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Original article

SOXHLET EXTRACTION CYCLE-DEPENDENT DIVERSITY IN PHENOLIC PROFILE AND ANTIOXIDANT POTENCY OF RED CABBAGE (BRASSICA OLERACEA VAR. CAPITATA F. RUBRA)

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Abstract

Introduction: The Brassicaceae vegetables are a rich source of secondary metabolites that exhibit several health benefits and protection against numerous degenerative diseases.

Objectives: The current study was performed to investigate the effect of Soxhlet extraction cycles on the phenolic profile of red cabbage (*Brassica oleracea* var. capitata *F. rubra*) and its biological activities.

Materials and Methods: The red cabbage sample was harvested from the research fields of Ayub Agricultural Research Institute Faisalabad, Pakistan. The ethanol extracts were prepared using 1-, 2-, 4-, and 8-cycles Soxhlet extraction technique. The antioxidant potential of red cabbage extracts was estimated by evaluation of total phenolic contents (TPC), total flavonoid contents (TFC), DPPH radical scavenging capacity and reducing power. The identification and quantification of polyphenols were carried out by RP-HPLC. The antibacterial activity of red cabbage extracts was determined against *Escherichia coli* and *Staphylococcus aureus* and the antiproliferative activity was carried out by MTT mitochondrial viability assay against the human A549 cancer cell line.

Results: Gallic acid, *p*-hydroxyl benzoic acid, chlorogenic acid, and *p*-coumaric acid were the major phenolic acids, whereas catechin and quercetin were the major flavonoids detected in the red cabbage extracts. The extraction cycles were found to have significant ($p \leq 0.05$) effects on the phenolic profile of the red cabbage extracts. TPC of extracts ranged from 5.22-11.72 mg/g dry matter, measured as gallic acid equivalent, while the TFC ranged from 1.64-5.19 mg/g dry matter; measured as catechin equivalent. The 4-cycles extract of red cabbage exhibited the maximum TPC, TFC, and DPPH free-radical scavenging and reducing activity.

Conclusion: The study concluded that the Soxhlet extraction cycles could exert a considerable effect on the yield and polyphenol composition of red cabbage extracts as well as their antioxidant potential. Antibacterial and antiproliferative activities were observed by all the extracts of red cabbage.

INTRODUCTION

Functional foods have considerable nutritional values and offer health benefits by protecting against oxidative stress and various diseases such as stomach ulcer, acid reflux, hemorrhoids, diabetes, heart diseases and cancer (Granato et al., 2020; Saeed et al., 2021). Polyphenols are organic compounds, found abundantly in plants, have become an emerging field of interest in nutrition in recent decades (Rad et al., 2020). Polyphenols may play a vital role in health through the regulation of metabolism, weight, chronic disease, and cell proliferation (Dominic et al., 2021; Iftikhar et al., 2022). Foods with high contents of polyphenols are considered as potential functional food (Rad et al., 2020; Iftikhar et al., 2022).

Isolation and extraction of polyphenols are influenced by several factors including extraction technique, solvent system, time, particle size, liquid to solid ratio and temperature (Zhang et al., 2018). Ideal extraction protocols should have the ability to ensure complete extraction of compounds of interest without any chemical moderation and degradation (Majeed et al., 2016). Soxhlet extraction is one of the most widely used extraction techniques for the extraction of natural products due to its efficiency, simplicity, cost-effectiveness, and reproducibility (Słomińska et al., 2012). However, as compared to other conventional techniques like shaking, maceration, boiling and stirring, Soxhlet extraction is an accelerated temperature extraction technique (Barnes et al., 2012). In Soxhlet extraction, several portions of the warm solvent are being passed by the sample of interest that may overheat the extracted compounds resulting in the degradation of heat labile compounds. To avoid the modification of compounds due to heat, single cycle Soxhlet extraction is also in practice. However, the yield of extracts in a single cycle is too low due to incomplete extraction. Therefore, optimization is needed for the extraction of all the bioactive components with optimized yield and without degradation (Laque et al., 2004).

The Brassicaceae vegetables are a reservoir of soluble fiber, vitamins, secondary metabolites, and phytochemicals that offer several health benefits and protection against numerous diseases. It was demonstrated by various studies that these vegetables are excellent sources of natural antioxidants as they produce polyphenols, tocopherols, and carotenoids (Poschner et al., 2019). Red cabbage (*Brassica oleracea* var. *capitata F. rubra*) is one of the cool season Cruciferous vegetables and known as red kraut, purple cabbage, and blue kraut (Burda et al., 2021). It contains different bioactive components such as vitamins A, B, and C, anthocyanins, isothiocyanates and excellent antioxidant activities (Zhang *et al*., 2021). Many studies revealed that red cabbage extract helps to minimize oxidative stress and exhibits anticancer, analgesic, and antibacterial activities (Drozdowska *et al*., 2020).

To the best of our knowledge, no study has ever been performed on Soxhlet extraction cycle-dependent diversity in the phenolic profile and antioxidant potential of red cabbage. This study aimed to develop the Soxhlet extraction processes (1-cycle, 2-cycles, 4-cycles, and 8-cycles) for the extraction of polyphenols from red cabbage, evaluation of antioxidant activity and antiproliferative activity. The identification and quantification of polyphenols were carried out by RP-HPLC. The antioxidant potential was estimated by various antioxidant assays including DPPH radical-scavenging capacity, evaluation of total phenolic (TPC), total flavonoid contents (TFC), and determination of the reducing power of obtained extracts. Red cabbage extracts also showed good antiproliferative activity against the human A549 cancer cell line and adequate antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*.

EXPERIMENTAL

Collection and pretreatment of plant material

The red cabbage (*Brassica oleracea* var. capitata *F. rubra*) was harvested from the experimental research area of Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan in January 2022. The authentication and identification of red cabbage sample was carried out by the taxonomist from the department of Botany, Government College University, Faisalabad, Pakistan. Edible leaves of red cabbage were washed, air dried under the shade at room temperature and ground to a fine powder (80-mesh) by a commercial electric grinder and stored in airtight polythene bags. All the chemicals used for this research work were of Analytical grade and purchased from Sigma Chemical Co. (St Louis, MO, USA) and Merck (Darmstadt, Germany).

Extraction of plant material

Cycle-dependent Soxhlet extraction such as 1-cycle, 2-cycles, 4-cycles, and 8-cycles was performed using the ethanol solvent as reported by Khanam *et al*. (2022). Extraction was carried out by taking 50 g fine powder (80-mesh) of red cabbage in a thimble of 500 mL Soxhlet extractor and 300 mL of absolute ethanol in its round bottom flask. Extracts obtained from three batched of each cycle were filtered by the filter paper Whattman filter paper (No. 1) and concentrated by vacuum rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd., Tokyo, Japan). The concentrated extracts were weighed to calculate the yield and stored at -4^{o}C.

Chromatographic analysis of extracts

All the red cabbage extracts (10 mg/mL) were analyzed for phenolic acids and flavonoids on High-Performance Liquid Chromatography (HPLC) as reported by Dominic *et al*. (2021). Chromera HPLC (Perkin Elmer, 520 South Main St., Suite 2423, Akron, OH, USA) equipped with C-18 column (250 × 4.6 mm internal diameter, 5 µm particle size) and UV/Vis LC detector was used. A non-linear gradient system containing two solvent systems like solvent A (methanol: acetonitrile; 30:70) and solvent B (distilled water and 0.5% glacial acetic acid and distilled water) was selected for separation. UV/Vis spectra were recorded at 275 nm. The identifications of compounds were done by matching the retention times with the standards whereas; quantifications were performed by spiking the known concentrations of authentic standards in the samples in standard addition method.

Estimation of total phenolic content (TPC)

Total phenolic contents of all the extracts of red cabbage were determined by the Folin Ciocalteu reagent as described by Dominic *et al*. (2021). Briefly, 0.5 mL of Folin-Ciocalteu reagent was mixed with 0.5 mL of extract solution (10 mg/mL ethanol) of red cabbage along with 7.5 mL distilled water and made the solution up to 10 mL. Then the mixture of each sample was kept at room temperature for 10 min and later 1.5 mL of sodium carbonate (20% w/v) was added to each solution. The mixture of all samples was then heated for 20 minutes at 40^{o}C in a water bath and then this mixture was cooled in an ice bath. The absorbance was recorded at 755 nm by UV/Vis spectrophotometer (Bio Tek Instrument, Inc., Winooski, VT, USA). TPC was calculated by comparing with the calibration curve of gallic acid (0.1, 0.2, 0.4, and 0.8 mg/mL) as a standard. The results of TPC were reported as mg/g of dry plant material, measured as gallic acid equivalent (GAE).

Estimation of total flavonoid content (TFC)

Total flavonoid contents of red cabbage extracts were measured by the procedure reported by Dominic *et al*. (2021). Each extract solution (1 mL) of red cabbage (10 mg/mL of dry material) and 5 mL distilled water was mixed in a volumetric flask followed by the addition of 0.3 mL of 5% $NaNO_2$. 0.5 mL of 10% $AlCl_3$ was added after 5 minutes in this mixture and then after another five minutes, 2 mL of 1 M NaOH was added and then distilled water was added to make the volume up to 10 mL. The absorbance was recorded at 510 nm by UV/Vis spectrophotometer (Bio Tek Instrument, Inc., Winooski, VT, USA). TFC was calculated by the standard calibration curve of catechin (0.1, 0.2, 0.4, 0.8 mg/mL). TFC results were expressed in mg/g of dry plant material, expressed as catechin equivalent (CE).

Measurement of DPPH radical scavenging capacity

Free-radical scavenging 2, 2-diphenyl, 1-picryl hydrazyl (DPPH) assay of red cabbage extracts was performed by the procedure reported previously (Iftikhar *et al.,* 2022). Briefly, 0.5 mL of each extract solution of the different cycle such as 1-cycle, 2-cycles, 4-cycles, and 8-cycles extracts of red cabbage (10 mg/mL) and 3mL of newly formed (90 µM) 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was mixed. Furthermore, after 30 minutes of incubation of the solution at room temperature, the absorbance was measured at 517 nm by UV/Vis spectrophotometer (Bio Tek Instrument, Inc., Winooski, VT, USA). Butylated hydroxytoluene (BHT) was used as positive control while the DPPH solution was used as blank. The percent radical scavenging was determined by the following formula:

$$\text{Radical Scavenging (\%)} = 100 \times (A_{blank} - A_{sample} / A_{blank})$$

A_{sample} is the absorbance of sample and A_{blank} is the absorbance of the DPPH solution. Exact calculations representing 50% scavenging (IC_{50}) was determined by plotting the percent scavenging activity against the extract concentration.

Measurement of reducing potential

The reducing potential of red cabbage extracts was determined according to the method reported previously by Gaafar *et al*. (2014). Briefly, 1 mL of each red cabbage extract solution (10 mg/mL) was mixed with the 5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 5 mL of 1% potassium ferricyanide. Then the mixture was incubated at 50 ^{o}C for 20 minutes by using a water bath. After this, 5 mL of 10% trichloroacetic acid was also added and centrifuged the mixture at 3000 rpm at 5^{o}C for 10 minutes in a refrigerator centrifuge (CHM-17; Kokusan Deriki, Tokyo, Japan). The upper layer (5 mL) of the mixture was diluted with 500 µl of distilled water and 1 mL of 1% ferric chloride was added into it and the absorbance was checked at 700 nm with a double beam spectrophotometer (Hitachi U-2001, Hitachi, Tokyo, Japan) and the BHT was used as a reference standard.

Antibacterial activities

Antibacterial activity of all red cabbage extracts was measured by well diffusion method against two bacterial strains (Gram-negative; *Escherichia coli* and Gram-positive; *Staphylococcus aureus*) according to the reported method (Hussain *et al*., 2017) with modification. Medium plates were seeded with 12-48 h already cultured microbial inocula (0.5 McFarland used as the reference standard (2×10^7 CFU/mL) was uniformly spread over ager surface in the patri plate by sterile cotton swab. Stock solution (40 mg/mL) of each red cabbage extract was prepared in the DMSO. Wells were made in the agar plates and then 20 µl of extracts of red cabbage were poured into each relevant well. All the plates were incubated at 37^{o}C for 24 h and then the zone of inhibition was noticed in mm.

Cell culture

Human A549 lung cancer cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 100 IU/mL penicillin, 100 µg/mL streptomycin, and 10% Fetal Bovine serum (FBS). Cancer cells were grown in a CO_2 incubator, maintained at 37^{o} C temperature and 5% CO_2 was also supplied (Rasul *et al*., 2021).

MTT mitochondrial viability assay

The antiproliferative activity of red cabbage extracts was carried out by MTT assay. Briefly, 96-well plates were seeded with human A549 lung cancer cells. After 12-24 h 100 µL of plant extracts (40 mg/mL) were added into a 96-well plate. Moreover, the cancer cells were incubated for 24 h at 37^{o}C temperature by the addition of 10 µL MTT (5 mg/mL). The media was aspirated and DMSO (100 µL) was added. After this, absorbance was recorded at 490 nm on an ELISA plate reader (Thermo Scientific) (Rasul *et al*., 2021). The percentage of cell inhibition was calculated by the following formula:

Percentage cell inhibition = 100 (Absorbance of treated cells /Absorbance of control)

Statistical analysis

Each experiment was performed in triplicate and the data were presented as the mean values ± standard deviation. To compare the difference between values, one way Analysis of Variance (ANOVA) followed by Bonferroni/Dunnett (all mean) post hoc test were applied using statistical software (Statistica 5.5; Stat Sift Inc, Tulsa, OK, USA) and SPSS (IBM, SPSS Inc.). The probability value *(p)* ≤ 0.05 was considered significantly different.

RESULTS AND DISCUSSION

Extracts yield

The red cabbage extracts yields (g/100 g), obtained by 1, 2, 4, and 8 Soxhlet extraction cycles are given in the Table 1. The maximum extract yield (10.2 g/100g of dry matter) was obtained with 8-cycles of Soxhlet

extraction, while the minimum yield (4.1 g/100g of dry matter) was obtained with 1-cycle Soxhlet extraction. The amount of extracted components was greatly influenced by the different extraction cycles and the effect was found to be significant ($p \leq 0.05$). The present study revealed that the extract yield depends on the extraction cycles. More the number of extraction cycles, more would be the yield because some compounds could take longer time to release the matrix. But the nutritional values might also be affected due to the continuous contact to the high temperature in Soxhlet (Nawaz *et al*., 2018). The difference in the yield of red cabbage extracts might be associated with differences like extractable components, condition and time of extraction and some components may be extracted at high temperatures while some at more extraction cycles (Zafar *et al*., 2022).

Table 1. Effect of Soxhlet cycles on the yield, total phenolics contents (TPC), Total flavonoids contents (TFC), DPPH (2,2-Diphenyl-1-Picrylhydrazyl) and Radical scavenging capacity of red cabbage extracts.

Assays	Soxhlet extraction cycles				Butylated Hydroxytoluene (BHT)
	1-cycle	2-cycles	4-cycles	8-cycles	
Yield (g/100g)	4.1±0.21^{d}	5.6±0.28^{c}	8.4±0.42^{b}	10.2±0.51^{a}	---
TPC (mg/g dry material)	5.22±0.31^{c}	9.78±0.52^{b}	11.72±0.67^{a}	10.61±0.62^{a}	---
TFC (mg/g dry material)	1.64±0.09^{b}	2.59±0.14^{ab}	5.19±0.29^{a}	4.84±0.28^{a}	---
DPPH IC_{50} (µg/mL)	660±24.6^{a}	110±6.6^{d}	30±0.96^{c}	77±4.62^{b}	19±1.17^{e}

Values are mean ± standard deviation of three different experiments and different letters (a, b, c, d, e) in superscript represents significant difference ($p \leq 0.05$) among different red cabbage extracts.

Quantification of phenolics compounds from red cabbage extracts

HPLC profiles of red cabbage extracts obtained by different extraction cycles are presented in Table 2 and separation of compounds from 4-cycle extract are shown in Figure 1. Variations in the quantity of phenolic compounds were observed in the different extracts. Gallic acid, *p*-coumaric acid, and chlorogenic acid were observed as the major phenolic compounds in red cabbage extracts, while catechin was the major flavonoid found in 4-cycles extract of red cabbage. The effect of extraction cycles on the quantification of phenolic acids and flavonoids was found to be significant ($p \leq 0.05$) and observed differently against different compounds. However, the 4-cycles extraction had the highest phenolic compounds detected followed by 8-cycles, 2-cycles, and 1-cycle extracts of red cabbage. Gallic acid was found to be in maximum amounts in 4-cycles extract (119.5 mg/100 g of dry plant material), while 1-cycles extract showed the minimum amount of gallic acid (19.71 mg/100 g of dry plant material). *p*-Coumaric acid, chlorogenic acid, and *p*-hydroxy benzoic acid were also found in major amount in 4-cycles extracts. Catechin was the major flavonoid in 4-cycles extracts (18.62 mg/100 g of dry plant material), whereas 1-cycle extract showed the minimum amount of catechin (0.08 mg/100 g of dry plant material). Gaaffar *et al*. (2014) reported the quantification of polyphenolic compounds in red cabbage with the higher contents of rutin (58.36 mg/100g DW), benzoic acid (14.40 mg/100g DW), luteolin (119.65 mg/100g DW) and quercetin (36.33 mg/100g DW). Another report published by Zafar *et al*. (2022) explained that the red cabbage extract is rich in phenolic acids and flavonoids including gallic acid, *p*-coumaric acid, benzoic acid and chlorogenic acid, ellagic and catechin. The chemical structure of compounds can also affect the polyphenolic contents which can cause resistance to heat. Polyphenol compositions of extracts are influenced by the extraction temperature, time, and polarity of the solvents (Ghafoor *et al*., 2019. There is a strong correlation between phenolic contents and antioxidant activity (Ghafoor *et al*., 2019).

Figure 1. Typical HPLC Chromatogram showing the separation of phenolic acids and flavonoids from 4-Cycles extract of red cabbage.

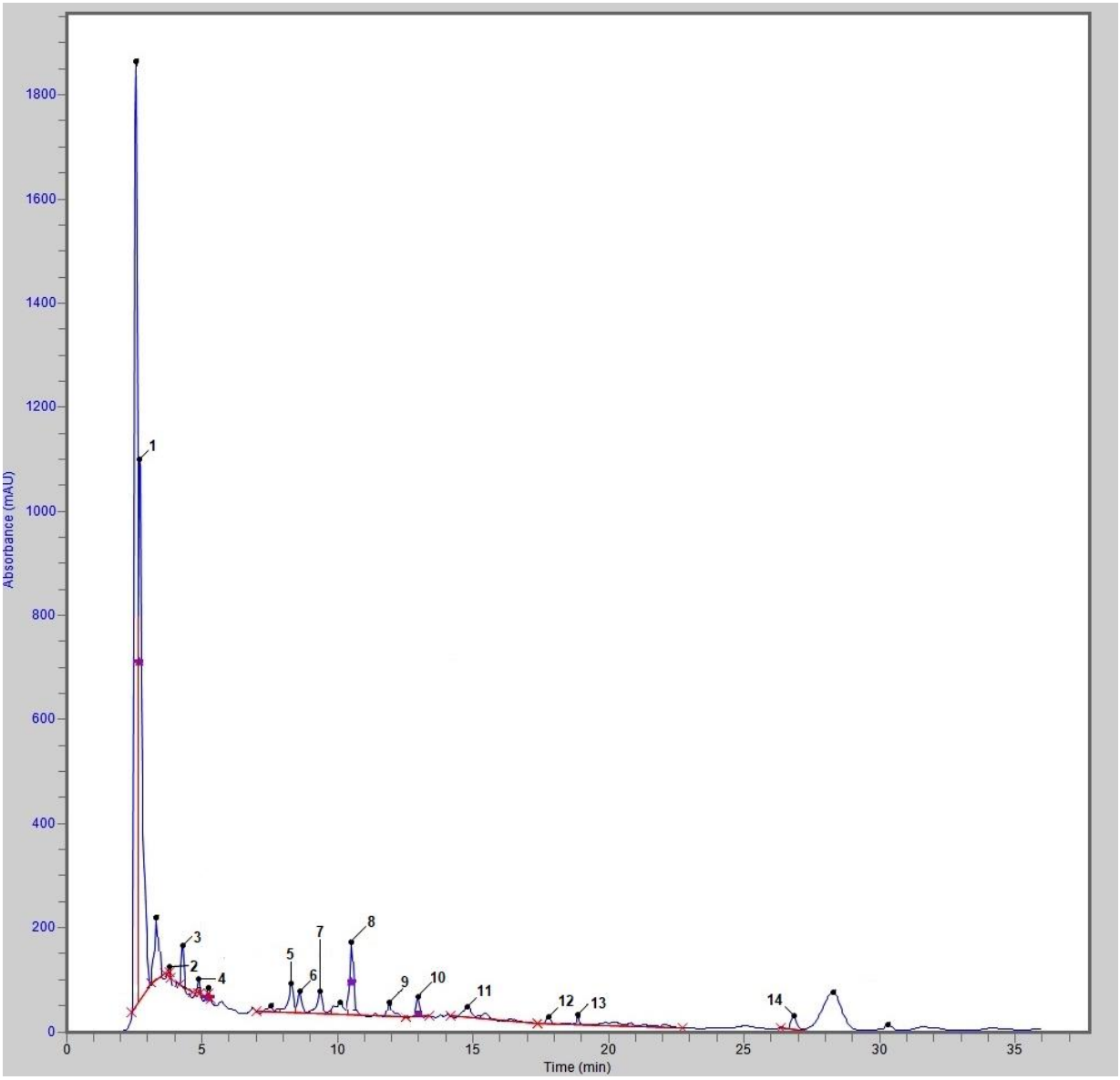


Table 2. Quantification of phenolic acids and flavonoids of red cabbage extracts.

Compounds	Retention time (minutes)	mg/100g of dry plant material			
		1-cycle extract	2-cycles extract	4-cycles extract	8-cycles extract
1. Gallic Acid	2.700	19.71±1.18^{a}	21.26±1.27^{de}	119.5±7.17^{a}	113.3±6.79^{a}
2. Para-hydroxyl benzoic acid	3.810	0.48±0.03^{d}	0.48±3.32^{a}	3.95±0.24^{b}	3.32±0.19^{c}
3. Catechin	4.301	1.49±0.09^{d}	6.13±0.37^{b}	18.62±6.13^{b}	15.19±0.91^{b}
4. Chlorogenic acid	4.890	0.08±0.01^{c}	1.85±0.11^{c}	4.85±0.29^{b}	2.58±0.15^{d}
5. Caffeic acid	8.305	0.44±0.03^{d}	0.82±0.05^{ef}	1.09±0.06^{d}	0.12±0.01^{c}
6. Syringic acid	8.600	0.29±0.02^{d}	_	0.70±0.04^{d}	_
7. Vanillic acid	9.350	0.41±0.03^{d}	0.61±0.04^{f}	0.78±0.05^{d}	0.06±0.01^{c}
8. *p*-coumaric acid	10.484	0.15±0.01^{d}	3.86±0.23^{b}	11.08±0.66^{c}	5.41±0.33^{e}
9. Sinapic acid	11.920	0.12±0.01^{d}	1.33±0.08^{d}	0.05±0.01^{d}	0.19±0.01^{c}
10. Ferulic acid	12.978	2.19±0.13d	0.02±0.01^{g}	0.18±0.01^{d}	0.67±0.04^{c}
11. Ellagic	14.790	_	0.11±0.01^{g}	0.56±0.04^{d}	0.32±0.02^{c}
12. Benzoic acid	17.771	0.08±0.01^{d}	0.04±0.01^{g}	0.06±0.04^{d}	2.72±0.16^{c}
13. Myricetin	18.834	_	0.09±0.01^{g}	0.19±0.01^{d}	_
14. Quercetin	26.830	0.58±0.04^{d}	1.04±0.06^{f}	0.13±0.01^{d}	0.42±0.03^{c}

Values are reported as mean ± standard deviation of three different experiments and different letters (a, b, c, d) in superscript represents significant difference ($p \leq 0.05$) among different red cabbage extracts.

Total phenolic and flavonoid contents of red cabbage extracts

The total phenolic contents (TPC) and total flavonoid contents (TFC) different red cabbage extracts are presented in Table 1. The TPC ranged from 5.22-11.72, mg/g of the dry weight of plant material, measured as Gallic acid equivalent (GAE). The maximum TPC was found in 4-cycles of red cabbage extracts (11.72 mg/g of the dry weight of plant material) while the minimum TPC was found in 1-cycle extract of red cabbage (5.22 mg/g of the dry weight of plant material). While the TFC ranged from 1.64-5.19 mg/g of the dry weight of plant material and was measured as catechin equivalent (CE). The maximum TFC was also found in 4-cycles extract of red cabbage (5.19 mg/g of the dry weight of plant material) while the minimum TFC was in 1-cycle extract of red cabbage (1.64 mg/g of the dry weight of plant material) as shown in Table 1. Statistical analysis showed that different extraction cycles exerted a significant effect ($p \leq 0.05$) on TPC and TFC.

Present results are varied from the results carried out previously by Gaafar *et al*. (2014) and Zafar *et al.* (2022), where the authors reported the higher TPC and TFC of red cabbage extract. Another report was also published by Tajali *et al*. (2020) who explained the lesser TPC in the red cabbage extract. The

variations in the phenolic contents might be due to variation in the extraction solvents, extraction time and temperature (Zhang *et al*., 2018; Iftikhar *et al*., 2022).

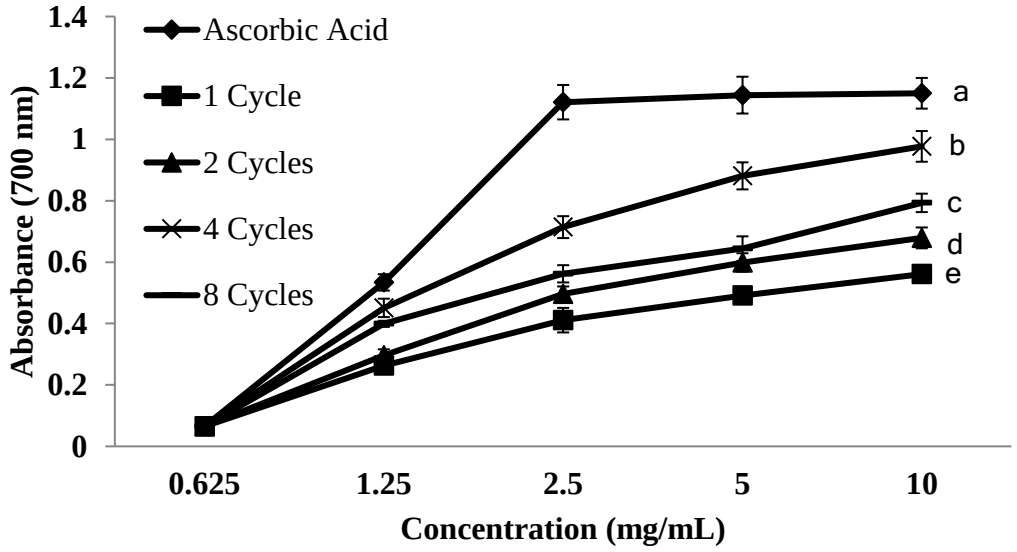
DPPH radical scavenging capacity of red cabbage extracts

Radical scavenging activity of red cabbage extracts was observed in terms of DPPH radical scavenging assay and the results are displayed in Table 1. Free radical scavenging activity of red cabbage extracts increased in a concentration-dependent manner and the concentration of extract providing 50% of scavenging (IC_{50}) is listed in Table 1. Red cabbage extract from 4-cycles extraction technique showed the best radical scavenging activity with IC_{50} 30 µg/mL as compared to other extracts (Table 1). Synthetic antioxidant, BHT showed the highest antioxidant activity with the smallest IC_{50} value (19 µg/mL) then all the red cabbage extracts. It was reported by Gaafar *et al*. (2014) that 5, 10, 20 and 40 µg/mL red cabbage extract concentration showed 70.52, 81.07, 88.83 and 93.71% radical scavenging activity, respectively. Another report published by Liang *et al*. (2019) on the antioxidant activity of red cabbage showed 69.82% radical scavenging activity. Significant variation ($p \leq 0.05$) was observed in the IC_{50} values of different red cabbage extracts. 4-cycles extract of red cabbage showed the highest radical scavenging activity, TPC and TFC (Li *et al*., 2018).

Reducing power of red cabbage extracts

The antioxidant activity in terms of reducing power of different cycles of red cabbage extracts is presented in Figure 2. Reducing potential of all the red cabbage extracts was measured up-to 10 mg/mL extraction, exhibited a general increase in antioxidant activity as the concentration increased. It was observed by above results that among other cycles of red cabbage extract, the 4-cycles showed maximum reducing potential. No earlier reports are available regarding reducing power of different cycles by Soxhlet extraction technique of red cabbage extracts. However, our results are comparable with the results reported by Gaafar *et al*. (2014) who explained that reducing potential of red cabbage extract is higher than the white cabbage extract. The results of the present investigation regarding the reducing potential of red cabbage extract are in line with the findings of Zafar *et al*. (2022) who reported the reducing power of extracts of all the species of cabbage, including red cabbage.

Figure 2. Reducing power of different red cabbage extracts.



Antibacterial activity of red cabbage extracts

The well diffusion method was used to carry out the antibacterial activity of red cabbage extracts against the two bacterial strains; *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) and inhibition zones are reported in the Table 3. All the red cabbage extracts showed considerable antibacterial activity. 4-cycles of soxhlet extraction of red cabbage extract showed higher antibacterial activity with the inhibition zone of (16.3 mm, and 11.4 mm) followed by 8-cycles, 2-cycles and 1-cycle extracts against the *Escherichia coli* and *Staphylococcus aureus* bacterial strains, respectively. Positive control, gentamycin exhibited the highest antibacterial activity with the inhibition zone of (17.0 mm and 19.0 mm) against the *Escherichia coli* and *Staphylococcus aureus* bacterial strains, respectively. There are no previous reports published on the effect of extraction cycles on the antibacterial activity of red cabbage extract. Like the present results, Zafar *et al*. (2022) also reported the antibacterial activity of red cabbage extract against *Escherichia coli*. The results of the present study were similar with the finding of Wang *et al*. (2016) who performed antibacterial activity of different *Brassica* species including red cabbage against *Escherichia coli* and *Staphylococcus aureus*. Bioactive components like phenolic acids and flavonoid present in medicinally active plants are effective antimicrobial agents against a broad range of microorganisms (Waghulde *et al*., 2018).

Table 3. Antibacterial and anti-proliferative activities of red cabbage extracts.

Extracts	Inhibition zone (mm)		% Inhibition A549 cells (40 mg/mL)
	Escherichia. coli	*Staphylococcus aureus*	
1-Cycle	12.0 ± 0.72^{e}	10.2 ± 0.61^{c}	35.8 ± 1.79^{d}
2-Cycles	13.4 ± 0.81^{d}	11.3 ± 0.67^{b}	40.9 ± 2.04^{c}
4-Cycles	16.3 ± 0.97^{b}	11.4 ± 0.68^{b}	58.2 ± 2.91^{a}
8-Cycles	14.5 ± 0.87^{c}	11.3 ± 0.67^{b}	42.2 ± 2.11^{b}
Gentamycine	17.0±1.02^{a}	19.0 ± 1.14^{a}	---

Values are reported as mean ± standard deviation of three different experiments and different letters (a, b, c, d) in superscript represents significant difference ($p \leq 0.05$) among different red cabbage extracts.

Antiproliferative activity of red cabbage extracts

The antiproliferative activity of red cabbage extracts was carried out using an *in-vitro* MTT mitochondrial viability assay against the Human A549 lung cancer cell line at 40 mg/mL extract concentration. A considerable difference was observed between the sensitivity and cytotoxicity of various red cabbage extracts tested in this study (Table 3). Among different red cabbage extracts, 4-cycles of red cabbage extracts exhibited the strongest inhibitory effect (58.2%) while the 1-cycle of red cabbage extract showed the lowest (35.8%) inhibitory effect against the Human A549 cancer cell line. Usually, it is believed that the major bioactive components present in vegetables ascertain biological properties like antioxidant and anticancer properties. The Brassicaceae vegetables are proven to be an excellent source of anticancer agents (Gaafar *et al*., 2014; Wang *et al*., 2016). Only a few reports are published about the antiproliferative activity of red cabbage extracts against the human A549 lung cancer cell line. The results of the present study agree with the findings of a previous study on cauliflower, broccoli, white cabbage, Chinese cabbage, and red cabbage extracts (Zafar *et al*., 2022). Li *et al*. (2007) reported a linear correlation between anticancer and antioxidant properties of different herbs against the human A549 lung cancer, and human breast cancer MCF-7 cell lines. Tajali *et al*. (2020) reported the anti-proliferative activity of red cabbage extract against cancerous and normal cell lines.

CONCLUSION

In the present study, it was reported first time the effect of Soxhlet extraction cycles (1-cycle, 2-cycles, 4-cycles, and 8-cycles) on the phenolic profiles and biological potentials of red cabbage extract. It can be concluded from the data obtained that 4-cycles of Soxhlet extraction was proved to be the most effective in terms of phenolic contents, antioxidant, antimicrobial and antiproliferative activities. All the red cabbage extracts possess significant ($p \leq 0.05$) antioxidants, high anti-proliferative activity against the human A549 cancer cell line, and antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The current study can be useful for the development of soxhlet extraction protocol for the preparation of extracts from plant materials, which can be useful for industrial extraction processes. Further study can be planned for the extraction of targeted compounds and evaluation of other biological activities *in vivo*.

Ethics approval

Not applicable

Informed consent

This article does not contain any studies with human participants or animal performance by any of the authors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of interest

The authors declare that they have no conflict of interest.

Authors Contribution Statement

Conceptualization: A.I.H and L.N.; Methodology: I. Z. and N.I.; Validation: A.I.H. and S.D.S.; Formal Analysis: A.K., I. Z. and N. I.; Investigation: I. Z. N.I. and A. K.; Original draft preparation: I.Z. and N.I.; Review and editing: L. N. and S. D. S. All authors read and approved the final version of the manuscript.

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