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A Comparative Study on the Active Constituents, Antioxidant Capacity and Anti-Cancer Activity of *Cruciferous* Vegetable Residues

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Abstract:

This study is pointed out to estimate the effectiveness of two solvents in the extraction and evaluating the active ingredients and their antioxidant activity as well as anti-cancer efficiency. Therefore, residues from four different *Brassica* vegetables viz. broccoli, Brussels sprout, cauliflower, and red cherry radish were extracted using two procedures methods: methanolic and water crude extracts. Methanol extracts showed the highest content of total phenolic (TP), total flavonoids (TF), and total tannins (TT) for broccoli and Brussels sprouts residues. Methanolic extract of broccoli and Brussels sprouts residues showed the highest DPPH· scavenging activity (IC_{50} = 15.39 and 18.64 µg/ml). The methanol and water extracts of Brussels sprout residues showed the highest chelating activity (IC_{50} = 11.77 and 5.94 µg/ml) and exhibited the highest reducing power (EC_{50} =14.38 and 20.18 µg/ml) with broccoli respectively. The HPLC analysis of phenolic compounds confirmed that the methanol extract of all the residues examined possessed high amounts of catchine, rutin, cumaric, benzoic, and luteolin. The methanol extract at 100 µg/ml of Brussels sprouts residues displayed a rise cytotoxic effect on HePG2 (80.40%), MCF7 (75.49%) and HCT116 (22.74%) followed by broccoli and red cherry radish, respectively. This result confirmed that Brussels sprouts residue contain effective chemical compounds that can inhibit the proliferation of cancer cells. Therefore, these results proposed that those *Brassica* vegetable residues might be beneficial as a potent antioxidant and anticancer agents and strongly recommended as fixing in constituent's food applications and pharmaceutical industries.

Keywords: Antioxidant, *Brassica* vegetable residues, Cytotoxic effect, HPLC, Phenolics.

Introduction:

For a long time, vegetables have been an important dietary source of natural antioxidants. Cruciferous vegetables are "vegetables of the *Brassicaceae* family. *Brassica* vegetables have medicinal, pharmaceutical as antioxidant and anticancer properties that are beneficial to the general human health, due to their content of diverse and widely useful natural compounds such as secondary metabolite compounds include phenols, flavonoids, terpenes, vitamins (1,2), and plant pigments which include carotenoids (3), anthocyanins, lycopene, tocopherol, etc. (4,5). Vegetable residue extracts contain compounds that have the restraining effects of free radicals. Antioxidants in vegetables protect against the serious effects of free radicals by reducing and curbing oxidative reactions. Natural antioxidant compounds reduce oxidative damage to the cell, and therefore they protect from many diseases for example cancer diseases (6). Cancer is one of the most serious problems for the general health of humans causing high mortality rates worldwide. Increased feeding on cruciferous vegetables, such as broccoli, cabbage, and cauliflower, is associated with positive inhibitory effects of cancer cell growth (7, 8). The inquiry of the underlying cellular mechanisms and the identification of dietary compounds that may exert anti-inflammatory and chemopreventive actions is an important future challenge for improving cancer prevention. Therefore, *Brassica* vegetable residues are important cheap sources of some phytochemicals that have potency as an antioxidant, and anticancer activity. For this purpose, many industrial vegetable residues were studied as a safe source of natural antioxidants such as phenols. Plant phenols play an

important role in protecting against lipid oxidation at the cellular level (9). There are many factors affecting the content of antioxidant compounds in *Brassica* vegetables including cultivars, agriculture conditions, agriculture practices and type of fertilization. Regarding these conditions, the previous study indicated that there is a good margin for enhancing antioxidant compounds of broccoli for economic production using organic fertilization. Also, they indicated the potential application of broccoli as a potent natural source of antioxidants as nutraceuticals (10). This increased interest has led to the discovery of antioxidants compounds in many vegetable residues to be an important and rich source of phytochemical compounds (9). Thus, the objective of this research was to evaluate the efficiency of different solvent extracts on the contents of phytochemicals and their antioxidants and anticancer activities in four *Brassica* vegetable residues. Further, *in-vivo* anticancer studies will be done.

Material and Methods:

Preparation of samples

Cruciferous (*Brassicaceae*) vegetable residues, broccoli (*Brassica oleracea* var. *Italica*), Brussels sprout (*Brassica oleracea* var. *gemmifera*), cauliflower (*Brassica oleracea* var. *botrytis*) and red cherry radish (*Raphanus sativus*) were obtained from the local market in Giza- Egypt (Fig. 1). *Cruciferous* residues were air-dried at room temperature (14 days) and dried at 40 °C in an air-circulation oven for 12-48 h, ground in a knife mill, and vacuum-packed and stored at – 4 °C until analysis.

Preparation of extracts

The dried powder (100 g) of various vegetable residues was soaked separately in two solvents (1 liter): distilled water and methanol (3 times), at room temperature using a shaker (Stewart, orbital shaker SSL1, UK) at 140 rpm for 48 hours. Whatman No.1 was used to filter the mixture. at room temperature using a shaker (Stuart, Orbital shaker SSL1, UK) at 140 rpm for 48 h. Whatman No.1 was used to filtrate the mixture. The supernatant was dried at 40 °C in a rotary evaporator under vacuum. The dried extracts were stored in the refrigerator until used.

Chemical studies

Total phenolic

The total phenolic (TP) of *Brassica* vegetable residue extracts was determined by Folin Ciocalteu reagent assay at 750 nm by spectrophotometer (Unicum UV 300), using Gallic acid as a standard (11). Total phenolics were expressed as mg Gallic acid equivalents (GAE)/g dry weight. Samples were analyzed in triplicates.

Samples	Whole plant	Edible parts	Residues
Broccoli (*Brassica oleracea* var. *Italica*)			
Brussels sprout (*Brassica oleracea* var. *gemmifera*)			
Cauliflower (*Brassica oleracea* var. *botrytis*)			
Red cherry radish (*Raphanus sativus*)			

Figure 1. Different parts of Cruciferous (*Brassicaceae*) vegetables.

Total flavonoid

Total flavonoid (TF) of *Brassica* vegetable residue extracts was determined by the aluminum chloride method at 510 nm by spectrophotometer (Unicum UV 300), using quercetin as a standard (12). Total flavonoids were expressed as mg quercetin equivalents (QE)/ g dry weight.

Total tannins

Total tannin (TT) of *Brassica* vegetable residue extracts was measured by Folin Ciocalteu`s reagent at 775 nm by spectrophotometer (Unicum UV 300), using tannic acid as a standard (13). Total tannins were expressed as mg tannic acid equivalent (TAE)/g dry weight.

Ascorbic Acid Determination:

The ascorbic acid content in fresh *Brassicaceae* vegetable residues was measured colorimetrically assay by spectrophotometer (Unicum UV 300), according to (14).

Identification and quantitation of phenolic compounds by HPLC

The dried crude methanolic extract (10 mg) of *Brassica* vegetable residues were dissolved in 2 ml methanol. HPLC spectral grade by vortex mixing for 30 min. The HPLC system is Agilent 1100 series coupled with a DAD detector following the method of (15). Sample injections of 5 µl were made from auto-sampler. The chromatographic separations were performed on a C18 column (4.6×250 mm, particle size 5 µm). A constant flow rate of 1 ml/min was used with mobile phases: (A) 0.5% acetic acid in distilled water at pH 2.65; and solvent (B) 0.5% acetic acid in 99.5% acetonitrile. The elution gradient was linear starting with A and ending with B over 50 min, using a DAD detector set at wavelength 280 nm. The results expressed as mg phenolic/100 g dry weight.

Antioxidant activity

Preparation of extracts:

The dried crude methanolic and water extracts (10 mg) of *Brassica* residues were dissolved in 10 ml methanol by vortex mixing for 30 min. for all assays.

DPPH· Free radical scavenging assay

Determination of DPPH· (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of vegetable residues extracts at different concentrations (25, 50, 75, 100 µg/ml) was measured spectrophotometrically (Unicum UV 300) at 515 nm according to (16). The capacity to scavenge the DPPH· radical was calculated using the following equation:

DPPH· scavenging activity %

$$= [(Ac - As (/ Ac] \times 100$$

Where: (Ac) was the absorbance of the control reaction and (As) the absorbance in the presence of the extracts. The results were expressed as IC_{50} (the concentration µg/ml of the grape pomace extracts that scavenge 50 % of DPPH· radical).

Chelating activity on Fe^{2+}

Chelating activities on ferrous ions of vegetable residues extracts or EDTA solution as a positive control at different concentrations (25, 50, 75, 100 µg/ml) was carried out spectrophotometrically (Unicum UV 300) at 562 nm according to (17). The chelating ability was calculated using the following equation:

Chelating activity (Inhibition %)

$$= [(Ac - As) / Ac] \times 100$$

Where: (Ac) was the absorbance of the control reaction and (As) the absorbance in the presence of the plant extracts. The results were expressed as IC_{50} (the concentration µg/ml of the *Brassica* residues extracts that.

Reducing power

The reducing power of vegetable residues extracts at different concentrations (25, 50, 75, 100 µg/ml) was assayed spectrophotometrically (Unicum UV 300) (18). The results were expressed as EC_{50} (the concentration µg/ml of the *Brassica* residues extracts that provided the reading of 0.5 absorbance at 700 nm).

Biological studies

Cytotoxic effect on the human cancer cell line (liver HePG2 – Breast MCF7 – Colon HCT116)

Three human carcinoma cell lines, (HePG2), (MCF-7) and HCT116) were obtained from the Karolinska Institute, Stockholm, Sweden. All cells were maintained in DMEM medium (Lonza Biowahittkar, Belgium). Media were supplemented with 1% antibiotic-antimycotic mixture (10,000 U/ml potassium penicillin, 10,000 µg/ml streptomycin sulphate, 25 µg/ml amphotericin B and 1% L-glutamine).

MTT assay

Cell viability was investigated using MTT [3-(4, 5- dimethylthiazol – 2 – yl) – 2 , 5-diphenyl-tetrazolium bromide] assay (19). Cell lines were incubated at 37 °C (Sheldon, USA). Cells were placed into 96-well microplates at a concentration of 10^4 cells/well and allowed to stand for 24 h. The medium was aspirated and fresh medium (without serum) was added to the cells with different concentrations (1.25 - 100 µg/ml) of methanolic and water extracts of *Brassica* residues dissolved in DMSO. After 48h incubation, the medium was aspirated and 40 µl MTT solution (2.5 µg/ml DMSO) was added to each well and incubated for further 4 h. The formazan crystals formed were dissolved and the reaction was stopped by adding 200 µl of 10% sodium dodecyl sulfate (SDS) to

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each well for overnight at 37°C. The amount of formazan produced was measured at 595 nm with a reference wavelength of 620 nm as a background using a microplate reader (Bio-Rad Laboratories, model 3350, USA). A positive control (Doxorubicin) which composed of 100 µg/ml was used as a known cytotoxic agent who gives 100% lethality under the same conditions (20). Data were expressed as growth inhibition (%) using the following formula: Growth inhibition (%)

$$= 100- (A_s/A_c) \times 100.$$

Where A_s is the absorbance of treated cells with extract, and A_c is the absorbance of untreated cells.

Statistical analysis

The data collected were subjected to analysis of variance (ANOVA) using Costat Statistical Analysis software version 6.303, Cohort, 2004, all analyses were done in triplicate and the averages were presented with their standard deviation. The differences between means were evaluated using least significant differences (LSD) at $P \leq 0.05$ (21).

Results and Discussion:

Chemical studies

Total phenolic

There are considerable major factors that affect total phenolic content 1- The polarity of the extracting solvent and 2- The solubility of chemical constituents in the solvent. As shown in Table 1, two solvent extracts have been used. The results revealed that methanol was better than the water extract in the extraction of phenolic compounds. The *Brassica* residues displayed the existence of significant amounts of phenolic. The methanol extract of broccoli residue was found to be the highest TP, while, the water extract of cauliflower residue showed the least. TP methanol extract of broccoli, Brussels sprout, cauliflower, and red cherry radish had 1.14, 1.15, 1.44 and 1.12 times higher TP than those of vegetable residues water extract, respectively. Broccoli methanol extract had 1.87, 1.79, and 1.69 times higher TP than those of Brussels sprout, cauliflower, and red radish methanol extracts. The phenolic contents in this study were similar to those notified formerly with (22). The amount of polyphenolic in this work is in agreement with previous studies (10,23). Phenolic compounds in *Brassica* vegetables appear to dissolve readily in methanol but in water extracts, they melt little and are compatible with other authors (24,25). TP of cabbage ranged between 110.2 to 153.3 mg per 100 g FW, while TP ranged from 133.4 to 140.13 mg for each 100 g (FW) for Brussels sprout (26).

Table 1. Phenol, flavonoids, tannins and vitamin C of *Brassica* Residues

Residue samples	Phenolic mg GAE/g DW		Flavonoids mg QE /g DW		Tannins mg TAE/g DW		Vitamin C mg/100g FW
	Methanol	Water	Methanol	Water	Methanol	Water	
Broccoli	21.47^{d} ± 0.07	18.91^{d} ± 0.05	16.96^{c} ± 0.14	14.94^{c} ± 0.07	5.51^{d} ± 0.03	4.11^{d} ± 0.02	72.4^{d} ± 0.23
Brussels sprouts	11.46^{a} ± 0.06	9.94^{b} ± 0.03	6.72^{b} ± 0.07	4.50^{b} ± 0.11	3.18^{a} ± 0.02	2.30^{b} ± 0.01	61.61^{c} ± 0.29
Cauliflower	12.02^{b} ± 0.06	8.32^{a} ± 0.04	5.31^{a} ± 0.09	3.26^{a} ± 0.17	3.36^{b} ± 0.02	1.66^{a} ± 0.02	50.91^{a} ± 0.33
Red cherry radish	12.65^{c} ± 0.05	11.29^{c} ± 0.06	5.22^{a} ± 0.15	4.37^{b} ± 0.14	3.62^{c} ± 0.01	2.96^{c} ± 0.01	52.78^{b} ± 0.14
LSD at 0.05	0.18	0.43	0.36	0.30	0.07	0.05	0.60

Results are mean values ± standard deviations (n=3). Means followed by the different letters in a column are significantly different ($P \leq 0.05$). GAE, gallic acid equivalents. QE, quercetin equivalent. TAE, tannic acid equivalents.

These results have shown that methanol is better than the water solvent in the extraction of phenolic compounds. Methanol extraction efficiency was the best for the extraction of phenolic compounds from the four *Brassica* residues, which could be due to the polarity that is suitable for phenolic compounds as well as the ability to dissolve phenolic compounds from within plant cells (27).

Total flavonoids

The estimated concentrations of flavonoids (16.96mg/g DW) were acquired from broccoli methanol extract had 2.52, 3.19, and 3.25 times higher TF than those of Brussels sprouts, cauliflower, and red radish, respectively as shown

in Table 1. All the vegetable residues showed the presence of considerable amounts of flavonoid. Broccoli residue methanol extract had a significantly ($P< 0.05$) higher amount of TF followed by Brussels sprouts, cauliflower, and red radish, respectively. The total flavonoid content of 11 vegetables from West Java, Indonesia were varied from 0.3 to 143 mg/100 g FW (28). Whilst, TF was ranged from 4.1 to 133.1 mg/100 g FW (29). The flavonoid content in this study corresponded well with those determined by others.

Total tannins

Phytochemicals and their derivatives are naturally present in vegetables and also have nutritional and medicinal benefits. Genus *Brassica* contains various phytochemicals like tannins, terpenoids, flavonoids, glycosides, and steroids (30). High-molecular-weight (>500) polyphenols are also known as plant tannins (31). The total tannins of vegetable residues revealed that significant diversity between two solvent extracts ($P \leq 0.05$) Table (1). The amount of TT observed in vegetable residues extracts ranged from (3.18-5.51 mg/g DW) of methanol extracts and (1.66-4.11 mg/g DW) of water extracts, respectively. The greatest amount of tannins was obtained from Broccoli (methanol extract) higher than those of Brussels sprouts, cauliflower, and red radish, respectively.

3.1.4. Vitamin C

Epidemiological studies have shown a direct correlation between increased consumption of *Brassica* vegetables and protection against cancer. This protective effect is largely due to the presence of phytochemicals, found in *Brassica* vegetables, which include vitamins C and E, phenols, carotenoids, and glucosinolates (32). The highest concentration of vitamin C was determined in broccoli residues (72.40mg/100g FW). The lowest was recorded for cauliflower (50.91 mg/100g FW). The results are compatible with studies showed that the total concentration of vitamin C ranged (64.87 to 44.25 mg /100g FW) of fresh red and white cabbage, and (18–129 mg /100g FW) of *Brassica* vegetables under study (33).

High-performance Identification of Phenolic components of *Brassica* vegetable residues

The HPLC analysis of methanol extracts of *Brassica* vegetable residues showed in Table 2. The methanolic extract was used for HPLC fractionation to increase its content of phenolic compounds compared to the water extract.

Table 2. HPLC profile of phenolic compounds of *Brassica* residues extracted by methanol

Phenolics mg/100g DW	Broccoli	Brussel sprouts	Cauliflower	Red cherry radish
Pyrogallol	7.39	-	-	-
Gallic acid	1.83	0.23	0.37	10.22
Chlorogenic acid	0.47	0.29	3.24	0.20
Vanillic	0.35	1.15	0.35	0.49
Catechins	114.77	54.50	38.19	17.79
Caffeic acid	1.26	2.51	3.83	0.24
Rutin	3.83	2.08	1.61	10.27
P-Coumaric acid	2.53	1.26	1.67	12.44
Ferulic	2.44	2.10	2.03	1.72
Benzoic	12.14	9.11	4.47	1.47
Acacetin	4.72	0.78	3.65	2.37
Myricetine	1.06	0.47	-	1.27
Coumarin	0.42	0.80	0.64	0.37
Luteolin	7.79	5.26	4.20	3.71
Quercetin	21.47	-	-	-
Cinnamic acid	0.29	0.44	0.29	0.31
Genistein	2.23	0.42	-	0.60
Kaempferol	0.50	4.31	3.21	2.77

Moreover, the results obtained from HPLC confirmed the similarity with the total estimates of phenols, flavonoids, and tannins. Methanolic extract of broccoli and Brussels sprout displayed higher content in most phenolic compounds predominately catechins, benzoic, and luteolin, respectively. Whereas, Methanolic extract of cauliflower and red cherry radish demonstrated lower content in phenolic compounds principally in vanillic, coumarin, and cinnamic acid, respectively. Data shown in (Table 2) reveals that methanol extract of all tested residues possesses the elevated amounts of

catechins, quercetin, benzoic, luteolin, and pyrogallol acids. It has been noted that pyrogallol and quercetin not perceptible in Brussels sprout, cauliflower, and red radish, respectively. In the previous study, it was confirmed that the content of total phenolics and flavonoids depends on the type, concentration, and degree of polarity of the solvent used (34).

Antioxidant activity

In the existing research three distinct methods (DPPH·) free radical scavenging capability, chelating activity on Fe^{2+}, and power reducing assay were deliberated.

Scavenging capability assay (DPPH·) on the rebating of the steady radical

The DPPH· is a common method for the determination of antioxidants in plant extracts due to their accuracy and ease of application. In addition to being a free radical, DPPH· is a chemically stable compound during the estimation and economical (35,36). The highest antioxidant scavenging activity of plant extracts against synthetic DPPH· free radical means the lowest amount IC_{50} (Inhibition concentration fifty for DPPH· free radical *in-vitro*). Oxidative stress causes many damages to the human body, which leads to many diseases and can be prevented by consuming foods rich in natural antioxidants such as vegetables and fruits (37). Antioxidants can intervene with the oxidation process by interacting with free radicals, chelating catalytic metals, and also by acting as oxygen scavengers. The results of the radical scavenging activity of two extracts of *Brassica* vegetable residues using DPPH· assays compared to synthetic antioxidants (BHT) are shown in Table 3.

Table 3. IC_{50} of *Brassica* vegetable residues extracts against DPPH· radical

Residue samples	IC_{50} µg/ml	
	Methanolic	Water
Broccoli	$15.39^{e} \pm 0.05$	$26.32^{b} \pm 0.09$
Brussels sprouts	$18.64^{b} \pm 0.12$	$52.66^{d} \pm 0.42$
Cauliflower	$26.59^{d} \pm 0.12$	$35.68^{c} \pm 0.13$
Red cherry radish	$22.10^{c} \pm 0.08$	$56.51^{e} \pm 0.43$
BHT	$3.30^{a} \pm 0.08$	$3.30^{a} \pm 0.08$
LSD at 0.05	0.24	0.61

Results are mean values ± standard deviations (n=3). Means followed by the different letters in a column are significantly different ($P \leq 0.05$).

The highest scavenging activity expressed as IC_{50} appeared with methanolic extract of broccoli and Brussels residues (15.39 and 18.64 µg/ml), while the water extract of cauliflower and red cherry radish residues presented the lowest scavenging ability (35.68 and 56.51 µg/ml), the high activity of antioxidant may be due to the fact that broccoli and Brussels sprouts residues have the greatest amount of phenolics, which increase their efficiency to give the hydrogen ion to restrain free radicals. Our results are in accordance with (22) who notified that antioxidant activity varied and differed based on the type and polarity of the solvent used in extracting vegetable residues, they found that the antioxidant activity of the ethane extract was relatively higher. These results are consistent with the results of peanut residues (skin and peel) extracts (38).

Chelating activity on Fe^{2+}

During food processing, some foods may be contaminated by the transfer of iron ions or other minerals to processed or preserved foods. These ions play an important role as initiators of the processes of oxidation and formation of free radicals (39). Contamination by iron ions or other metals can be reduced by the addition of plant extracts or other compounds that have the potential to chelate iron or other metals. All the vegetable extracts possess noteworthy ferrous particle chelating limit, however, in all states, it was all together ($P<0.05$) lower than that of EDTA. The results obtained were shown in Table 4.

Table 4. IC_{50} of *Brassica* vegetable residue extracts as chelating of ferrous ion

Residue samples	IC_{50} µg/ml	
	Methanolic	Water
Broccoli	$23.11^{d} \pm 0.06$	$16.16^{d} \pm 0.09$
Brussels sprouts	$11.77^{b} \pm 0.08$	$5.94^{b} \pm 0.11$
Cauliflower	$17.24^{c} \pm 0.09$	$9.43^{c} \pm 0.19$
Red cherry radish	$25.27^{e} \pm 0.18$	$17.62^{e} \pm 0.05$
EDTA	$2.43^{a} \pm 0.16$	$2.43^{a} \pm 0.16$
LSD at 0.05	0.23	0.21

Results are mean values ± standard deviations (n=3). Means followed by the different letters in a column are significantly different ($P \leq 0.05$).

Amongst the vegetable by-product extracts which conferred the highest Fe^{2+}-chelating capacity was established in Brussels sprouts water extract (5.94 µg/ml) and methanolic (11.77 µg/ml), followed by cauliflower (9.43 and 17.24 µg/ml, respectively). The lowest activity was found of red cherry radish (17.62 µg/ml) for water extract and (25.27 µg/ml) for methanolic extract. The process of iron casting depends on the concentration of both the extract and the EDTA. The absorbance value of the Fe^{2+}-ferrozine complex is linearly reduced by increasing the ability of Brussels sprouts and cauliflower extracts on the iron chelation expressed as IC_{50}. Some studies propose that there is no

correlation between increased phenolic content and antioxidant efficiency, which has been demonstrated by several factors including the presence of other effective compounds and the synergistic effects. These results can be explained as vegetable residues contain high levels of phenolics, flavonoids, and strong antioxidant compounds such as sulfur compounds (glucosinolates), as previously reported by (40).

Reducing power

The ability of the active chemical compounds in plant extracts in the test solution to reduce ferric in the Fe^{3+}/ferricyanide complex to Fe^{2+}/ferrous can be measured by measuring Perl's Prussian blue color at 700 nm, which acts as a potential indicator of their ability as antioxidants (41). Thus, the reducing ability of a compound is a significant indicator of its potential antioxidant activity. The methanol and water extracts of the four *Brassica* residues show a clear concentration-dependent reduction ability that corresponds to the total content of phenols, flavonoids, and tannins (Table 1). Broccoli methanol extract was significantly higher than those of the remaining residues (Table 5). It was observed that methanol extract and water extract of cauliflower possess the least reducing power. Solvent has low density, viscosity, and high diffusivity that lead to facilely prevalent, in-plant pores materials to make their way out the bioactive matters (42).

Table 5. Reducing power activity of *Brassica* vegetable residues extracts

Residue samples	EC_{50} µg/ml	
	Methanolic	Water
Broccoli	$14.38^{b} \pm 0.03$	$20.18^{b} \pm 0.08$
Brussels sprouts	$21.48^{c} \pm 0.05$	$28.32^{c} \pm 0.50$
Cauliflower	$32.62^{e} \pm 0.12$	$50.68^{e} \pm 0.27$
Red cherry radish	$26.96^{d} \pm 0.07$	$37.81^{d} \pm 0.18$
BHT	$3.29^{a} \pm 0.14$	$3.29^{a} \pm 0.14$
LSD at 0.05	0.19	0.50

Results are mean values ± standard deviations (n=3). Means followed by the different letters in a column are significantly different ($P \leq 0.05$).

The high reducing ability at low concentrations of the used extracts referred to high antioxidant activity. Regarding this concept, Broccoli and Brussels sprout residues showed reducing power close to that of BHT. Previous studies have notified that the reducing power of bioactive compounds was linked with antioxidant activity (43,44). Thus, to elucidate the relationship between the antioxidant effects of selected vegetable residues and their reducing power it is necessary to determine their reducing power. Based on the results with this study, the extract with the maximum antioxidant activity had the highest concentration of phenols and had significant antioxidant activity since vegetables are powerful sources of bioactive compounds (45). So, the vegetable residues recycling and the utilization of such residues will be helpful for avoiding environmental pollution (46,47).

Biological studies

Cytotoxic effects

This study is an attempt to examine the *Brassica* vegetable residues extracts for their cytotoxic effect against different human cancer cell lines liver HepG2, breast MCF-7, and colon HCT116 compared with doxorubicin as control positive drug at 100 µg/ml, as shown in Table (6). From the results, it is evident that Brussels sprouts methanol extract displayed potent growth inhibitory activity against cancer cell line of liver HepG2, breast MCF-7, and colon HCT 116 followed by broccoli, cauliflower, and red cherry radish, respectively. The remarkable cytotoxic activity of methanolic extract of Brussel sprouts and broccoli could be a result of their high contents of phenolics, flavonoids, and tannins (Table 1,2) and this is also confirmed by their high antioxidant activities (Table 3,4,5). There are previous studies confirm our results and the cytotoxic effect of *Brassica* vegetable extracts against the proliferation of cancer cell lines. Four cruciferous vegetables cabbage, cauliflower, kohlrabi, and radish showed antiproliferative activity expressed as IC50 against three cell lines (MCF7, DL, and NIH-3T3), cabbage (92.5, 189.7, 589.7 µg/ml) followed by cauliflower (378.7, 398.9, 597.9 µg/ml), kohlrabi (389.5, 396.9, 619.7 µg/ml) and radish (415.4, 423.3, 703.6 µg/ml), respectively by MTT assay (48). Furthermore, the water extract of green and red cabbage exhibited the cytotoxic effect (as IC_{50}) on MCF 7 cell lines 176, 40 µg/ml, and 1125, 342 µg/ml at 24 and 48 h, respectively by using Alamar Blue Assay (49). The leaves of different cultivars of broccoli by-products (Kyoyoshi, Myeongil 96, and SK3-085) possess the highest Inhibition activity against (NCI-H1299) lung cancer cell line followed by florets, leaf stems and stems (50). Additionally, the antiproliferation effects of purple Kohlrabi peel (*Brassica oleracea* var. *gongylodes*) on human cancer cells (HepG2 liver, HCT-116 colons, and A549 lung cancer cells) were investigated in a dose-dependent manner. The antiproliferation activity of Kohlrabi extracts exceeded by 40% in colon cancer cells. These results indicated that Kohlrabi may contain bioactive compounds such as flavonoids that may facilitate cancer prevention (51).

Table 6. Cytotoxicity effect of *Brassica* Residue extracts

Residue samples	Remarks % at 100 ppm					
	HePG2		MCF7		HCT116	
	Methanol	Water	Methanol	Water	Methanol	Water
Broccoli	42.82	25.44	60.43	39.46	31.28	28.37
Brussels sprouts	80.42	51.28	75.94	45.87	22.74	11.52
Cauliflower	15.81	15.73	22.45	18.61	23.43	12.58
Red cherry radish	37.56	13.79	29.16	19.82	26.79	22.72
Doxorubicin as a positive control	100 %					

The potency of *Brassica* vegetable residues extracts as a cytotoxic effect on different cancer cell may be mainly dedicated to the presence of phenolics, flavonoids, vitamins and sulfur compounds as glucosinolates (GLSs) secondary plant compounds which have functions in inhibition of the cancer cell development, and on the immune system as reported by (52,53). Previous bioactive ingredients inhibit oxidative stress, activates detoxification enzymes, stimulate the immune system, reduces the risk of cancer, inhibit malignant transformation and cancer-causing mutations, and minify the proliferation of cancer cells. These substances through the induction of enzymatic systems I and II phases of xenobiotics metabolism may affect the elimination or neutralization of cancer development and mutagenic factors of DNA (54). As a result, phenolic compounds may support the protective of chronic diseases, such as chronic inflammation, atherosclerosis, and cancer by delaying oxidative degradation and stimulating enzymes that detoxify carcinogens and also blocking the formation of cancer by inhibiting at least 30 types of agents that may cause cancer (52, 55, 56,). Based on the results obtained in this research on the scope *in-vitro*, we will try very hard to prove these results *in-vivo*.

Conclusions:

The results suggest that *Brassica* vegetable residues may be beneficial as a potent antioxidant and anticancer agents and effectively appointed as natural materials in constituent's food applications and pharmaceutical industries in addition to recycling residues to reduce the environmental pollution and improve human health.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given permission for re-publication attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in the National Research Centre, Egypt.

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دراسة مقارنة على المحتوي من المكونات الكيميائية النشطة، والقدرة المضادة للأكسدة والنشاط المضاد لنمو الخلايا للسرطانية المستخلصة من مخلفات الخضروات الصليبية

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الخلاصة:

أجريت هذه الدراسة لتقييم كفاءة وفعالية مخلفات أربعة أنواع من خضروات العائلة الصليبية (البراسيكا) تم ستخلاصهم بمذيبين (الميانولي - المائي) وهم كاتالي البروكولي ، كرنب بروكسل ، القرنبيط ، الفجل الأحمر. وتم تقييم المستخلصات من حيث محتواها الكلي من المركبات الفعالة مثل الفينولات و الفلافونيدات والتانينات وفيتامين سي واتعرف عليها بالـ HPLC ودراسة نشاطاتها المضادة للأكسدة بأكثر من طريقة مثل الـ ·DPPH والـ Fe^{2+}-chelating والـ Reducing power وكذلك دراسة كفاءتها في كبح جماح ومنع إنتشار وتكاثر ثلاثة أنواع من الخلايا السرطانية للإنسان علي النطاق المعملي مثل خلايا سرطان الكبد HePG2 و خلايا سرطان القولون HTC116 وخلايا سرطان الثدي MCF7. أظهرت النتائج أن المستخلص الميثانولي لمخلفات البروكولي وكرنب بروكسيل أعطي أعلي محتوي من المركبات الفعالة و أعلي نشاط مضاد للأكسدة في كبح جماح الشق الحر الـ ·DPPH و وأعلي قدرات إختزلية ضد الحديديك الـ Reducing power. بينما أظهر المستخلص المائي لكل من كرنب بروكسيل والقرنبيط اعلى نشاط في عملية خلب الحديدوز الـ Fe^{2+}-chelating. أكد تحليل HPLCللتعرف علي المركبات الفينولية الموجودة في مستخلصات مخلفات خضروات البراسيكا التي تم التعرف عليها أن المستخلص الميثانولي يمتلك كميات مرتفعة من الكاتشين والروتين والكيومماريك والبنزويك و الليتيولين. كما أظهر المستخلص الميثانولي لمخلفات كرنب بروكسيل تأثيراً مثبطاً و مانع للإنتشار وقاتل مع كافة أنواع الخلايا السرطانية وكان ذلك متبوعاً بالبروكولي والفجل الاحمر على التوالي. أكدت هذه النتائج أن مخلفات كرنب بروكسيل تحتوي على مركبات كيميائية فعالة يمكنها أن تمنع تكاثر الخلايا السرطانية. لذلك ، اقترحت هذه النتائج أن مخلفات خضروات البراسيكا يمكن إستخدامها كمضادات أكسدة قوية ومضادة لإنتشار الخلايا السرطانية وذلك في تطبيقات الصناعات الغذائية و الدوائية.

الكلمات المفتاحية: مضادات نمو الخلايا للسرطانية، مضادات الأكسدة، مخلفات خضروات العائلة الصليبية، HPLC.