antibacterial activity.

Conclusion

The aqueous extracts of leaves, flesh and peel of *B. oleracea* var. *gongylodes* cultivated in Tunisia showed statistically significant differences in the total phenols, flavonoids, flavonols and tannins contents. These compounds were more concentrated in the aqueous extract from peel. The leaves contained phenolic acid (chlorogenic acid) and flavonoids (catechol, epigallocatechin and epicathechin-3-*O*-gallate). Accordingly, we suggest using water as the extractor to isolate phenolic acid and flavonoid constituents from green kohlrabi leaves. Flavonol, isorhamnetin-3-*O*-glucoside was present only in flesh and peel. The peel and leaves were richer in ascorbic acid than flesh, which is a frequently consumed part of kohlrabi. Water, crude protein, nitrogen, hemicellulose, chlorophylls, sodium, phosphorus and microelements (Fe, Zn, Mn and Ni) were more condensed in leaves. However, flesh was the richest in potassium. Flesh and peel contained equal content of soluble sugars and the sucrose, which is the main sugar, was more concentrated in peel. Thus, peel had the highest linoleic and palmitic acids level and oleic acid amount was elevated in leaves.

Table 6 Antibacterial activity (IZD; MIC and MBC) of *B. oleracea* var. *gongylodes* leaves, flesh and peel aqueous extracts

Plant part/antibiotic	Parameter	*Bacillus anthracis*	*Bacillus subtilis*	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella enterica*	*Pseudomonas aeruginosa*
Leaves	IZD (mm)	6.33 ± 0.57^a	7.16 ± 0.35^a	6.16 ± 0.28^a	11.33 ± 0.7^b	6.33 ± 0.57^a	6.16 ± 0.28^a
	MIC (mg/mL)	> 5	5	5	0.625	> 5	> 5
	MBC (mg/mL)	> 5	> 5	5	2.5	> 5	> 5
Flesh	IZD (mm)	6.33 ± 0.57^a	7.16 ± 0.35^a	6.16 ± 0.28^a	6.16 ± 0.28^a	6.16 ± 0.28^a	6.16 ± 0.28^a
	MIC (mg/mL)	> 5	5	> 5	2.5	> 5	> 5
	MBC (mg/mL)	> 5	> 5	> 5	> 5	> 5	> 5
Peel	IZD (mm)	6.33 ± 0.57^a	7.16 ± 0.35^a	6.33 ± 0.57^a	6.16 ± 0.28^a	6.33 ± 0.57^a	6.33 ± 0.57^a
	MIC (mg/mL)	5	5	> 5	5	> 5	> 5
	MBC (mg/mL)	> 5	> 5	> 5	5	> 5	> 5
Gentamicin	IZD (mm)	31.8 ± 0.35^b	20.33 ± 0.7^b	18.33 ± 0.7^b	30.16 ± 0.35^c	22.33 ± 0.7^b	21.33 ± 0.7^b
	MIC (µg/mL)	0.5	0.5	0.062	0.5	0.125	4
	MBC (µg/mL)	3.9	1.9	3.9	15.6	12.5	50

Means in each column followed by different letters are significantly different ($p < 0.05$)

IZD inhibition zone diameter (mm), *MIC* minimum inhibitory concentration (mg/mL), *MBC* minimum bactericidal concentration (mg/mL)

Regarding the bioactivities and summarized data recorded by the three antioxidant assays, it appears that the aqueous extract of leaves was the most active. Different plant parts exhibited antibacterial activity against some studied bacteria and leaves aqueous extract was the most active against *S. aureus*. Finally, these results suggest that the flesh, and specially the peel and leaves of *B. oleracea* var. *gongylodes,* are a potential source of high value components for nutritional and pharmaceutical purposes, as well as functional foods and nutraceutical applications.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest for publishing this work.

References

1. T. Ćosić, B. Vinterhalter, D. Vinterhalter, N. Mitic, A. Cingel, J. Savić, B. Bohanec, S. Ninković, In Vitro Cell. Dev. Biol. Plant **49**, 294–303 (2013)
2. J.W. Lee, E.H. Han, J.G. Cho, N.I. Baek, Y.H. Lee, Annual Meeting of the Korean J Hort Sci Technol. Gyeongsang National University, Gyeong-nam, Korea. Abstract No. 162 (2009)
3. M.E. Cartea, M. Francisco, P. Soengas, P. Velasco, Molecules **16**, 251–280 (2011)
4. V. Rasal, B. Shetty, A. Sinnathambi, S. Yeshmaina, P. Ashok, Internet J. Pharmacol. **4**(2), 7659 (2005)
5. D.B. Kim, J.W. Oh, G.H. Shin, Y.H. Kim, J.S. Lee, I.J. Park, J.H. Cho, O.H. Lee, FASEB J. **28**(1), 394 (2014)
6. E.A. Hassan, A. Hussein, M.E. El-Awadi, Nat. Sci. **9**(8), 149–157 (2011)
7. W.T. Park, J.K. Kim, S. Park, S.W. Lee, X. Li, Y.B. Kim, M.R. Uddin, N.I. Park, S.J. Kim, S.U. Park, J. Agric. Food Chem. **60**, 8111–8116 (2012)
8. J.V. Higdon, B. Delage, D.E. Williams, R.H. Dashwood, Pharmacol. Res. **55**, 224–236 (2007)
9. A. Ben Sassi, F. Harzallah-Skhiri, N. Bourgougnon, M. Aouni, Indian J. Med. Res. **127**, 183–192 (2008)
10. Y. Velioglu, G. Mazza, L. Gao, B.D. Oomah, J. Agric. Food Chem. **6**, 4113–4117 (1998)
11. G. Miliauskas, P.R. Venskutonis, T.A. Van Beek, Food Chem. **85**(2), 231–237 (2004)
12. R. Julkunen-Tiitto, J. Agric. Food Chem. **33**(2), 213–217 (1985)
13. I. Bettaieb Rebey, S. Kefi, S. Bourgou, I. Ouerghemmi, R. Ksouri, M. Saidani Tounsi, B. Marzouk, Plant Foods Hum. Nutr. **69**(3), 189–290 (2014)
14. R. Lo Scalzo, T. Iannoccari, C. Summa, R. Morelli, P. Rapisarda, Food Chem. **85**, 41–47 (2004)
15. J. Kjeldahl, Z. Fresenius, Anal. Chem. **22**, 366–382 (1883)
16. P.J. Van Soest, J.B. Roberston, B.A. Lewis, J. Dairy Sci. **74**, 3583–3593 (1991)
17. M. Rinne, S. Jaakkola, P. Huhtanen, Anim. Feed Sci. Technol. **67**, 1–17 (1997)
18. A.O.A.C., Official Methods of Analysis of the Association of Official Analytical Chemists, 18th edn. (Association of Official Analytical Chemists, Arlington, Virginia, 2006)
19. R.E. Kitson, M.G. Mellon, Ind. Eng. Chem. Anal. Ed. **16**(6), 379–383 (1944)
20. D.I. Arnon, Plant Physiol. **24**(1), 1 (1949)
21. F. Bartolozzi, G. Bertazza, D. Bassi, G. Cristoferi, J. Chromatogr. A **758**, 99–107 (1997)
22. M. Dhibi, B. Mechri, I. Cheraif, M. Hammami, J. Agric. Food Chem. **58**, 12210–12215 (2010)
23. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Free Radic. Biol. Med. **26**, 1231–1237 (1999)
24. D. Huang, B. Ou, R.L. Prior, J. Agric. Food Chem. **53**, 1841–1856 (2005)
25. J. Pinela, L. Barros, A.M. Carvalho, I.C.F.R. Ferreira, Food Chem. Toxicol. **49**, 2983–2989 (2011)
26. M.B. Hlila, K. Majouli, H. Ben Jannet, M. Aouni, M. Mastouri, B. Selmi, Asian Pac. J. Trop. Biomed. **7**(7), 629–632 (2017)
27. W.M. Pak, K.B. Kim, M.J. Kim, B.K. Kang, S.W. Bark, B.R. Kim, N.K. Choi, S.R. Yoon, D.H. Ahn, J. Applied Biol. Chem. **57**, 353–358 (2014)
28. E.S. Chauhan, A. Tiwari, A. Singh, Int. J. Home Sci. **2**(3), 123–126 (2016)
29. H. Olsen, K. Aaby, G.I.A. Borge, J. Agric. Food Chem. **57**, 2816–2825 (2009)
30. C. Sousa, M. Taveira, P. Valentao, F. Fernandes, J.A. Pereira, L. Estevinho, A. Bento, F. Ferreres, R.M. Seabra, P.B. Andrade, Food Chem. **110**, 953–961 (2008)
31. A. Romani, P. Vignolini, L. Isolani, F. Ieri, D. Heimler, J. Agric. Food Chem. **54**, 1342–1346 (2006)
32. A. Crozier, I.B. Jaganath, M.N. Clifford, in *Plant Secondary Metabolites: Occurrence, Structure and Role in the Human Diet*, ed. by A. Crozier, M. Clifford, H. Ashihara (Blackwell, Oxford, UK, 2006), pp. 1–24
33. P.C.H. Hollman, I.C.W. Arts, J. Sci. Food Agric. **80**, 1081–1093 (2000)
34. P. Soengas, M. Cartea, M. Francisco, T. Sotelo, P. Velasco, Food Chem. **134**, 725–733 (2012)
35. E. Sikora, E. Cieślik, A. Filipiak-Florkiewicz, T. Leszczyńska, Acta Sci. Pol. Technol. Aliment. **11**, 45–51 (2012)
36. S.S. Cha, M.Y. Lee, J.J. Lee, Korean J. Food Preserv. **20**, 88–96 (2013)
37. E. Koh, K.M.S. Wimalasiri, A.W. Chassy, A.E. Mitchell, J. Food Compos. Anal. **22**, 637–643 (2009)
38. W. Biel, E. Jendrzejczak, A. Jaroszewska, R. Witkowicz, E. Piątkowska, A. Telesiński, Ital. J. Food Sci. **29**(4), 728–740 (2017)
39. I. Navarro-González, V. García-Valverde, J. García-Alonso, M. Jesús Periago, Food Res. Int. **44**(5), 1528–1535 (2011)
40. M.G. Ferruzzi, J. Blakeslee, Nutr. Res. **27**, 1–12 (2007)
41. O.K. Chun, N. Smith, A. Sakawaga, C.Y. Lee, Int. J. Food Sci. Nutr. **55**, 191–199 (2004)
42. B. Giorgio, A. Giulia, F.S. Giuditta, D.S. Mauro, S. Maurizio, J. Food Prot. **69**, 2274–2279 (2006)