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# Phenolic Composition, Antioxidant Capacity and Antibacterial Activity of Selected Irish Brassica Vegetables

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Vegetables belonging to the Brassicaceae family are rich in polyphenols, flavonoids and glucosinolates, and their hydrolysis products, which may have antibacterial, antioxidant and anticancer properties. In the present study, phenolic composition, antibacterial activity and antioxidant capacity of selected Brassica vegetables, including York cabbage, Brussels sprouts, broccoli and white cabbage were evaluated after extraction with aqueous methanol. Results obtained showed that York cabbage extract had the highest total phenolic content, which was 33.5, followed by 23.6, 20.4 and 18.4 mg GAE/g of dried weight (dw) of the extracts for broccoli, Brussels sprouts and white cabbage, respectively. All the vegetable extracts had high flavonoid contents in the order of 21.7, 17.5, 15.4 and 8.75 mg QE/g of extract (dw) for York cabbage, broccoli, Brussels sprouts and white cabbage, respectively. HPLC-DAD analysis showed that different vegetables contain a mixture of distinct groups of phenolic compounds. All the extracts studied showed a rapid and concentration dependent antioxidant capacity in diverse antioxidant systems. The antibacterial activity was determined against Gram-positive and Gram-negative bacteria. York cabbage extract exhibited significantly higher antibacterial activity against *Listeria monocytogenes* (100%) and *Salmonella abony* (94.3%), being the most susceptible at a concentration of 2.8%, whereas broccoli, Brussels sprouts and white cabbage had moderate to weak activity against all the test organisms. Good correlation ($r^2$ 0.97) was found between total phenolic content obtained by spectrophotometric analysis and the sum of the individual polyphenols monitored by HPLC-DAD.

Keywords: Antibacterial activity, antioxidant capacity, Brassica vegetables, HPLC, polyphenols

Fruits and vegetables constitute an important part of a healthy human diet. A high intake of fruits and vegetables is positively associated with the prevention of cardiovascular diseases, cancer, aging, diabetes, osteoporosis, hypertension, and stroke. The European Prospective Study of Cancer (EPIC) estimated that an increase in fruits and vegetables intake of just 50 g per day has the potential of reducing the risk of premature death from any cause by 20% [1].

Brassica vegetables belong to the Brassicaceae or Cruciferae family, a large family comprised of approximately 3,500 described species apportioned among 350 genera. *Brassica oleracea* constitutes a number of vegetables like cauliflower, broccoli, kohlrabi, kale, cabbage and Brussels sprouts. These Brassica vegetables are rich in a number of biologically active compounds such as vitamins, phenolic acids, flavonoids and glucosinolates, which are associated with antioxidant, antibacterial and anticancer properties.

Free radicals and reactive oxygen species (ROS) are generated in the living system as a consequence of normal metabolism as well as several external factors such as smoking, excessive exercise and diet [2]. These free radicals are highly reactive and can react with most molecules in its vicinity. These include lipids, nucleic acids, proteins and carbohydrates and has been linked in the causation and progression of several chronic diseases, including ageing, cancer, and many other diseases [3]. In food systems, these free radicals react with fats and oils and cause oxidative deterioration, which is responsible for rancid odor and flavors, with a consequent decrease in nutritional quality, safety and shelf life of food.

All aerobic organisms, including human beings, have antioxidant defenses that protect against oxidative damage, and numerous damage removal and repair enzymes are present to either remove or repair damaged molecules [4]. However, this natural antioxidant mechanism can be inefficient; hence, dietary intake of antioxidant compounds becomes important [5]. Antioxidants have also been applied in the food industry to prolong the shelf life of foods, especially those rich in polyunsaturated fats. Synthetic antioxidant compounds, such as butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA) have been used commonly in processed foods to control lipid oxidation. Reports revealing that these synthetic chemicals could be toxic and the higher manufacturing costs with the increasing consciousness of consumers with regard to food additive safety, created a

need for identifying alternative low-cost, natural and probably safer sources of food antioxidants.

Numerous studies have highlighted the potential importance of Brassica vegetables as a source of antibacterial [6-9] and antioxidant substances [10-14] from different geographical areas. Growing evidence suggests that both genotype and growing conditions may alter the phytochemical composition and their different properties, including antioxidant and antibacterial activity. Ireland has a temperate climate which influences the production of a range of fruit and vegetables. Brassica vegetables account for almost half (47%) of all field vegetable production area in Ireland. Cabbage is the most important Brassica vegetable (by area), representing 19% of total field vegetable production area, followed by broccoli, with 13% of total production area [15].

The main objectives of the present study were to (i) determine the phenolic composition of aqueous methanolic extracts of Brassica vegetables; and (ii) examine the antioxidant capacity of Brassica vegetables against a number of reactive oxygen/nitrogen species and metal ions. In addition, this study was also designed to evaluate the antibacterial activity of Brassica vegetable extracts against several food spoilage and pathogenic bacteria.

The extraction yields of the various vegetable samples were 3.26, 5.08, 5.25 and 6.89% for York cabbage, white cabbage, broccoli and Brussel sprouts, respectively. The total phenolic content (TPC) of the various vegetable extracts varied between 18.7 ± 0.43 to 33.5 ± 1.50 mg GAE/g of extracts (dw). York cabbage extract exhibited significantly ($P < 0.05$) higher TPC compared with broccoli and Brussels sprouts, while white cabbage was the lowest among all the vegetables studied. Kaur and Kapoor [16] estimated the TPC of 33 commonly consumed vegetables and reported values for cabbage, broccoli and Brussels sprouts of 92.5 mg, 87.5 and 68.8 mg/100 g fresh weight (fw), respectively. Our results showed that Irish Brassica vegetables had higher TPC values (140.6, 126.8, 109.1 and 86.4 mg GAE/100 g (fw) for Brussels sprouts, broccoli, York cabbage and white cabbage, respectively).

All the vegetables showed the presence of considerable amounts of flavonoid. York cabbage had a significantly ($P < 0.05$) higher amount of TFC [21.7 ± 1.90 mg QE/g of extract (dw)], followed by broccoli [17.5 ± 1.25 mg QE/g of extract (dw)], Brussels sprouts [15.4 ± 0.72 mg QE/g of extract (dw)] and white cabbage [8.80 ± 1.25 mg QE/g of extract (dw)]. Andarwulan *et al*. [13] studied the flavonoid content of 11 vegetables from west Java, Indonesia and found that it varied from 0.3 to 143 mg/100 g (fw), whereas Lin and Tang [17] reported variation from 4.1 to 133.1 mg QE/100 g (fw). The flavonoid content found in this study [70.7 mg/100 g (fw) for York cabbage, 106.2 mg/100 g (fw) for Brussels sprouts, 94.0 mg/100 g (fw) for broccoli and 41.1 mg/100 g (fw) for white cabbage] corresponded well with those determined by others.

Five different groups of polyphenols were identified by comparing their UV-vis spectra with those of reference compounds and reported values. All five polyphenolic groups were quantified using the standard curves of the representative standards. The hydroxybenzoic acid (HBA) derivatives were quantified at 280 nm and expressed as gallic acid equivalents (GAE), hydroxycinnamic acid (HCA) derivatives at 320 nm and expressed as chlorogenic acid equivalents (CAE), flavones, polymethoxylated flavones (PMF), and glycosylated flavonoids (GSF) at 360 nm and expressed as rutin equivalents (RE), and anthocyanins at 520 nm and expressed as cyanidin-3-glucoside equivalents (Cn3GE).

Polyphenolic profiles at 280 nm for the studied Brassica vegetables showed that York cabbage contained a mixture of more than 20 phenolics. Six peaks were identified as HBA derivatives, five as HCA derivatives, seven as flavones, two as polymethoxy flavonoids and one peak of glycosylated flavonols and polymethoxylated flavonols, whereas white cabbage was a mixture of only two groups of phenolic compounds, namely HBA and HCA. Neilsen *et al*. [18] showed that white cabbage contains a mixture of more than 20 compounds of which seven have been identified as 3-*O*-sophoroside-7-*O*-glucosides of kaempferol and quercetin with and without further acylation with hydroxycinnamic acids. Our results showed that York cabbage had more than 20 phenolic compounds, while white cabbage had only 8. Broccoli and Brussels sprouts contain a mixture of three types of phenolic compounds, with the predominant one being a hydroxybenzoic acid derivative.

The results obtained showed significant qualitative variations in the polyphenolic profiles, as well as variation in the concentration of each individual compound. The concentration of individual polyphenols of the studied vegetables is summarized in Table 1; HBA ranged from 1.67 ± 0.01 to 8.15 ± 0.02 mg GAE/g of extract (dw). Broccoli had a significantly higher HBA content than the other vegetables tested. HCA varied from 0.57 ± 0.01 to 6.96 ± 0.01 mg CAE/g of extract (dw) and was highest in York cabbage. PMF varied from 0.24 ± 0.01 to 2.35 ± 0.12 mg RE/g of extract (dw) and was highest in Brussels sprouts. Only York cabbage showed the presence of flavones [7.84 ± 0.02 mg RE/g of extract (dw)] and GSF content [0.25 ± 0.01 mg RE/g of extract (dw)], whereas none of the extracts showed the presence of anthocyanin. The results showed a clear ranking order, in terms of phenolic content: York cabbage > broccoli > Brussels sprouts and > white cabbage.

All the vegetables extracts, at the tested concentrations, were capable of directly reacting with and quenching DPPH radicals. The extracts of broccoli were found to have the highest DPPH radical scavenging capacity, with an $EC_{50}$ value of 0.75 ± 0.07 mg/mL, followed by York cabbage (0.79 ± 0.05 mg/mL). The order of antioxidant activities depended on the extraction method, and on the

type of reactive species in the reaction mixture. In the case of Brussels sprouts and white cabbage, no significant difference ($P > 0.05$) was observed in DPPH radical scavenging capacity.

All the extracts showed a rapid and concentration dependent antioxidant capacity. The lipid peroxidation inhibition ability of York cabbage extract was superior to that of all the other tested vegetables with an $EC_{50}$ value of 4.78 ± 0.53 mg/mL, followed by Brussels sprouts (7.90 ± 0.22 mg/mL), broccoli (6.62 ± 0.97 mg/mL) and white cabbage (10.58 ± 0.11 mg/mL). All the vegetable extracts and the reference compound (ascorbic acid) showed concentration dependent lipid peroxidation inhibition ability, and significant differences ($P < 0.05$) were observed between all the vegetables and ascorbic acid.

All the vegetable extracts had significant ferrous ion chelating capacity, but in all cases it was significantly ($P < 0.05$) lower than that of EDTA. Among the vegetables studied, the highest $Fe^{2+}$ chelating capacity was found in York cabbage (1.49 ± 0.03 mg/mL), followed by broccoli (1.58 ± 0.10 mg/mL) and Brussels sprouts (2.14 ± 0.36 mg/mL). The lowest activity was found for white cabbage (3.45 ± 0.37 mg/mL). The metal chelating effects of all the extracts and EDTA were concentration dependent (not shown). The absorbance of the $Fe^{2+}$-ferrozine complex linearly decreased as concentration of the test sample increased.

All the vegetable extracts and the reference compound potently scavenged $H_2O_2$ in a dose-dependent manner (not shown). It appeared that among the vegetable extracts, York cabbage had the strongest $H_2O_2$ scavenging capacity (1.15 ± 0.06 mg/mL), followed by broccoli (1.29 ± 0.10 mg/mL), Brussels sprouts (2.14 ± 0.02 mg/mL) and white cabbage (2.84 ± 0.21 mg/mL). On the other hand, BHT had a significantly higher scavenging capacity compared with the vegetable extracts tested (from 6 to 20 times).

All the vegetable extracts examined reduced ferric iron to different extents. York cabbage had the highest reducing capacity.

In the present study, a range of concentrations (2.8 to 0.09%) of the vegetable extracts were checked for their antibacterial activity against selected organisms. Figure 1 shows a comparative study of the antibacterial activities of the first three dilutions for York cabbage, broccoli, Brussels sprouts and white cabbage extracts with the synthetic antibacterial agents, sodium benzoate and sodium nitrite, against *L. monocytogenes*, *P. aeruginosa*, *S. abony* and *E. faecalis*. Regardless of the organism, York cabbage extracts exhibited significantly higher antibacterial activity, followed by broccoli, whereas only weak to moderate activity was observed for Brussels sprouts and white cabbage extracts. Resistance to the York cabbage extract was not correlated with taxonomy, since *L. monocytogenes* (Gram-positive) (Figure 1a) and *S. abony* (Gram-negative) (Figure 1b) were the most sensitive, followed by *P. aeruginosa* (Figure 1c) and *E. faecalis* (Figure 1d), which were the most resistant.

An inhibition of 100% was achieved using York cabbage extract concentrations of 2.8% and 1.4% against *L. monocytogenes*. As the extract concentration was serially diluted, the antibacterial effect was seen to reduce, with the 0.7% concentration producing a reduction of 76% growth compared with the control. Broccoli extract showed moderate inhibition (69%) against *L. monocytogenes* at a concentration of 2.8%, whereas both Brussels sprouts and white cabbage showed weak inhibition (<50%). In the case of *S. abony*, York cabbage extracts showed an inhibition of 72%, broccoli 57%, Brussels sprouts 38% and white cabbage 34%.. York cabbage and broccoli extracts showed similar inhibition (50-55%) against *P. aeruginosa*, whereas only 25-40% was produced by Brussels sprouts and white cabbage. *E. faecalis* was the most resistant of all the organisms with growth inhibition in the range of 25-30% by York cabbage and broccoli.

Antibacterial activities of vegetable extracts were also compared with synthetic antibacterial agents, such as sodium benzoate and sodium nitrite. York cabbage extract showed significantly higher ($P < 0.05$) inhibition against *L. monocytogenes* (Fig. 1a) (16 and 4% higher) in comparison with sodium benzoate (84%) and sodium nitrite (96%) at 2.8% concentration, respectively, whereas broccoli showed almost 30% lower activity in comparison with the above mentioned synthetic antibacterial agents. *S. abony* (Figure 1b) was also inhibited to a higher extent with York cabbage extracts (27 and 10% higher) compared with sodium benzoate and sodium nitrite, respectively, whereas broccoli extracts showed lower inhibition activity.

A correlation analysis was carried out between phenolic compounds and antioxidant capacity [$EC_{50}$ value except FRAP, which is TE/g of extract (dw)] of Brassica vegetables extract. In the present study, TPC, TFC, HBA, HCA, flavones, PMF, and GSF was correlated with FRAP, DPPH, LPO, FIC, and $H_2O_2$ (Table 2). As can be seen, none of the phenolic compounds was correlated with any antioxidant capacity, other than TFC, which is highly correlated with FIC ($r^2$ 0.95) and $H_2O_2$ ($r^2$ 0.98). The absence of correlation between phenolic compounds and antioxidant capacity could be due to the following reasons: the antioxidant capacity observed was not only contributed by phenolic compounds, but could possibly be due to the presence of other constituents, such as ascorbic acid, tocopherol and pigments, as well as synergistic effects among them, which also contribute to the total antioxidant capacity [19]. Similar results were obtained by other authors from leafy vegetables [14]. Spectroscopic determination of TPC was positively correlated ($r^2$ 0.97) with the summation of the individual polyphenols as examined by HPLC-DAD.

**Table 1**: Hydroxybenzoic acid, hydroxycinnamic acid, flavone, polymethoxylated flavone, glycosylated flavonoid and anthocyanin contents of selected Brassica vegetables

| | HBA GAE/g (dw) of extract | HCA CAE/g (dw) of extract | Flavones RE/g (dw) of extract | PMF RE/g (dw) of extract | GSF RE/g (dw) of extract | Anthocyanins Cn3GE /g (dw) of extract |
|---|---|---|---|---|---|---|
| York cabbage | 1.67 ± 0.01[a] | 6.96 ± 0.01[a] | 7.84 ± 0.02 | 0.24 ± 0.01[a] | 0.25 ± 0.01 | ND |
| Broccoli | 8.15 ± 0.02[b] | 1.26 ± 0.01[b] | ND | 1.40 ± 0.68[b] | ND | ND |
| Brussels sprouts | 3.77 ± 0.09[c] | 3.45 ± 0.12[c] | ND | 2.35 ± 0.02[c] | ND | ND |
| White cabbage | 4.86 ± 0.43[d] | 0.57 ± 0.01[b] | ND | ND | ND | ND |

Data are expressed as mean ± SD (n=2).
HBA Hydroxybenzoic acid, HCA Hydroxycinnamic acid, PMF Polymethoxylated flavones, GSF Glycosylated flavonoid.
Means not sharing the same letter are significantly different (LSD) at $P < 0.05$ probability level in each row.

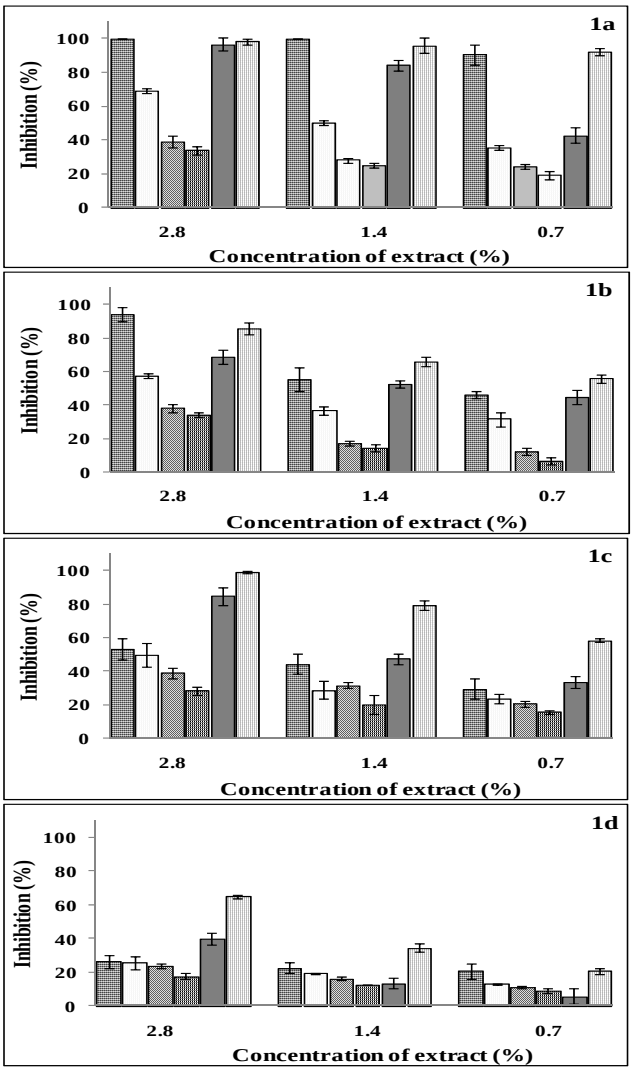


**Figure 1**: Comparative study of antibacterial activities of the first three dilutions for York cabbage (▦), broccoli (□), Brussels sprouts (▨) and white cabbage (▩) extracts with sodium benzoate (■) and sodium nitrite (▤) in different concentrations against (3a) *L. monocytogenes*, (3b) *S. abony*, (3c) *P. aeruginosa* and (3d) *E. faecalis*. (All the values are means ± SD of three parallel experiments in duplicate)

In conclusion, the present study showed that the Irish Brassica vegetable extracts are rich in polyphenols and have an appreciable amount of antioxidant and antibacterial properties and may be exploited as bio-preservatives in food applications and as neutraceuticals.

**Table 2**: Correlation matrix showing relationship between Brassica vegetables polyphenols and antioxidant capacity

| | DPPH | FIC | FRAP | H202 | LPO |
|---|---|---|---|---|---|
| TPC | -0.7113 | -0.7303 | 0.3570 | -0.8216 | -0.7282 |
| | (0.2887) | (0.2697) | (0.6430) | (0.1784) | (0.2718) |
| TFC | -0.7483 | -0.9572 | -0.0737 | -0.9890 | -0.9419 |
| | (0.2517) | (0.0428) | (0.9261) | (0.0110) | (0.0581) |
| HBA | -0.2430 | 0.0349 | -0.7868 | 0.1550 | 0.3420 |
| | (0.7570) | (0.9651) | (0.2132) | (0.8450) | (0.6580) |
| HCA | -0.2752 | -0.5512 | 0.5214 | -0.6551 | -0.7421 |
| | (0.7248) | (0.4488) | (0.4786) | (0.3449) | (0.2579) |
| Flavones | -0.4797 | -0.4980 | 0.6287 | -0.6205 | -0.5787 |
| | (0.5203) | (0.5020) | (0.3713) | (0.3795) | (0.4213) |
| PMF | -0.4797 | -0.4980 | -0.6287 | -0.6205 | -0.5787 |
| | (0.5203) | (0.5020) | (0.3713) | (0.3795) | (0.4213) |
| GSF | 0.1622 | -0.3521 | -0.7728 | -0.2395 | -0.4281 |
| | (0.8378) | (0.6479) | (0.2272) | (0.7605) | (0.5719) |

Value in bracket denotes *P* –Value

Further investigation can be carried out identifying and quantifying individual polyphenolic compounds and analysis of their antioxidant and antibacterial properties.

## Experimental

***Plant material and their preparation:*** Fresh Irish Brassica vegetables (York cabbage, white cabbage, broccoli and Brussels sprouts) were purchased from a local supermarket in Dublin. For the cabbage, immediately after purchase, the outer leaves were removed, the heads divided into 4 segments, and the central core removed. The segments were chopped into 0.5 × 5-6 cm pieces, using a vegetable cutting machine. For Brussels sprouts, most external leaves and 2 mm of the lower ends were removed, and the remaining portion was used for the analysis, whereas in the case of broccoli, the heads were obtained by cutting the main stalk. The florets, together with about 1 cm of the stalk, were cut off from the rest of the stalk and used as broccoli samples. Finally, the above prepared samples were crushed in a mortar and pestle in the presence of liquid nitrogen and stored at -20°C until used.

***Preparation of extracts:*** Extraction of phenolic compounds was carried out according to the method of Ganesan *et al.* [20]. Briefly, 5 g of crushed vegetable samples were added to 3 different flasks and extracted with 60% methanol with 1 min of nitrogen flushing at 20 psi. Flasks were kept in a shaking incubator (Innova 42, Mason Technology, Ireland) at 100 rpm and 40°C for 2 h. The infusions were filtered through Whatman #1 until a clear

extract was obtained. The extracts were evaporated to dryness in a multi evaporator (Syncore Polyvap, Mason Technology, Ireland) at 60°C at their respective pressure and stored at -20°C until used.

***Determination of total polyphenolic content:*** Total polyphenolic content (TPC) of vegetable extracts was determined by the method of Ganesan *et al.* [20] using Folin-Ciocalteau's phenol reagent (Sigma-Aldrich, Germany). Results were expressed as gallic acid (Sigma-Aldrich, Germany) equivalents per g (GAE/g) of extract (dw) through the calibration curve of gallic acid.

***HPLC-DAD analysis of polyphenolic compounds:*** The HPLC system consisted of a reversed-phase HPLC column on an Alliance HPLC (Waters, e2695 Separations modules) equipped with an auto sampler and controller with dual pump, a 2998 photodiode array detector (PDA) and Empower software. An Atlantis C18 column (250 mm × 4.6 mm, 5 µm particle size) from Waters (Waters, Milford, MA) was used for polyphenolic separation at 25ºC. The mobile phase was 6% acetic acid in 2 mM/L sodium acetate (Sigma-Aldrich, Germany) (Solvent A) and acetonitrile (Fischer Scientific, UK) (Solvent B). The system was run with a gradient program. The solvent gradient was as follows: 0-15% B in 45 min, 15-30% B in 15 min, 30-50% B in 5 min and 50-100% B in 5 min. A flow rate of 1 mL/min was used and the total run time for samples was 70 min. Samples and mobile phases were filtered through a 0.22 µm Millipore filter (Millipore, Bedford, MA) prior to HPLC injection and 20 µL of sample was injected. The chromatograms were monitored at 280 nm (hydroxybenzoic acid), 320 nm (hydroxycinnamic acids), 360 nm (flavones and flavonols) and 520 nm (anthocyanins); and complete spectral data were recorded in the range of 220-600 nm.

***Determination of total flavonoid content:*** The total flavonoid content (TFC) was determined according to the method of Liu *et al.* [21] and results were expressed as quercetin (Sigma-Aldrich, Germany) equivalents per g (QE/g) of extract (dw).

### *Antioxidant capacity analysis*

In the present study five different methods [2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity, Ferric reducing antioxidant potential (FRAP) assay, Lipid peroxidation in a hemoglobin-induced linoleic acid system (LPO), Ferrous ion chelating capacity (FIC) and Hydrogen peroxide ($H_2O_2$) scavenging assay] were used for the estimation of total antioxidant capacity of the Brassica vegetables. All the methods were carried out according to the existing protocols in our laboratory [22]. For the DPPH free radical scavenging capacity and lipid peroxidation inhibitory ability, ascorbic acid was used as a reference compound and the results were expressed as mg ascorbic acid equivalents per gram (AscE/g) (dw) of extract. Trolox (Sigma-Aldrich, Steinheim, Germany) was used as a standard for FRAP assay and the results were expressed as mg trolox equivalents per gram (TE/g) (dw) of extract; BHT was used as a reference compound for $H_2O_2$ scavenging capacity and the results were expressed as mg Butylated hydroxytoluene (BHT) equivalents per gram (BHTE/g) (dw) of extract whereas Ethylenediaminetetraacetic acid (EDTA) was used as a reference compound for FIC and the results were expressed as mg EDTA equivalents per gram (BHTE/g) (dw) of extract.

### *Antibacterial activity analysis*

***Bacterial strains and growth conditions:*** The bacterial strains used in this study included Gram-negative (*Salmonella abony* NCTC 6017, *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive (*Listeria monocytogenes* ATCC 19115, *Enterococcus faecalis* ATCC 7080) (Medical Supply Company, Dublin, Ireland) bacteria. All the cultures were maintained at -70ºC in 20% glycerol stocks and grown in Tryptic Soy Broth (TSB, pH 7.2, Scharlau Chemie, Spain) at 37°C, excluding *P. aeruginosa,* which was grown at 30°C for 18 h to obtain sub-cultures. To obtain a working concentration, a bacterial suspension was prepared in NaCl, 0.85% (BioMerieux, France) equivalent to McFarland standard 0.5 with the help of a Densimat photometer (BioMerieux, France). Finally, the suspension was diluted with TSB in order to get a concentration of $1\times10^6$ cfu/mL.

***Antibacterial activity assay:*** The antibacterial assay was carried out through a microtiter well method according to the protocol available in our laboratory [23]. Vegetable extracts were dissolved in TSB media and 200 µL was added to the first row of the plate. The first well of each row had the highest concentration of the tested extracts (2.8%). Serial dilutions along each column were made with the addition of 100 µL TSB. Test bacteria (100 µL) from the $10^6$ CFU/mL suspensions were added to all the wells. Wells containing cabbage extracts (100µL) and sterile TSB (100 µL) were treated as a negative control (sample blanks), while control wells contained sterile TSB (100 µL) and bacterial suspension (100 µL). One well of the microtiter plate containing sterile TSB (200 µL) served as a blank to check for contamination. The readings were taken immediately after the plate was inoculated with culture by a microtiter plate reader at 600 nm with 10 sec agitation before the measurement of optical density. The microtiter plates were incubated without agitation at 37°C for all the test organisms except *P. aeruginosa*, which was incubated at 30°C and the readings were taken after 24 h. Sodium benzoate (Sigma-Aldrich, Germany) and sodium nitrite (Sigma-Aldrich, Germany) were taken as positive controls. The antibacterial activity was determined in terms of percentage of inhibition calculated by the following formula.

$$I\% = \frac{(C_{24} - C_0) - (T_{24} - T_0)}{C_{24} - C_0} \times 100$$

where $I$ is the percentage inhibition of growth, $C_{24}$ is the blank compensated $OD_{600}$ of the control of the organism at 24 h, $C_0$ is the blank compensated $OD_{600}$ of the control of the organism at 0 h, $T_{24}$ is the negative control compensated $OD_{600}$ of the organism in the presence of test extract at 24 h and $T_0$ is the negative control compensated $OD_{600}$ of the organism in the presence of test sample at 0 h.

***Statistical analysis:*** All experiments were conducted in triplicate and replicated at least twice, unless otherwise stated. Results are expressed as mean values ± standard deviation. Analysis of variance (ANOVA) and multiple comparisons (Fisher's least-significant-difference test) were used to evaluate the significant difference among various treatments using the STATGRAPHICS Centurion XV. Differences at $P < 0.05$ were considered to be significant.

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