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Phenolic acid contents of kale (*Brassica oleraceae* L. var. *acephala* DC.) extracts and their antioxidant and antibacterial activities

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Abstract

Nine phenolic acids were identified and quantified by HPLC–MS in leaves and 10 in seeds of kale (black cabbage). The free, ester (methanol-soluble), glycoside and ester-bound (methanol-insoluble) phenolic acid contents of the leaves were 487, 532, 4989 and 6402 ng/g fresh weight, respectively. Ferulic and caffeic acids (total contents; 4269 and 4887 ng/g, respectively) were the most abundant. The seed contents of these fractions were 1993, 1477, 1231 and 4909 ng/g dry weight (DW), respectively, and sinapic acid was the most abundant (5037 ng/g DW). The fractions' total phenolic contents, determined colorimetrically, were highly correlated with their DPPH scavenging capacity, and in antimicrobial activity assays, with nine test organisms representing a wide array of taxa, all of the fractions were effective against *Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus subtilis* and (most strongly) *Moraxella catarrhalis*. Antimicrobial and antioxidant activities of kale phenolics in free and conjugated forms are discussed.

Keywords: Black cabbage; Kale; *Brassica oleraceae* var. *acephala*; Phenolic acid; Leaf; Seed

1. Introduction

Food plants with apparent cancer and cardiovascular disease-preventing (and antimicrobial) properties include several varieties of *Brassica oleraceae* (Beecher, 1994; Herrmann, 1989) and other cruciferous vegetables (Fahey & Stephenson, 1999). In addition, extracts of many species of the *Brassicaceae* family have been shown to have activities against various important pathogens, especially bacterial pathogens (de Saraviaa & Gaylardeb, 1998). Indeed, numerous surveys have highlighted the potential importance of cabbage as a source of antibacterial substances. Frequently-cited substances that appear to contribute to these health-related properties of brassicas, and other plants, include glucosinolates, flavonoids, phenolics and related analogues (de Saraviaa & Gaylardeb, 1998; Fahey & Stephenson, 1999; Herald & Davidson, 1983; Mithen, Dekker, Verkerk, Rabot, & Johnson, 2000).

Kale or black cabbage (*B. oleraceae* L. var. *acephala* DC., family: Brassicaceae) is an important constituent of the traditional diets of inhabitants of Northeastern Anatolia in Turkey. In the study reported here we investigated the antioxidant and antibacterial activities of phenolic fractions isolated from kale leaves and seeds. To our knowl-

Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; DW, dry weight; FW, fresh weight; HBAs, hydroxybenzoic acid derivatives; HCAs, hydroxycinnamic acid derivatives; HPLC–MS, high-performance liquid chromatography–mass spectrometry; IC_{50}, concentration causing 50% inhibition; PHAs, total content of phenolic acids.

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edge this is the first time the activities of such extracts from any *Brassica* species have been analysed, although the antioxidant and antibacterial activities of *Brassicas*, and their importance in human health, have been investigated (Beecher, 1994; Hollman, Hertog, & Katan, 1996). In addition, the total phenolic contents (measured using a modified Folin–Ciocalteau colorimetric method) and phenolic acid levels in four phenolic fractions (free, ester, glycoside and ester-bound) of kale leaves and seeds were determined by HPLC–MS.

2. Materials and methods

2.1. Plant material

Three mature kale (*B. oleraceae* L. var. *acephala* DC.) leaves were harvested from each of six fields in Trabzon (Turkey) in the winter of 2003, immediately cut into small pieces, and after thorough mixing three 10 g samples were frozen in liquid N_2 and stored at −80 °C until they were extracted and fractionated. Mature seeds were collected throughout July and August 2003 from the same fields, and triplicate 10 g air-dried seed samples were used for further analysis.

2.2. Chemicals and reagents

Standards of 3,5-dihydroxybenzoic, gallic, protocatechuic, *p*- and *m*-hydroxybenzoic, gentisic, *o*-, *m*- and *p*-coumaric, caffeic, ferulic, syringic, sinapic, chlorogenic, vanillic, salicylic and *trans*-cinnamic acids, 1,1-diphenyl-2-picrylhydrazyl (DPPH, 90%) and Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO). The formic acid and methanol used in the HPLC analyses were purchased from Merck (Darmstadt, Germany), Folin–Ciocalteau reagent from Jan Kulich (Hradec Králové, Czech Republic) and deionised water was prepared using a Simplicity 185 system (Millipore, Bedford, MA).

2.3. Extraction of leaf and seed phenolic acids

Frozen samples were powdered in liquid nitrogen using a mortar and pestle, then defatted by Soxhlet extraction with *n*-hexane and petroleum ether. Phenolic acids were extracted, isolated and purified according to the method of Krygier, Sosulski, and Hogge (1982), as modified by Zadernowski and Kozlowska (1983). The defatted samples were extracted with 80% methanol, containing 0.2 mg/ml DBC (2,6-di-*tert*-butyl-β-cresol) as an antioxidant, for 10 min at 5 °C. The homogenate was filtered and concentrated under vacuum, its pH was adjusted to 2 (6 M HCl) and free phenolic acids were extracted into diethyl ether. The aqueous phase remaining after the ether extraction was divided into two aliquots, one of which was hydrolysed with 2 M NaOH for 4 h under a nitrogen atmosphere at room temperature. After acidification to pH 2 (6 M HCl), phenolic acids released from the soluble esters were extracted again into diethyl ether. To the second aliquot, 6 M HCl was added and the medium was hydrolysed under a nitrogen atmosphere for 1 h in a boiling water bath. Phenolic acids released from soluble glycosides were also separated into diethyl ether. The solid residue obtained after filtration of the first homogenate was dissolved in 2 M NaOH for 4 h and, after acidification to pH 2 (6 M HCl), phenolic acids released from methanol-insoluble ester-bound acids were extracted into diethyl ether. All the ether fractions were evaporated under vacuum.

2.4. HPLC–MS instrumentation and conditions

Phenolic acids were analysed by HPLC–MS as previously described (Ayaz, Hayirlioglu-Ayaz, Gruz, Novak, & Strnad, 2005). *m*-Coumaric acid was added as an internal standard before the analysis to all fractions to a final concentration of 10^{-5} M. Ten microliters of each sample were injected onto a reversed phase column (Luna Phenyl–Hexyl, 5 μm, 250 × 2 mm; Phenomenex, Torrance, CA). HPLC–MS analyses were performed using an Alliance 2690 Separations Module (Waters, Milford, MA) coupled to a PDA 996 photodiode array detector (Waters) and a ZMD 2000 single quadrupole mass spectrometer, equipped with an electrospray interface (Micromass). Signals acquired from the MS were processed by MassLynx software (Data Handling System for Windows, version 4.0, Micromass) and analytes were quantified from the ratio of their respective peak areas to the peak area of the internal standard.

2.5. Total phenolic contents

Total phenolic contents of the fractions were determined using the Folin–Ciocalteau colorimetric method, as modified by Velioglu, Mazza, Gao, and Oomah (1998). Briefly, 5 μl of the tested fraction in distilled water were mixed with 100 μl of 10-fold diluted Folin–Ciocalteau reagent, incubated at room temperature for 5 min and then 100 μl Na_2CO_3 (75 g/l) were added to the mixture. Absorbance was measured at 725 nm after 90 min incubation at 30 °C and the results were expressed as gallic acid equivalents.

2.6. Antioxidant activity

Radical quenching activity was determined using a previously described DPPH scavenging assay with modifications (Deby & Magotteaux, 1970; Joyeux, Mortier, & Fleurentin, 1995). Briefly, 90 μl portions of a methanol solution of the tested sample at a minimum of five different concentrations were each mixed with 180 μl of a methanolic DPPH solution (20 mg/l). After 30 min, absorbance at 517 nm was measured and IC_{50} values were obtained from the resulting inhibition curves.

2.7. Antimicrobial activity tests

The following microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey): *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Moraxella catarrhalis* ATCC 25238, *Escherichia coli* ATCC 35218, *Enterobacter cloacae* ATCC 13047, *Pseudomonas aeruginosa* ATCC 10145, *Candida albicans* ATCC 60193 and *Candida tropicalis* ATCC 13803. The antimicrobial activities of each of the fractions obtained from the kale leaves and seeds were then assayed using the agar well diffusion method (Perez, Pauli, & Bazerque, 1990), as adapted by Ahmad, Mehmood, and Mohammad (1998). The test extracts were weighed and dissolved in methanol to prepare 1 mg/ml solutions. Each microorganism was suspended in Brain Heart Infusion (BHI) (Difco, Detroit, MI) broth and diluted to ca. 10^6 colony forming units (cfu) per ml. They were then "flood-inoculated" onto the surface of both BHI agar and Sabouraund Dextrose agar (SDA) (Difco) plates (except for *C. albicans* and *C. tropicalis*, for which only SDA was used) which were then dried. Five millimetre diameter wells were cut from the agar using a sterile cork-borer, and 100 μl of the extracts were placed in the wells. The plates were then incubated for 18 h at 35 °C, after which the antimicrobial activity of the test extract was evaluated by measuring the zone of inhibition against the test organism. Ampicilin (30 μg/ml), triflucan (5 μg/ml) and aqueous methanol (85%, v/v) served as controls. The results were expressed in terms of the diameter of the inhibition zone: $\leqslant$5.5 mm, inactive; 6.0–14 mm, partially active; 15–24 mm, active; $\geqslant$25 mm, very active.

2.8. Statistics

Data were analysed with one-way ANOVA using the StatView Statistical Package (SAS Institute, Inc., Cary, NC). Differences were considered statistically significant when $p < 0.05$. IC_{50} values and Pearson's correlation coefficients were obtained using MS Excel 2003.

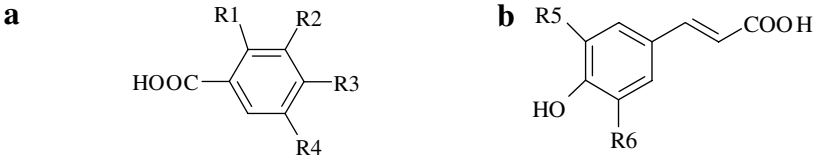
3. Results and discussion

3.1. Phenolic acid composition of kale leaves and seeds

In the kale leaves, nine phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, salicylic, *p*-coumaric, caffeic, ferulic and sinapic) were identified and quantified by HPLC–MS. The most abundant acids were ferulic (169 ng/g FW in free and 170 ng/g FW in ester forms) and caffeic acid (2040 ng/g FW in glycoside and 2640 ng/g FW in ester-bound forms). Five of these phenolic acids were hydroxybenzoic acid derivatives (HBAs, see Fig. 1a), at total concentrations of 143, 177, 243 and 334 ng/g FW in free, ester, glycoside and ester-bound forms, respectively and four were hydroxycinnamic acids (HCAs, Fig. 1b) present in the leaves at 343, 355, 4746 and 6069 ng/g FW in free, ester, glycoside and ester-bound forms, respectively. The kale leaves contained HBAs and HCAs at concentrations of 897 and 11,513 ng/g FW, respectively. The most abundant individual phenolic acids in the leaf extracts were caffeic (4887 ng/g FW, 39.4%) and ferulic (4269 ng/g FW, 34.4%) both of which are HCAs (Table 2), and jointly comprised almost 74% of the total content of phenolic acids (PHAs).

Total phenolic contents determined using the Folin–Ciocalteau colorimetric method were higher for all fractions than the corresponding PHA values determined by HPLC–MS, with differences ranging from 11-fold for ester-bound phenolic acids to 770-fold for ester forms. The total phenolic content in the kale leaves was determined to be 1366 ng/g FW, of which the identified phenolic acids accounted for only 0.9%.

In the kale seeds, 10 phenolic acids were identified and quantified. Salicylic acid was not detected in either the free or ester fractions, while the glycoside and ester-bound fractions contained this acid at concentrations of 27.1 and 110 ng/g DW, respectively. The most abundant phenolic acid in the seeds was sinapic acid at concentrations of 1076, 796, 661 and 2505 ng/g DW in free, ester, glycoside and ester-bound forms, respectively. Six of these phenolic acids were HBAs at total concentrations of 90.9, 45.5,



Acid	R1	R2	R3	R4
Gallic	H	OH	OH	OH
Protocatechuic	H	OH	OH	R
p-Hydroxybenzoic	H	H	OH	H
Vanillic	H	OCH_3	OH	H
Syringic	H	OCH_3	OH	OCH_3
Salicylic	OH	H	H	H

Acid	R5	R6
p-Coumaric	H	H
Caffeic	H	OH
Ferulic	H	OCH_3
Sinapic	OCH_3	OCH_3

Fig. 1. Structures of hydroxybenzoic (a) and hydroxycinnamic (b) acid derivatives found in kale leaves and seeds.

208 and 831 ng/g DW in free, ester, glycoside and ester-bound forms, respectively, and four were HCAs, at total concentrations of 1902, 1431, 1023 and 4078 μg/g DW in free, ester, glycoside and ester-bound forms, respectively. The black cabbage seeds contained HBAs and HCAs at total concentrations of 1176 and 8434 ng/g DW, respectively. The total phenolic acid contents in the seeds in free, ester, glycoside and ester-bound forms were 1993, 1477, 1231 and 4909 ng/g DW, respectively. The HPLC–MS analyses revealed that the most abundant phenolic acids were sinapic (5037 ng/g DW, 52.4%) and ferulic (1670 ng/g DW, 17.4%) acids, both of which are HCAs (Table 2), and jointly comprised 70% of the total PHAs.

As in the leaves, the total phenolic content of the seeds (6057 ng/g DW, see Table 1) was much higher than their phenolic acid contents determined by HPLC–MS; the identified phenolic acids accounted for only 0.16% of the seed phenolic content. This difference is probably due to the presence of other compounds besides the acids determined by HPLC–MS. The Folin–Ciocalteau reagent reacts with all forms of phenolic compounds containing at least one benzene ring with at least one OH-group, and various other compounds, such as ascorbic acid. Besides phenolic acids, various classes of flavonoids, lignan derivatives and condensed polyphenolic compounds are likely to be either initially present, or formed during the extraction procedures, in all fractions. These differences are in accordance with other data describing the antioxidant activity and phenolic contents of various foods and herbs (Heimler, Vignolini, Dini, & Romani, 2005; Proestos, Chorianopoulos, Nychas, & Komaitis, 2005; Velioglu et al., 1998; Valentová, Cvak, Muck, Ulrichová, & Šimánek, 2003; Zheng & Wang, 2001).

The comparative analyses of phenolic acids in the four phenolic fractions of both the leaves and seeds of black cabbage show that they contained lower amounts of free phenolic acids, in accordance with previous reports indicating that the majority of phenolic acids are present in plants in bound forms (Ayaz et al., 2005; Chen & Zuo, 2007; Robbins, 2003; Shahidi & Naczk, 1995; Zuo, Wang, & Zhan, 2002).

3.2. Antioxidant activity

The antioxidant activity of all of the fractions was determined in terms of the proportion (%) of DPPH scavenged by 10 μg of the dry fraction per ml of reaction mixture, and resulting IC_{50} values are shown, in μg/ml, in Table 2. All of the fractions were able to scavenge the radical, but they were all less active than Trolox, with IC_{50} values ranging from 3.86 to 400 μg/ml compared to 2.14 ± 0.06 μg/ml for Trolox ($p < 0.01$). The lowest activity was found for the free acid fraction from kale leaves. This finding is probably due to the presence of other ether-extractable compounds in that fraction, and is consistent with its relatively low total phenolic content (1.62%, compared to 16–67% in the other fractions). Overall, the antioxidant activities of the fractions were highly correlated with their total phenolic contents ($r = 0.969$). These results are also consistent with previous findings (Beta, Nam, Dexter, & Sapirstein, 2005; Bouaziz, Chamkha, & Sayadi, 2004; Kuti & Konuru, 2004; Liyana-Pathirana & Shahidi, 2006).

Phenolics from cruciferous vegetables, such as flavonols (present in *Brassica* species), reportedly have preventive properties against cardiovascular diseases and some types of cancer (Beecher, 1994; de Saraviaa & Gaylardeb, 1998; Fahey & Stephenson, 1999; Hollman et al., 1996; Mithen et al., 2000). Discarded parts of these cruciferous vegetables also contain phenolics and have shown antioxidant properties. For instance, flavonoid concentrations have been found to be much higher in discarded parts of cauliflowers than in their edible parts, where only trace amounts have been detected (Hollman & Arts, 2000). Furthermore, the discarded parts contained 3-fold higher flavonol levels than other *Brassica* species (Hollman & Arts, 2000). Llorach, Espin, Tomas-Barberan, and Ferreres (2003) showed that caffeic and sinapic acids were the main phenolic acids in cauliflower. As in cauliflower, in our study we found caffeic and sinapic acids to be the most abundant phenolic acids in the leaves and seeds, respectively, of black cabbage.

3.3. Antimicrobial activity of phenolic fractions of kale leaves and seeds

Since it is essential to assay the effects of candidate antimicrobial substances against specific target microorganisms, nine typical microorganisms were utilised as taxonomical representatives in an initial screening programme: the Gram-positive spore-forming bacillus, *B. subtilis*; the Gram-positive cocci *S. aureus* and *E. faecalis*; the Gram-negative coccus, *M. catarrhalis*; the Gram-negative bacilli *Escherichia coli*, *Enterobacter cloace* and *Pseudomonas aeruginosa*; and the yeasts *Candida albicans* and *C. tropicalis*.

As shown in Table 3, all of the phenolic fractions extracted from these leaves effectively inhibited growth of the Gram-positive bacteria *S. aureus*, *E. faecalis*, *B. subtilis* and the Gram-negative bacterium *M. catarrhalis*, which is known to be a major respiratory pathogen in a paediatric and adult patient populations (Sarubbi, Myers, Williams, & Shell, 1990).

In contrast, none of the fractions inhibited growth of the Gram-negative bacteria *P. aeruginosa* and *E. cloaca*, and only the ester and ester-bound phenolic fractions effectively inhibited the growth of the two yeast-like fungi (*C. tropicali* and *C. albicans*). Furthermore, no inhibition zone developed in the growth media around colonies of either *E. coli* or *E. faecalis*, when the ester-bound phenolic fractions were tested, around the Gram-negative *E. cloaceae* and *P. aeruginosa*, in tests with the free phenolic fraction, or around the two yeast-like fungi *C. tropicali* and *C. albicans*, in tests with the glycoside fraction. However, the Gram-negative bacterium *M. catarrhalis*, which is one of the most common agents of upper respiratory tract infec-

Table 1
Phenolic acids content of kale (*Brassica oleraceae* L. var. *acephala* DC.) leaf and seed[a]

Phenolic acids (ng/g)	Leaf (per fresh weight)					Seed (per dry weight)				
	Free	Ester	Glycoside	Ester-bound	Total[b]	Free	Ester	Glycoside	Ester-bound	Total[b]
Gallic acid	4.6 ± 0.6	5.1 ± 0.8	1.2 ± 0.2	2.9 ± 0.3	13.8	0.9 ± 0.0	0.5 ± 0.0	2.3 ± 0.0	7.2 ± 0.0	10.9
Protocatechuic acid	17.8 ± 1.2	22.0 ± 0.3	38.5 ± 1.3	52.4 ± 2.1	131	7.2 ± 0. 3	4.1 ± 0.0	10.5 ± 0.5	59.2 ± 1.0	81
p-Hydroxybenzoic acid	92.2 ± 1.8	122 ± 2.3	171 ± 1.9	239 ± 3.4	624	71.8 ± 2. 4	34.4 ± 0.8	74.4 ± 6.1	292 ± 3.5	473
Vanillic acid	24.1 ± 0.4	22.3 ± 0.6	31.7 ± 0.6	36.8 ± 0.3	115	7.6 ± 0. 5	4.0 ± 0.3	69.2 ± 2.6	271 ± 4.3	351
Syringic acid	ND[d]	ND	ND	ND		3.4 ± 0.0	2.5 ± 0.0	24.8 ± 2.1	91. 6 ± 2.4	123
Salicylic acid	4.6 ± 1.4	5.1 ± 0.6	1.2 ± 0.3	2.9 ± 0.3	13.8	ND	ND	27. 1 ± 0.3	110 ± 1.2	138
Total HBAs[c]	143	177	243	334	897	90.9	45.5	208	831	1176
HBAs (%)	29.5	33.2	4.9	5.2	7.2	4.6	3.1	16.9	16.9	12.2
p-Coumaric acid	59.3 ± 3.8	59.1 ± 2.6	950 ± 6.3	1240 ± 8.1	2308	300 ± 7. 1	220 ± 7.1	99.3 ± 3.2	487 ± 9.1	1106
Caffeic acid	96.5 ± 2.5	110 ± 4.1	2040 ± 7.3	2640 ± 10.0	4887	55.7 ± 1. 3	51.5 ± 3.7	93.4 ± 1.8	420 ± 4.2	621
Ferulic acid	169 ± 2.7	170 ± 5.1	1749 ± 4.3	2180 ± 8.4	4269	470 ± 7. 1	365 ± 5.3	169 ± 11.2	666 ± 7.4	1670
Sinapic acid	18.7 ± 0.8	15.5 ± 0.6	6.5 ± 0.8	8.7 ± 0.4	49.4	1076 ± 12. 1	796 ± 6.3	661 ± 1.8	2505 ± 12.1	5037
Total HCAs [c]	343	355	4746	6069	11,513	1902	1431	1023	4078	8434
HCAs (%)	70.5	66.8	95.1	94.8	92.8	95.4	96.9	83.1	83.1	87.8
Total PHAs [c]	487	532	4989	6402	12,410	1993	1477	1231	4909	9609
Total phenolic content (ng/g)	46.7 ± 0.1	409 ± 13.6	834 ± 46.9	72.5 ± 5.4	1366	2149 ± 150.1	2264 ± 78.7	1127 ± 68.2	518 ± 5.9	6057

HBAs – hydroxybenzoic acid derivatives, HCAs – hydroxycinnamic acid derivatives, PhA – phenolic acids.

[a] Values are means ± SD ($n = 3$).

[b] Total is sum of phenolic fractions.

[c] Total is sum of individual forms of the phenolic acids identified.

[d] Not detected.

Table 2
DPPH scavenging activities of phenolic fractions extracted from kale (*Brassica oleraceae* L. var. *acephala* DC.) leaf and seed[a]

Fraction	Leaf		Seed	
	Scavenging (%)[b]	IC_{50}[c] (μg/ml)	Scavenging (%)[b]	IC_{50} (μg/ml)
Free	−2.14 ± 0.05	400 ± 12.4	44.3 ± 2.12	3.86 ± 0.18
Ester	37.8 ± 1.60	4.39 ± 0.21	44.8 ± 1.46	3.68 ± 0.04
Glycoside	19.7 ± 0.27	7.37 ± 0.22	18.8 ± 2.29	7.70 ± 0.22
Ester-bound	4.06 ± 0.70	24.6 ± 0.77	25.4 ± 2.82	6.49 ± 0.22
Trolox	70.4 ± 0.38	2.14 ± 0.06		

[a] Values are expressed as mean ± SD, $n = 4$.
[b] % of the DPPH radical (20 mg/l) scavenged by 10 μg/ml of the fractions tested (or Trolox).
[c] Concentration of the fraction needed for 50% scavenging.

Table 3
Antimicrobial activity of phenolic fractions of kale (*B. oleraceae* L. var. *acephala* DC.) leaf and seed (measured as inhibition zone diameter in mm)[a]

Phenolic fraction	Microorganism								
	S. aureus ATCC 25923	*E. faecalis* ATCC 29212	*B. subtilis* ATCC 6633	*M. catarrhalis* ATCC 25238	*E. coli* ATCC 35218	*E. cloaceae* ATCC 13047	*P. aeruginosa* ATCC 10145	*C. tropicali* ATCC 13803	*C. albicans* ATCC 10231
Leaf									
Free	16.1 ± 1.0[b]	12.3 ± 1.2	22.0 ± 1.2	20.2 ± 1.2	14. 4 ± 1.2	–	–	–	
Ester	20.3 ± 1.2	20.1 ± 1.2	24.0 ± 1.2	20.0 ± 0.2	–	–	–	12. 1 ± 1.2	12 ± 1.2
Glycoside	18.4 ± 1.2	20.0 ± 1.2	20.3 ± 1.2	20.1 ± 1.2	20.3 ± 1. 2	–	–	–	–
Ester-bound	14.2 ± 1.2	–	20.1 ± 1.2	20.4 ± 1.2	–	–	–	12.3 ± 1. 2	12.1 ± 1.2
Seed									
Free	–	–	–	20.3 ± 1.2	–	–	–	–	–
Ester	30.5 ± 1.2	–	–	20.1 ± 1.2	18 ± 1.2	–	–	–	–
Glycoside	12.4 ± 1.2	–	16.2 ± 1.2	20.0 ± 1.2	–	–	–	–	
Ester-bound	32.3 ± 1.2	20.2 ± 1.2	34.0 ± 1.2	24.2 ± 1.2	–	–	–	14. 3 ± 1.2	14.1 ± 1.2

Phenolic acid compositions and the levels of the fractions were given in Table 1.
[a] Effect of the fractions at 1 mg/ml. Values are means ± SD ($n = 3$).
[b] 5.5–10 mm, partially active; 11–19 mm, active; ⩾20 mm, very active; −, inactive.

tion, was found to be sensitive to all tested fractions (Table 3).

Phenolic fractions extracted from the seeds were less effective than the leaf phenolic acid fractions although, as with the leaf extracts, *M. catarrhalis* was found to be the most sensitive of the tested microorganisms to them. The ester-bound fraction was found to have the widest spectrum of antimicrobial activity, being effective against both Gram-positive (*S. aureus*, *E. faecalis*, *B. subtilis*) and Gram-negative (*M. catarrhalis*) bacteria, and the two yeast-like fungi (*C. tropicali* and *C. albicans*), for which the sizes of the inhibition zones were very similar. The growth of *E. coli*, *S. aureus* and *M. catarrhalis* was also inhibited by the soluble ester fraction. Similarly, the glycoside fraction inhibited the growth of *B. subtilis*, *S. aureus* and *M. catarrhalis*. In contrast, the free phenolic acid fraction did not effectively inhibit any of the tested organisms, except *M. catarrhalis* (Table 3).

A particularly promising finding is that the black cabbage extracts tested were effective against Gram-negative bacterial strains, since many antibiotics are less active against Gram-negative than Gram-positive bacteria, probably due to the former's more complex cell wall structure, which has additional lipopolysaccharides on the outer surface, which generally reduce the ability of most antibiotics and extracts to penetrate the bacterial cells (Rang & Dale, 1987). The antimicrobial activities of various natural compounds present in plant leaves, flowers, stems, roots or fruits have been reported by many workers, but to our knowledge, there have been no previous reports on the antimicrobial activities of phenolics in the four phenolic fractions of black cabbage leaves and seeds investigated here. Since the phenolic isolates of the plant parts (leaves and seeds) used in this study produced inhibition zones against test organisms, it is expected that they could be used to treat infections and diseases caused by these organisms, and if the active compounds of the extracts are isolated and identified, alternative therapeutic antibiotics could be produced from these cruciferous plants.

4. Conclusion

In conclusion, the results of the present investigation indicate that kale leaves and seeds can be rated as good dietary sources of natural phenolic antioxidants and other compounds with high or moderate antimicrobial activity.

They contain many phytochemicals that can potentially be utilised for pharmaceutical research. Further work is needed to identify the active principle(s) from various leaf and seed extracts of black cabbage (kale). Therefore, further phyto-pharmaceutical studies using similar or more advanced analytical approaches will be required.

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