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Phytochemical characterization, and antioxidant and antimicrobial activities of white cabbage extract on the quality and shelf life of raw beef during refrigerated storage†

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Brassica vegetables are well-characterized, containing a wide-spectrum of phenolic compounds that are responsible for their diverse biological activities like antioxidant and antimicrobial activities. This study explored the preservative effect of *Brassica oleracea* var. *capitate* f. *alba* (white cabbage; WC) on beef under refrigerated conditions for 16 days. The antimicrobial activities of WC were evaluated against foodborne pathogenic bacteria and fungi. The antioxidant activity was determined on the basis of total phenolic and flavonoid contents, through employing DPPH and ABTS assays. The chemical composition was analyzed by GC-MS analysis. The results indicated that among the different solvent extracts, white cabbage chloroform extract [WCCE] exhibited outstanding bioactive properties due to the presence of 4-nitro-3-(trifluoromethyl)phenol, and the effects of WCCE at different levels (A and B) on the quality and shelf life of beef in storage were evaluated. The color parameters (lightness, yellowness, and redness), texture analysis, and pH values were monitored constantly with 4 days interval, and microbial analysis was conducted. The results showed that WCCE-A treatment significantly reduced the total viable counts, psychrotrophic bacteria, and yeast-molds when compared with WCCE-B and control during refrigeration storage, with the activity varying in a dose-dependent manner ($p < 0.05$). Significantly, the WCCE-A treatments had better appearance compared with the control after 16 days of storage. All results confirmed that WCCE which is rich in bioactive compounds, effectively maintains the quality of beef compared to the control by retarding lipid oxidation and microbial growth at refrigeration temperature and also emphasize the potential applications of this plant in different industrial sectors.

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1. Introduction

Meat is a staple food, providing proteins of high nutritional value and a high content of essential minerals and vitamin B.[1] Fresh meat and meat products are generally merchandised at cold temperatures (2–5 °C).[2] Meat products commonly spoil during refrigeration because of microbial growth and oxidative rancidity together with biochemical and enzymatic deterioration.[3,4] This microbial growth and oxidative rancidity brings about undesirable changes in organoleptic properties, off-flavors, discoloration, gas production, and alteration in pH.[3,5] Antimicrobial and antioxidant agents are used to delay, retard, or prevent microbial spoilage and other deteriorations. So, nowadays people are showing greater interest in safety foods that contain bioactive or functional components which will give additional benefits to their health status. The antimicrobials and antioxidants added in meat products are mainly synthetic.[6] Despite the proven efficiency of these chemical preservatives in the food and feed chain, the acquisition of microbial resistance to the utilized chemicals and unsightly side effects of those chemicals on human health.[7] Unlike synthetic compounds, natural extracts obtained from plants rich in phenolic compounds have been reported to be more active and can enhance the overall quality of food by decreasing lipid oxidation and microbial growth.[7,8] In this regard, various plants have been investigated scientifically as antimicrobial and antioxidants in foodstuff.[9–11] In addition, these plant extracts considered nutritionally safe and easily degradable.[8] At present, vegetables attain substantial importance among the possible added

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natural antimicrobials and antioxidants due to the presence of a wide range of plant secondary metabolites such as phytochemicals that have been used for centuries in ancient or traditional medicine. Plants have an amazing ability to produce a wide variety of plant secondary metabolites. Among secondary metabolites, phenolics, tannins, terpenoids, alkaloids, and glucosinolate have been reported as major compounds liable for the bioactive properties ascribed to vegetables.[12] More specifically, the activity of these metabolites against meat-dwelling microorganisms has been described.[1] According to the literature[1,13–15] and, different compounds of plant origin can effectively inhibit the growth of pathogenic and spoilage microorganisms. Therefore, assessment of the prospective antimicrobial and antioxidant potential of vegetables could be a novel approach for food technologists and scientists to work on. Among these bioactive compounds, one of the most important groups consists of members of the Brassicaceae family, which are one of the most extended food crops in numerous countries.[16] Many vegetables of the Brassicaceae family have been well-characterized to possess bioactive properties towards a wide range of microorganisms of clinical importance.[17,18] White cabbage (WC; *Brassica oleracea* var. *capitata* f. *alba*) is one of the main Brassicaceae crops, which have been widely described as a source of phenolic acids, flavonoids, tannins, terpenoids, and fiber and antioxidant substances.[8,19] In addition, it is a rich source of vitamins (such as K, A, C, B6 folate, thiamine, and riboflavin), minerals (calcium, potassium, and magnesium), and tryptophan amino acids.[20] Brassica vegetables such as cabbage and Chinese cabbage are known with antimicrobial and antioxidant properties.[19,21] Currently, traditional solvent extraction is an effective process to extract the phytochemical components. Cabbage is one of the most widely consumed vegetables worldwide due to its common availability in local markets, affordability, and consumer preference. The WC is a prosperous source of nutrients and rich in health-promoting phytochemicals. The consumption of WC varies widely around the world and is used in many ways such as raw or stir-fried and in the form of cuisines. Pickling is one of the most popular ways of preserving cabbage, creating dishes such as sauerkraut. In addition, it has an important place in traditional remedies to cure hangovers, headaches, fevers, and to prevent sunstroke.[22] Further, it has been extensively used in the mitigation of symptoms related to gastrointestinal disorders (gastritis, peptic and duodenal ulcers, irritable bowel syndrome) as well as treatment of minor injury, rheumatism, and sore throat.[20] To our knowledge, no investigation has addressed the use of WC as a natural preservative in beef meat. Hence, this study aimed to evaluate the antimicrobial, antioxidant, and cytotoxic activities of WC extracts *in vitro* using different solvents. In addition, we showed for the first time that WC extracts have deleterious effects on a wide range of pathogenic microorganisms. Based on these *in vitro* results, extract with higher antimicrobial and antioxidant activity was selected and its effectiveness was also evaluated at different levels on physicochemical properties of raw beef meat at refrigeration storage.

2. Results and discussion

2.1. *In vitro* antimicrobial activity

This current study showed the antimicrobial activity of WC and its application in raw beef meat. Results showed that WCCE was seen to have greater potential compared to other extracts of WC. The results of antimicrobial activity of Brassica

Table 1 Screening of antimicrobial activity of WC extract against tested microorganisms[a,b]

	WC extracts (zone of inhibition; mm, Conc. 33 mg mL^{-1})					
Test microorganism's	CE	TE	DE	EEE	EtE	ME
Gram-negative bacteria						
ATCC 14028	11.00 ± 0.02^{c}	14.00 ± 0.01^{a}	10.00 ± 0.03^{d}	11.00 ± 0.03^{c}	13.00 ± 0.05^{b}	10.00 ± 0.06^{d}
ATCC 35150	13.00 ± 0.03^{b}	14.00 ± 0.02^{a}	11.00 ± 0.04^{c}	09.00 ± 0.06^{d}	12.00 ± 0.05^{c}	09.00 ± 0.05^{d}
ATCC 43894	12.00 ± 0.01^{b}	14.00 ± 0.04^{a}	11.00 ± 0.03^{c}	09.00 ± 0.03^{d}	11.00 ± 0.05^{c}	09.00 ± 0.07^{d}
Gram-positive bacteria						
ATCC 13150	12.00 ± 0.03^{c}	14.00 ± 0.02^{a}	12.00 ± 0.03^{c}	11.00 ± 0.05^{d}	13.00 ± 0.07^{b}	11.00 ± 0.05^{d}
ATCC 12600	10.00 ± 0.04^{d}	15.00 ± 0.07^{a}	11.00 ± 0.02^{c}	11.00 ± 0.03^{c}	12.00 ± 0.06^{b}	—
ATCC 19118	13.00 ± 0.01^{b}	15.00 ± 0.03^{a}	11.00 ± 0.05^{d}	11.00 ± 0.07^{d}	12.00 ± 0.05^{c}	—
ATCC 14579	11.00 ± 0.03^{b}	16.00 ± 0.05^{a}	11.00 ± 0.02^{b}	10.00 ± 0.06^{c}	11.00 ± 0.07^{b}	—
Fungi						
KCTC 7965	14.00 ± 0.01^{b}	—	13.00 ± 0.03^{c}	15.00 ± 0.05^{a}	—	—
KCTC 6145	—	—	—	—	—	—
KCTC 6143	10.00 ± 0.04^{a}	—	10.00 ± 0.03^{a}	09.00 ± 0.05^{b}	—	—
KCTC 6317	09.00 ± 0.02^{b}	—	09.50 ± 0.05^{a}	09.00 ± 0.03^{b}	—	—

[a] CE: chloroform extract, TE: toluene extract, DE: dichloromethane extract, EEE: ethyl ether extract, EtE: ethanol extract, ME: methanol extract, DWE: distilled water extract. [b] —: no inhibition zone, a–d letters are according to increasing mean values, different letters in each row for each extract's antimicrobial activity represent statistically significant difference ($p < 0.05$), same letters in each row for each extract's antimicrobial activity represents non-significant difference ($p > 0.05$).

vegetable extracts against foodborne pathogens (including Gram-positive and Gram-negative bacteria) and fungi were assessed by visualizing the presence or absence of inhibition zone and measuring the zone diameter (Table 1). As shown in Table 1, all extracts of WC exhibited a moderate to weak activity against all test organisms, with higher effectiveness towards Gram-positive bacteria as compared to Gram-negative bacteria. All bacterial strains were susceptible to WC extracts. The results of antifungal activity assays showed that the WC extracts moderately reduced the growth of *C. albicans*, *A. flavus* var. *flavus*, *A. niger*. However, the extracts were significantly not active against *A. fumigatus*. Overall, the inhibitory effects of all extracts on fungal strains were lower compared to bacterial strains. Out of the seven different solvents used for extraction for WC, toluene and chloroform extracts exhibited the highest activity towards the tested microorganisms, followed by dichloromethane, ethyl ether, ethanol, and methanol extracts. The WC toluene extract was most active against only the bacterial strains with a zone of inhibition (14–16 mm) whereas chloroform extract was active against all the microorganism studied with a zone of inhibition ranging from 9–15 mm, with the highest zone of inhibition observed for ATCC 35150 (15 mm), followed by ATCC 13150 (14 mm). The least zone of inhibition was observed for KCTC 6145 (08.50 mm). The antimicrobial activity of the extract may be attributed to the presence of flavonoid and phenolic content, which have been reported to be involved in antibacterial and antifungal activity of plant extracts.[10,21,23,24] Further, the chloroform extract of WC was heated at 95 °C for three different times (5, 45, and 90 min) to evaluate the thermostability of the extract. The bioactive compounds present in WCCE showed heat stability and the antimicrobial activity enhanced with an increase in heating time (Table 2). WC chloroform extract has the highest activity against all the test microorganisms and WCCE may contain thermo-tolerant antimicrobial compounds. Indeed the study showed the presence of such compounds that maintained activity at 95 °C even for 90 min of heating. Comparing the antimicrobial activity of WC extracts, it could be noted that the bacteria were more sensitive to all extracts than fungi. However, further increase leads to no change in antimicrobial efficacy (data not shown). Hence the active compound present in the WCCE is found to be heat stable and may not be protein in nature.

For a more accurate determination of antimicrobial activity, a growth inhibition assay was conducted. The susceptibility of *S. aureus* ATCC 13150 and *E. coli* ATCC 35150 against WCCE was estimated and results are presented as MICs (Fig. 1a and b). WCCE displayed the MIC values of about 11.5 mg mL^{-1} against ATCC 35150 and ATCC 13150. The MIC analysis of WCCE showed activity against Gram-positive and Gram-negative bacteria, which indicate that WCCE contains several antimicrobial compounds. The antimicrobial activity of the WC extract is supported by the studies performed by Hu *et al.*,[25] that exhibited moderate to the good activity of WC aqueous and methanolic extract against Gram-positive and Gram-negative. As per our knowledge, there is no report about the detrimental effect of WC crude extracts against pathogenic fungi.

Table 2 Thermostability of the WCCE against tested microorganisms[a]

Test microorganism's	Thermostability of extracts at 95 °C for different times (min) by measuring zone of inhibition (8 mm)		
	5	45	90
Gram-negative bacteria			
ATCC 14028	13.00 ± 0.01^{b}	14.50 ± 0.05^{a}	19.00 ± 0.05^{a}
ATCC 35150	09.00 ± 0.01^{b}	12.00 ± 0.03^{a}	10.00 ± 0.04^{a}
ATCC 43894	12.00 ± 0.03^{b}	13.00 ± 0.03^{a}	13.00 ± 0.05^{a}
Gram-positive bacteria			
ATCC 13150	14.50 ± 0.01^{a}	14.00 ± 0.02^{a}	16.00 ± 0.04^{a}
ATCC 12600	12.00 ± 0.02^{a}	12.00 ± 0.01^{a}	14.00 ± 0.03^{a}
ATCC 19118	15.00 ± 0.02^{a}	15.00 ± 0.02^{a}	20.00 ± 0.06^{a}
ATCC 14579	15.00 ± 0.02^{b}	18.00 ± 0.03^{a}	21.00 ± 0.02^{a}
Fungi			
KCTC 7965	08.50 ± 0.03^{b}	08.50 ± 0.03^{b}	08.50 ± 0.05^{bc}
KCTC 6145	—	08.00 ± 0.03^{b}	10.50 ± 0.05^{a}
KCTC 6143	11.30 ± 0.02^{a}	11.00 ± 0.01^{a}	12.00 ± 0.03^{b}
KCTC 6317	—	09.30 ± 0.03^{a}	09.50 ± 0.04^{a}

[a] —: no inhibition zone, a–c letters are according to increasing mean values, different letters in each row for each extract's antimicrobial activity represent statistically significant difference ($p < 0.05$), same letters in each row for each extract's antimicrobial activity represents non-significant difference ($p > 0.05$).

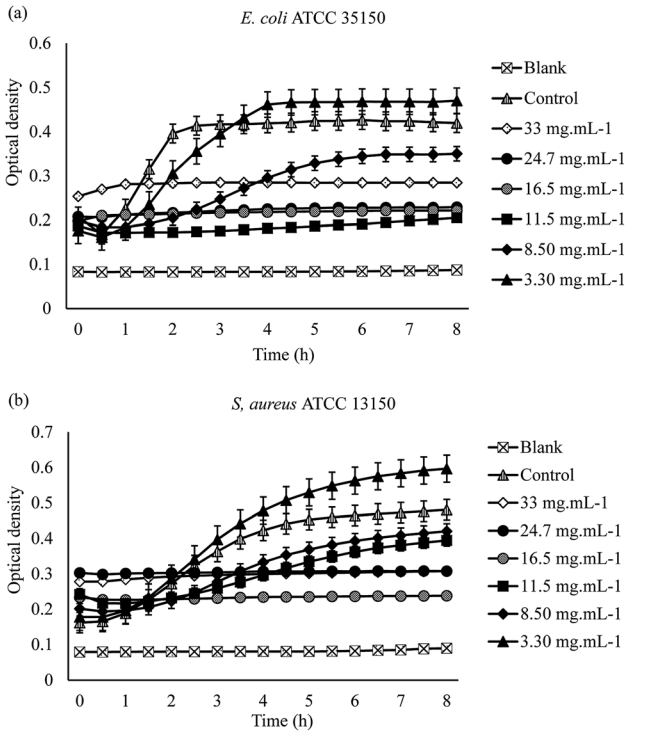

Fig. 1 Growth inhibitory activity of different extracts on growth of *E. coli* ATCC 35150 (a), and *S. aureus* ATCC 13150 (b).

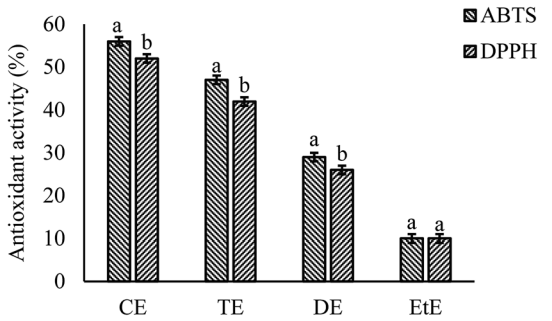

Fig. 2 Antioxidant activity of different extracts of WC; CE: chloroform extract, TE: toluene extract, DE: dichloromethane extract, and ETE: ethyl ether extract.

2.2. Phenolic and flavonoid contents

The total phenolic content (TPC) of the WC chloroform, dichloromethane, ethyl ether, toluene, ethanol, and methanol extracts, calculated from the calibration curve was 82, 78, 78, 65, 81, and 75 mg GAE 100 g^{-1}, respectively. The total flavonoid content (TFC) of the WC chloroform, dichloromethane, ethyl ether, toluene, ethanol, and methanol extracts, calculated from the calibration curve was 17.4, 16.9, 16.5, 15.1, and 14.8 mg GAE 100 g^{-1}, respectively. The main compounds responsible for the antimicrobial activity of Brassica vegetables are known as phenolic and flavonoid compounds.[15,26] Furthermore, TPC and TFC were found higher in WCCE. Applications of phenolic and flavonoids are spreading rapidly in the food industry to enhance the safety and quality of food.[8] The choice of solvent plays a significant role in the GAE level in plants, however, the GAE level decreased by plant age, as reported in previous studies.[27] Overall, chloroform extract exhibited the highest TPC and TFC and our result was in agreement with the previously reported studies.[28,29]

2.3. Antioxidant activity

DPPH radical scavenging activity of WC solvent extracts are shown in Fig. 2. The reducing scavenging activity is related to the amount and composition of different phenolic compounds present in the extracts. Among the WC extracts (Fig. 2), chloroform had the highest antioxidant activity in both ABTS and DPPH assay with 56 and 52%, respectively, followed by toluene,

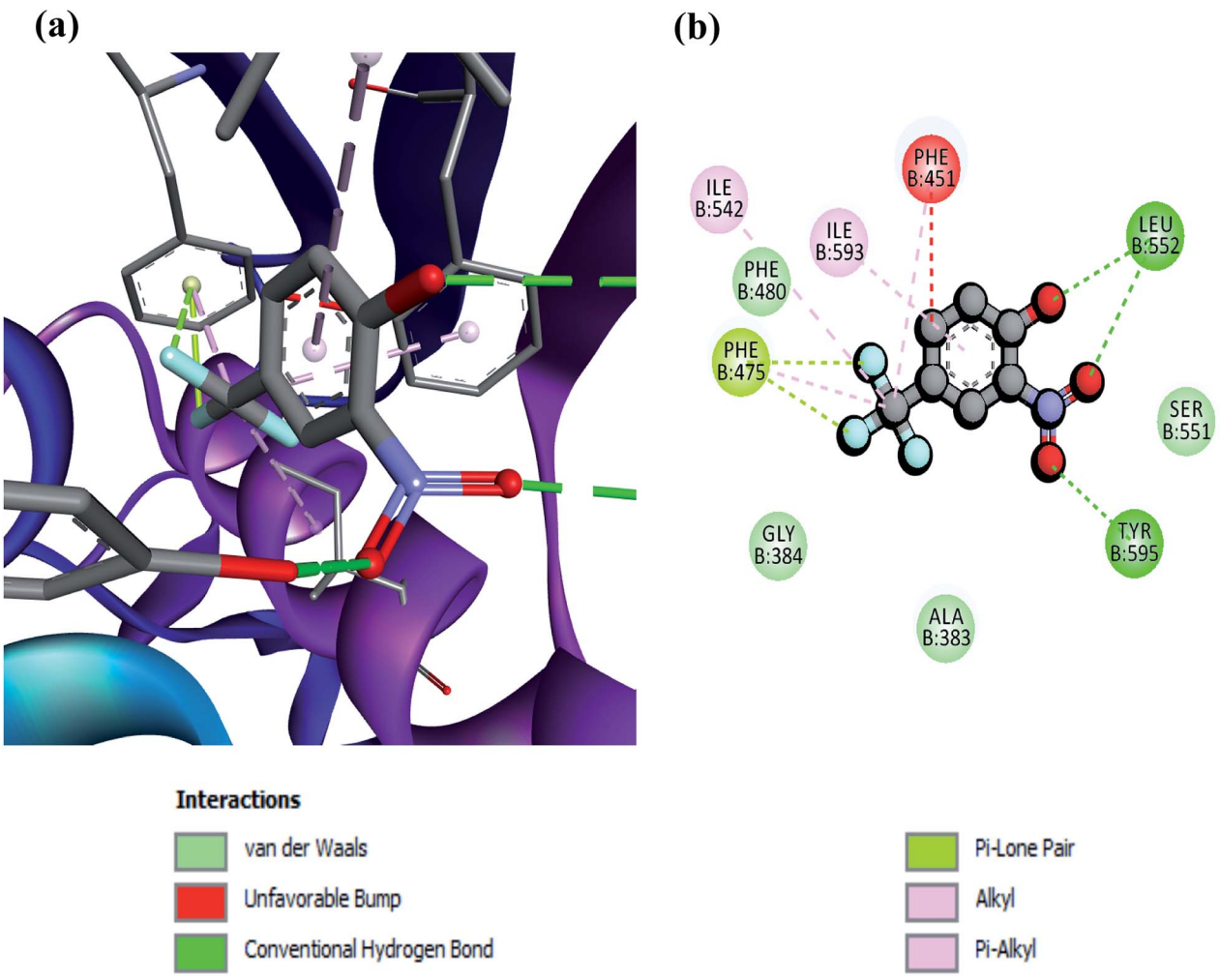

Fig. 3 2D and 3D structures showed the molecular interaction of LpxC with 4-nitro-3- of (trifluoromethyl)phenol.

dichloromethane, and ethyl ether extract, however, methanol, ethanol, and distilled water extracts did not exhibit pronounced activity as compared to other solvents. The antioxidant activity of extracts varied markedly with different solvents due to different antioxidant potentials of compounds with different polarities[30] and are highly specific and sensitive to temperature and incubation period.[31] For a broader evaluation of the antioxidant capacity, two assays (ABTS and DPPH) were carried out. In both assays, chloroform extract exhibited higher antioxidant activity and our results are in agreement with previously reported studies of Brassica vegetables.[22] The observed antioxidant activity may be attributed to the presence of various antioxidant compounds identified in GC-MS analysis. Each vegetable studied has its antioxidant compounds that might influence their antioxidant and free radical scavenging activities, and total phenolic and flavonoid content.[32]

2.4. GC-MS analysis

The isolated compounds from crude WCCE are shown in Table 3 and the chromatogram depicted in S1.† Different classes of organic chemical constituents were identified by the GC-MS analysis. The GC-MS result shows medicinally valued phytochemicals are present in the plant extracts. A total of 4 compounds were recognized based on the peak area, retention time, and the chemical formula with reported literature. These bioactive compounds including phenolic acids, flavonoids are well recognized as a plant-derived antimicrobial and antioxidant agents.[8,17] In addition, the GC-MS analysis was also performed for other extracts of WC extracts (S2†). The isolated compounds in WCCE which are considered as a responsible compound for its antimicrobial activity was not found in other extracts. To date, few studies have focused on cabbage's antimicrobial and antioxidant activity and none of these studies have specified the compounds responsible for its activity. The different antimicrobial and antioxidant potency of *Brassica* could depend on numerous factors, such as genotype, growth conditions, and time of harvest, with these influencing the content of secondary metabolites (bioactive compounds) responsible for the antimicrobial and antioxidant activity of *Brassica*. Several authors have reported that phenolic compounds play an important role in the antimicrobial and antioxidant activity in cruciferous vegetables.[25,33] The WC most likely contains more than a single compound with antimicrobial and antioxidant properties indicating that the use of different solvents could exploit the properties of the active

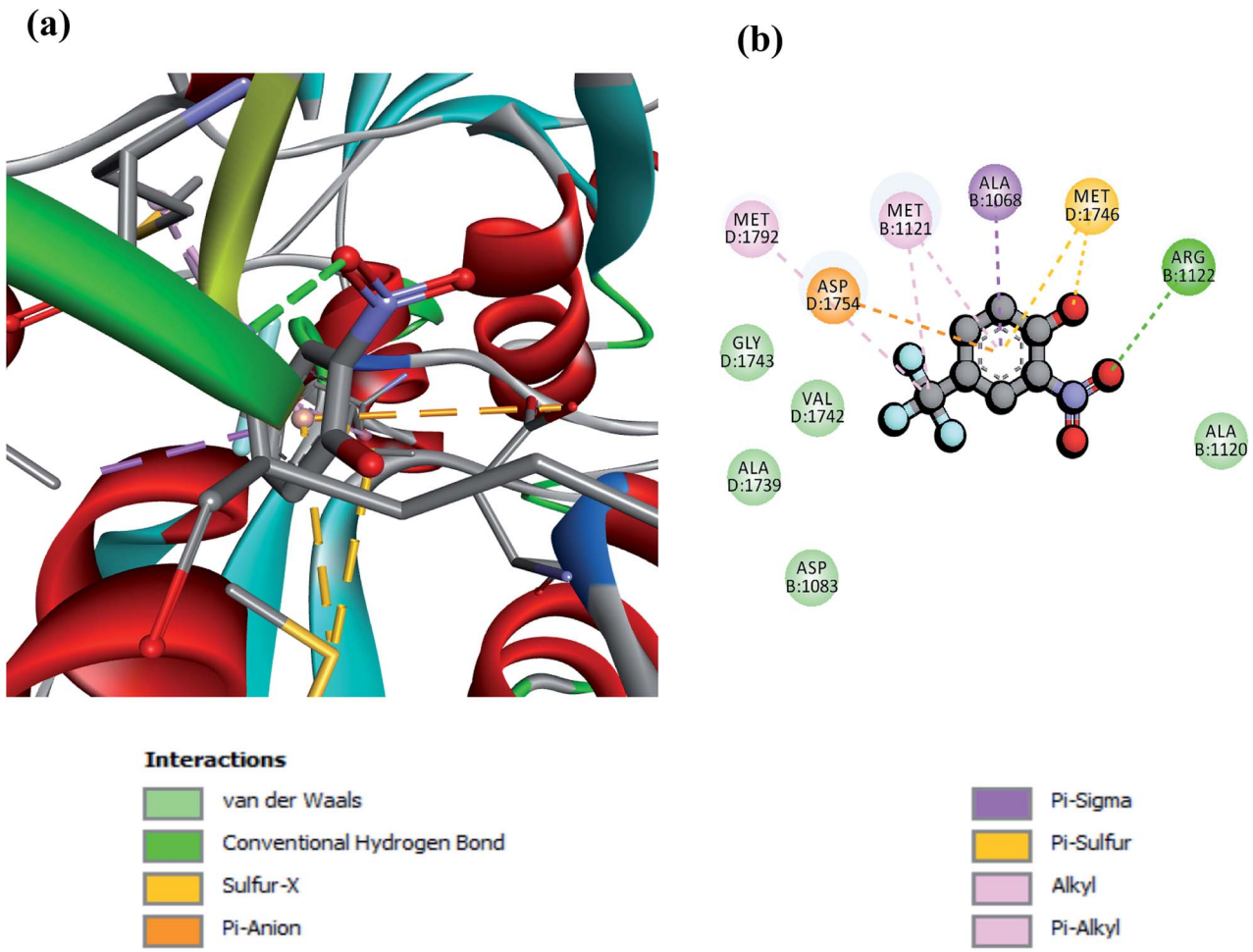

Fig. 4 2D and 3D structures showed the molecular interaction of 4PLB with 4-nitro-3-(trifluoromethyl)phenol.

compounds present in *Brassica*. Chloroform was the choice of solvent in the present investigation because it shows the highest activity among all extracts. Further, a molecular docking study was employed against target proteins involved in antimicrobial mechanisms to investigate to understand the stereoelectronic features responsible for the bioactive activity.

2.5. Docking study

The binding interaction and docking score of the isolated compounds towards LpxC and 4PLB are shown in Fig. 3 & 4 and Table 3. Among the isolated compounds, 4-nitro-3-(trifluoromethyl)phenol showed good docking scores towards both LpxC and 4PLB with −6.90 and −6.87 kcal mol^{-1}, respectively. The binding model of 4-nitro-3-(trifluoromethyl) phenol with LpxC and 4PLB was depicted in Fig. 3 and 4, respectively. The 4-nitro-3-(trifluoromethyl)phenol established strong interactions on the active sites of LpxC and showed two hydrogen bond interactions (Leu 552 and Tyr 595) also with pi-Alkyl and pi-Lone pair of Ile 542, Ile 593 and Phe 475, respectively as van der Waals of Phe 482, Gly 384, Ala 383 and Ser 551. With 4PLB, 4-nitro-3-(trifluoromethyl)phenol also established strong interactions on the active sites and showed one hydrogen bond interaction (Arg 1122), as well as van der Waals of Gly 1743, Val 1742, Ala 1739, Asp 1083, and Ala 1120. In addition form interactions with pi-anion, pi-sulfur, pi-sigma, and pi-alkyl of Asp 1754, Met 1746, Ala 1068, and Met 1792, and Met 1121, respectively, justifying the powerful antibacterial activity of WCCE. Since our identified compounds are well-known to possess antimicrobial activity, the compounds were evaluated for their antimicrobial activity by the disc diffusion method. They were evaluated for antimicrobial activity towards three Gram-negative bacteria, four Gram-positive bacteria, and four fungal strains (S2†). All the compounds were active towards all the microorganisms studied. The results indicated that among the identified compounds from WCCE, 4-nitro-3-(trifluoromethyl)phenol was the most active compounds with higher docking scores against LpxC, and 4PLB and established strong interactions in amino acids. Thus, the binding model reported here, indicated that 4-nitro-3-(trifluoromethyl)phenol compounds behave as LpxC and 4PLB inhibitors and could be a potential source of natural antimicrobial agent.

It is well recognized that plants with antimicrobial and antioxidant properties have gained substantial attention industrially and commercially as a natural food preservative. Antimicrobial and antioxidant potential of plants are difficult to correlate to specific compounds because of their complexity and variability, however, the antimicrobial and antioxidant potential of cruciferous vegetables could be attributed to the phenolic and flavonoid compounds with proven antimicrobial and antioxidant effects.[33,34]

2.6. Cytotoxicity assay

The cytotoxic effect of WCCE on *C. elegans* is shown in Fig. 5. The *C. elegans* show a lower preference towards OP50 combined with WCCE which could be attributed to the presence of the secondary metabolites in WC. However, the CI of worms fed with OP50 combined with WCCE was comparable to the worms fed with OP50, which is a normal food for the growth of *C.*

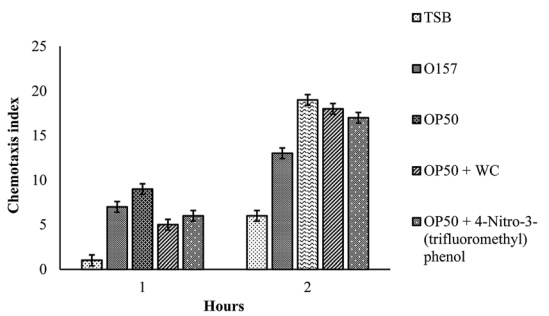

Fig. 5 Chemotaxis assay of WCCE.

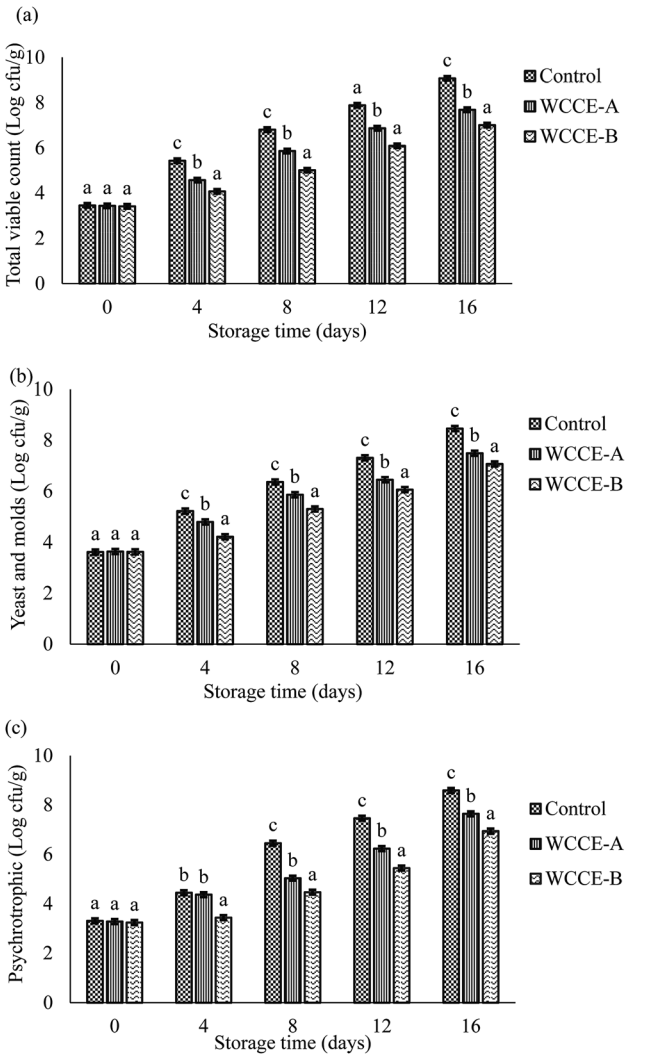

Fig. 6 Effect of white cabbage extract A and B on (a) total viable count, (b) yeast and molds and (c) psychrotrophic aerobic bacteria in beef meat during storage at 4 °C. Values representing mean ± SD of three replicates; different letters indicate significant difference ($p < 0.05$) for each time, same letters indicate no significant difference ($p > 0.05$) for each time.

Table 3 Chemical composition of the WCCE and the physicochemical properties of the identified compounds

Name of the compound	Chemical formula	Molecular weight (Da)	Area (%)	Docking score (kcal mol^{-1}) 4PLB	LpxC	Activity	References
2-Furancarboxaldehyde	$C_5H_4O_2$	96.084	0.14	−5.84	−5.70	Antibacterial	48
4-Nitro-3-(trifluoromethyl) phenol	$C_7H_4F_3NO_3$	207.107	0.01	**−6.87**	**−6.90**	Antimicrobial and antioxidant	49
4*H*-Pyran-4-one	$C_5H_4O_2$	96.084	0.04	−5.40	−5.91	Pharmacological activity	50
Methanesulfonic acid	CH_4O_3S	96.106	0.02	−6.51	−6.56	Antioxidant	51

elegans. The identified compound, 4-nitro-3-(trifluoromethyl) phenol showed the comparable CI to OP50. Regarding the cytotoxic activity, the behavioral characteristics of WCCE showed no cytotoxic activity against *C. elegans*. As we described in Sections 3.4 and 3.5, 4-nitro-3-(trifluoromethyl)phenol is a potential compound responsible for the antimicrobial and antioxidant potential of WC. To the author's best knowledge there are no previous reports regarding the cytotoxic activity of WC.

2.7. Application of extract on raw beef meat

2.7.1. Microbiological analysis of beef meat. The growing awareness and concern regarding the safety and quality of meat and meat products have led to numerous developments for the preservation of meat in the meat industry. For this reason, the number of chemical preservatives used, however, some of these chemical preservatives are considered carcinogenic and attributed to the risk of hypertension and heart diseases.[7] Thus, the employment of natural preservatives substitute these chemical preservatives and may be of great interest to the meat industry. Thus, we evaluate the ability of WC extract for the preservation of beef meat under refrigeration storage. The WCCE was selected for the shelf-life study as a result they exhibited excellent antimicrobial, antioxidant activities in both analysis with thermostability. The microbial changes (total viable count (TVC), psychrotrophic bacteria, and yeast and molds) obtained by studying the beef meat treated with WCCE-A and WCCE-B during storage at refrigerated temperatures are shown in Fig. 6a–c. The results showed that the microbial population (TVC, psychrotrophic bacteria and yeast and molds) decreased significantly with the incorporation of the natural extract and longer storage time. Under the same storage conditions, meat samples treated with WCCE-B showed better results compared to WCCE-A and control groups. Control samples exhibited the most rapid increase in the level of microorganisms. By the end of storage day, the microbial population increased to 7 log CFU per g which is considered as the upper acceptable microbiological limit for fresh meat.[5] The incorporation of the natural extract was effective against TVC and shows a similar tendency towards psychrotrophic bacteria, however, it was less sensitive towards yeast and molds. Antimicrobial and antioxidant activities are affected by many factors, including pH, microorganism, food component, temperature, and natural component properties. Taken together, the mean values of all the tested microorganisms for control as well as treated samples increased significantly ($p < 0.05$) at each subsequent interval. However, all the microorganisms tested in this study were susceptible to WCCE and microbial counts of all analyzed microorganisms were lower in beef meat samples treated with WCCE-B and they could be used for delaying the bacterial growth and prolong the shelf life of beef meat.

Table 4 Effects of WCCE on the pH, TBARSs and moisture values of beef meat during storage at 4 °C[a]

Quality attributes	Treatments	Storage time (days) at 4 °C 0	4	8	12	16
pH	Control	5.84 ± 0.02^{a}	5.88 ± 0.02^{a}	6.12 ± 0.06^{a}	6.45 ± 0.05^{a}	6.59 ± 0.02^{a}
	WCCE-A	5.82 ± 0.03^{a}	5.94 ± 0.01^{a}	5.96 ± 0.01^{a}	5.92 ± 0.01^{b}	5.68 ± 0.02^{b}
	WCCE-B	5.79 ± 0.02^{a}	5.79 ± 0.01^{a}	5.84 ± 0.01^{a}	5.78 ± 0.01^{b}	5.62 ± 0.01^{b}
Moisture (%)	Control	43.74 ± 0.02^{a}	41.00 ± 0.03^{a}	39.10 ± 0.02^{bc}	37.06 ± 0.07^{a}	31.25 ± 0.09^{b}
	WCCE-A	43.39 ± 0.06^{a}	41.99 ± 0.0^{a}	39.69 ± 0.01^{b}	36.69 ± 0.05^{b}	31.95 ± 0.05^{b}
	WCCE-B	43.92 ± 0.04^{a}	41.25 ± 0.05^{a}	40.22 ± 0.02^{a}	37.26 ± 0.05^{a}	33.08 ± 0.07^{a}
TBARs (mg MDA per kg)	Control	0.13 ± 0.01^{a}	0.43 ± 0.02^{a}	1.49 ± 0.02^{a}	1.95 ± 0.05^{a}	1.69 ± 0.06^{a}
	WCCE-A	0.12 ± 0.03^{a}	0.22 ± 0.01^{b}	0.30 ± 0.04^{b}	0.61 ± 0.03^{b}	0.55 ± 0.04^{b}
	WCCE-B	0.12 ± 0.04^{a}	0.26 ± 0.07^{b}	0.316 ± 0.03^{b}	0.52 ± 0.03^{b}	0.46 ± 0.03^{b}

[a] All values are expressed as mean ± SD of three replicates, TBARSs: thiobarbituric acid reactive substances, a–c letters are according to increasing mean values, different letters in each column for each quality analysis represent statistically significant difference ($p < 0.05$), same letters in each column for each quality analysis represents non-significant difference ($p > 0.05$).

2.7.2. Changes in pH. The results for the pH of raw beef meat samples treated with WCCE-A and WCCE-B measured during the shelf-life study for 16 days are given in Table 4. The initial pH values of control, WCCE-A, and WCCE-B treated samples ranged from 5.79 to 5.84. During storage, the pH value differed significantly ($p < 0.05$) among samples. The pH values of control samples displayed a significantly higher ($p < 0.05$) and a rise in pH values until 16 days of storage. On the other hand, the pH values of WCCE-A and WCCE-B treated beef samples slightly increased until 8 days as storage processed and then began to decrease 16 days after storage (Table 4). At the end of the storage period, the pH value was 6.59, 5.68, and 5.62 for control, WCCE-A, and WCCE-B, respectively. In general, the pH of samples treated with WCCE-A and WCCE-B remained lower than the control samples probably because of the antimicrobial activity exerted with the control of microbial growth. The results are in agreement with the previous results.[4,35] Regarding pH, the pH gradually increased in all samples. This increase in pH could be attributed to the degree of meat spoilage due to protein breakdown resulting in the accumulation of metabolites mainly amines due to microbial action during storage or endogenous enzymes such as lipase and protease that produces deamination of protein and ultimately increase in pH.[4] However, the pH of WCCE-A and WCCE-B was maintained throughout the storage period and no big changes were observed.

2.7.3. Moisture analysis. Moisture content is one of the major components of most food products including the meat were analyzed. Alteration in moisture content may cause significant variations in the stability and quality of food. Overall, moisture content gradually decreases in all the meat samples with and without WCCE treatment during the storage period (Table 4). This decrease in moisture content could be attributed to the evaporation of moisture from meat in refrigeration and our result is in agreement with the previously reported studies of Abdolghafor and Saghir,[36] in buffalo meat.[37] Overall the moisture generally decreased during refrigeration irrespective of the type of meat.[37,38] However, the moisture loss was significantly minimized ($p < 0.05$) in WCCE-B treated samples as compared to control and WCCE-A.

2.7.4. Lipid oxidation. The effect of WCCE on lipid oxidation of beef meat was investigated during storage of 16 days at 4 °C, as depicted in Table 4. The values of TBARS gradually increased with the storage time in all samples, however, the application of WCCE caused a significant ($p < 0.05$) reduction of TBARS value as compared to control samples. In particular, TBARS values of samples treated with WCCE-B remained stable during the first 8 days of storage and far from control values. These results illustrate the efficacy of WCCE towards lipid oxidation in beef meat. The TBARS values of all treatments were significantly lower (remains below the minimum threshold value, *i.e.* 1 mg of malonaldehyde per kg of meat) than in the control, demonstrating that the extracts effectively protected towards lipid oxidation of raw beef. This increase in TBARS values might be due to lipid oxidation and the production of volatile metabolites. Meat and meat products are vulnerable to lipid oxidation because the process involves the oxidation of membrane-abundant polyunsaturated fatty acids resulting in the formation of malonaldehyde. The WC extract used in this study were rich in phenolic compounds such as phenolic acids, flavonoids, glucosinolate, and their hydrolytic products.[33,39] The phenolic compounds of WC extract might be attributed to the inhibition of the lipid oxidation process in foodstuffs, because of the inhibitory activity of phenolic compounds towards the formation and propagation of free radical reaction through chelation of transition metal ions, specifically those of iron and copper.[40] Similar results for the effective activity of phenolic compounds towards inhibiting the lipid oxidation were provided by Lee and Ahn[41] and Khan *et al.*[4] for the storage of turkey.

2.7.5. Color deterioration during refrigerated storage of beef meat. Color plays a vital role in both quality and consumer preference in meat and meat products. The color (redness, yellowness, and lightness) of meat products considered to be one of the most enlightening parameters by which consumers judge their acceptability. Among the color values, redness is the most important parameter in evaluating meat oxidation, as a decrease in redness reduces the acceptability of the meat products to the consumer. Effect of WCCE-A and WCCE-B on

Table 5 Changes in color parameters of beef meat treated with WCCE during storage at 4 °C[a]

		Storage time (days) at 4 °C				
Treatments	Parameters	0	4	8	12	16
Control	L^*	54.97 ± 0.06^a	50.30 ± 0.04^a	45.35 ± 0.05^a	39.29 ± 0.03^a	35.41 ± 0.02^a
WCCE-A		55.61 ± 0.01^a	53.94 ± 0.02^b	46.60 ± 0.03^b	42.96 ± 0.04^b	39.84 ± 0.01^b
WCCE-B		55.25 ± 0.03^a	54.23 ± 0.05^b	48.83 ± 0.01^c	45.16 ± 0.01^c	42.49 ± 0.06^c
Control	a^*	15.39 ± 0.02^a	12.17 ± 0.07^b	10.59 ± 0.04^a	7.18 ± 0.03^b	5.33 ± 0.01^b
WCCE-A		15.69 ± 0.05^a	11.24 ± 0.02^a	7.53 ± 0.02^c	3.77 ± 0.07^a	1.27 ± 0.04^a
WCCE-B		15.59 ± 0.05^a	11.29 ± 0.02^a	8.07 ± 0.05^b	5.26 ± 0.03^a	3.89 ± 0.07^a
Control	b^*	6.10 ± 0.02^a	4.36 ± 0.02^a	03.56 ± 0.06^a	02.14 ± 0.01^a	-01.26 ± 0.02^a
WCCE-A		6.51 ± 0.07^a	5.34 ± 0.02^b	05.72 ± 0.04^b	02.48 ± 0.06^a	0.65 ± 0.06^b
WCCE-B		6.14 ± 0.06^a	5.68 ± 0.04^b	05.57 ± 0.02^b	04.58 ± 0.03^a	2.81 ± 0.04^b

[a] All the values are mean ± SD, L^*: lightness, a^*: redness, b^*: yellowness, letters a–c are given according to the increasing mean values, values for each parameter sharing same letter in each column represents non significance difference at ($p > 0.05$), values for each parameter sharing different letter in each column represents significance difference at ($p < 0.05$).

Table 6 Texture profile analysis of WCCE on muscle of beef meat during storage at 4 °C[a]

Days	Treatments	Texture parameters				
		Hardness	Cohesiveness	Springiness	Chewiness	Gumminess
0th	Control	405 ± 0.02[a]	0.64 ± 0.03[a]	1.57 ± 0.01[a]	4.00 ± 0.05[a]	259 ± 0.03[a]
	WCCE-A	897 ± 0.04[c]	0.53 ± 0.01[a]	1.71 ± 0.03[a]	8.58 ± 0.06[c]	530 ± 0.04[c]
	WCCE-B	637 ± 0.05[b]	0.59 ± 0.03[a]	1.51 ± 0.03[a]	5.60 ± 0.04[b]	381 ± 0.05[b]
4th	Control	230 ± 0.04[a]	0.74 ± 0.00[a]	3.57 ± 0.02[b]	5.00 ± 0.00[a]	400 ± 0.00[a]
	WCCE-A	640 ± 0.01[b]	0.65 ± 0.00[a]	4.40 ± 0.04[c]	9.00 ± 0.00[b]	430 ± 0.00[b]
	WCCE-B	935 ± 0.03[c]	0.67 ± 0.00[a]	2.35 ± 0.05[a]	10.10 ± 0.02[c]	470 ± 0.00[c]
8th	Control	260 ± 0.03[a]	0.65 ± 0.05[a]	1.92 ± 0.01[a]	3.50 ± 0.03[a]	183 ± 0.05[a]
	WCCE-A	1085 ± 0.05[c]	0.50 ± 0.04[a]	1.54 ± 0.03[a]	10.2 ± 0.05[b]	674 ± 0.06[c]
	WCCE-B	770 ± 0.04[b]	0.67 ± 0.05[a]	1.88 ± 0.02[a]	10.1 ± 0.03[b]	546 ± 0.07[b]
12th	Control	1410 ± 0.06[b]	0.55 ± 0.03[a]	1.94 ± 0.04[a]	16.4 ± 0.01[b]	862 ± 0.03[b]
	WCCE-A	1410 ± 0.03[b]	0.55 ± 0.01[a]	1.94 ± 0.03[a]	16.4 ± 0.04[b]	862 ± 0.03[b]
	WCCE-B	775 ± 0.04[a]	0.58 ± 0.03[a]	1.44 ± 0.05[a]	7.1 ± 0.03[a]	503 ± 0.02[a]
16th	Control	595 ± 0.05[b]	0.59 ± 0.02[a]	2.93 ± 0.03[c]	10 ± 0.01[a]	348 ± 0.04[a]
	WCCE-A	1225 ± 0.06[c]	0.52 ± 0.06[a]	1.65 ± 0.01[b]	10.3 ± 0.06[a]	637 ± 0.05[b]
	WCCE-B	495 ± 0.03[a]	0.49 ± 0.02[a]	0.74 ± 0.05[a]	08.0 ± 0.03[a]	650 ± 0.05[b]

[a] All the values are mean ± SD, letters a–c are given according to the increasing mean values, values for each parameter sharing same letter in each column represents non significance difference at $p > 0.05$, values for each parameter sharing different letter in each column represents significance difference at $p < 0.05$.

color stability (lightness (L^*), redness (a^*), and yellowness (b^*)) of raw beef were depicted in Table 5. The L^* value of the raw beef sample was altered slightly by the addition of WCCE-A and WCCE-B extract. The initial L^* values for the meat sample were documented 54.97–55.61 for all samples and it was found to be decreased ($p < 0.05$) as storage time progressed (Table 5). The final L^* values for the beef meat samples were in the range of 35.41–42.49 for all samples. The redness (a^*) values of beef meat samples was observed initially as 15.39–15.69 and decreased to 1.27–5.33 during storage for all sample as depicted in Table 6. A significant decrease in the L^* values was observed for all samples at the end of storage. Few authors have described that L^* values in meat products are mainly attributed to the moisture and fat content (the water and free fat in the surface affects the light reflection) because both factors make the product lighter colored. Like redness and lightness, the yellowness also had a declining trend with an increase in storage time and this decrease was more pronounced for control as compared to treated samples. Similar data for the storage period were reported by Khan *et al.*[4] and Bazargani-Gilani *et al.*[5] After the 8th day of storage, most of the color values of the WCCE-B treated meat sample was steadily changed as compared to WCCE-A and control samples. The change in color values could be attributed to the oxidation of meat. Although the addition of WCCE caused initial changes in the surface of the color of the raw beef as compared to control, these changes may be acceptable to consumers because many herb extracts are traditionally used for coloring and curing meat in Asian countries.

2.7.6. TPA analysis. Table 6 depicts the effect of WCCE on the textural properties of meat. For the five textural parameters analyzed, the control sample and those treated with WCCE exhibited similar values ($p < 0.05$). This result shows that the WCCE treatment did not have any effect on the textural properties of meat. The only textural parameter affected was hardness, the meat sample treated with WCCE-A and control sample being softer than the others, while WCCE-B samples exhibited significantly higher hardness value. The hardness abruptly drops during the 4th days of storage in all meat samples. In the next days of storage until day 8th, all samples show a small decrease in hardness. Refrigeration of meat slows down the decrease in all samples, however, it was significantly less in meat samples treated with WCCE-B. Based on the meat textural properties the results showed gradual softening of meat, hardness if the sample decreases during storage, while the most significant decrease of hardness observed during the first 4 days of storage. Hardness is one of the main factors deciding the commercial value of meat. The change in hardness directly influences the chewiness which in turn influences the springiness of meat during texture profile analysis.[42] The decrease in chewiness directly related to the softening of meat due to the decrease in hardness, however, lower chewiness contributes to better consumer perception. Taken together, during the 8 days of storage WC-B treatment was sufficiently successful in maintaining the meat quality by preventing microbial growth.

Recently, the use of natural preservatives has become more popular, as compared to synthetic antimicrobial and antioxidant agents. The increased demand for naturally occurring preservatives has led to the manipulation of more effective and safe compounds in the meat industry. Therefore, WCCE could be a potential natural antimicrobial and antioxidant agent in the meat industry. However, the use of high concentrations of natural extract may cause adverse effects on the organoleptic properties of meat and meat products. Thus, further studies are required to determine the effective concentrations of these extracts to achieve antimicrobial and antioxidant activities in

meat and meat products without adversely affecting the organoleptic properties.

3. Materials and method

3.1. Plant materials

Fresh WC was acquired from a local supermarket (Chuncheon, South Korea). The fresh WC (leaves and stem) was washed and cut into pieces and dried in a drying oven at 75 °C for 24 h. The dried WC was then finely ground using an electric blender (Shinil Mixer model SFM-40WS, Wonikov Co., Ltd. Korea) and stored at room temperature until extracted.

3.2. Preparation of vegetable extract

The dried WC was extracted with six solvents, independently *viz.* ethyl ether, ethanol, methanol, chloroform, dichloromethane, and toluene. Concisely, 2 g of the dried powder was soaked into the respective solvent for 24 h at 37 °C with 200 rpm. After incubation, glass bottles were kept in a desiccator for approximately 2 days to evaporate the solvent. After solvent evaporation water was added into polar solvents and DMSO in non-polar solvents and the final extract was collected as described in our previous study.[33]

3.3. TPC, TFC and antioxidant activities of vegetable extracts

3.3.1. Total phenol content (TPC). TPC was analyzed spectrophotochemically (at 765 nm) using the Folin-Ciocalteu method[43] and expressed as mg of gallic acid equivalents (GAE) per g dried vegetable, based on a standard curve generated with gallic acid. All measurements were performed in triplicate.

3.3.2. Total flavonoid content (TFC). TFC was analyzed using the colorimetric assay method.[33] The absorbance readings were taken at 415 nm and TFC was expressed as mg of quercetin equivalents (QE) per g dried vegetable. Quantification was based on a standard curve generated with quercetin. All measurements were performed in triplicate.

3.3.3. Gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis was conducted by using An Agilent 7890 network system and 5975C inert mass selective detector according to the method of Rubab *et al.*[33]

3.3.4. Antioxidant activity. The antioxidant activity of the WC extract was assessed by two commonly used assay for plants, 1-diphenyl-2-picrylhydrazyl (DPPH) assay, and ABTS free radical assay according to the previously reported methods with some modifications.[33]

3.4. Docking method

The molecular docking studies were performed using Argus Lab 4.0.1 (Mark Thompson and Planaria Software LLC) and BIOVIA Discovery Studio 2016 (Accelrys Software Inc., San Diego, CA, USA) software. The X-ray crystallographic structure of LpxC (PDB ID: 3U1Y) and novel bacterial topoisomerases inhibitors (PDB ID: 4PLB) were downloaded from the protein data bank (https://www.rcsb.org/) and the compounds (ligands) structure was prepared using the ACD/ChemSketch based on the canonical SMILES procured from NCBI (https://www.ncbi.nlm.nih.gov/pccompound). The co-crystalized ligand or site finder protocol was used to define the active site for docking. The poses are chosen according to the interaction between the ligand and the receptor protein which depends on the number of hydrogen bonds, distance, and binding energy.

3.5. *In vitro* antimicrobial activity

3.5.1. Microorganism and culture preparation. A total of seven bacterial strains and four fungal strains obtained from the American Type Culture Collection (ATCC) and Korean Collection for Type Culture (KCTC) were tested to analyze the antimicrobial potential of WC extracts. The test microorganisms are; three Gram-negative pathogen bacteria (*Salmonella enterica typhimurium* ATCC 14028, *Escherichia coli* ATCC 43894, *Escherichia coli* ATCC 35150), four Gram-positive pathogens (*Staphylococcus aureus* ATCC 13150, *Staphylococcus aureus* ATCC 12600, *Listeria monocytogens* ATCC 19118, and *Bacillus cereus* ATCC 14579) and four fungi (*Aspergillus fumigatus* KCTC 6145, *Aspergillus flavus* var. *flavus*. KCTC 6143, *Aspergillus niger* KCTC 6317 and *Candida albicans* KCTC 7965). Each bacterial strain was subculture in TSB at 37 °C and fungal strains in MRS broth at 30 °C for 16–18 h. All microorganism's growth was harvested using 0.1% sterilized BPW, and its absorbance was measured at 600 nm and diluted to obtain a final concentration of 10^8 CFU mL^{-1} using a spectrophotometer.

3.5.2. Disk diffusion test. The antimicrobial assay was performed using a disc diffusion assay with some modifications.[44] Briefly, 100 μL of all bacterial strains were spread on the MHA and fungal strains on the MRS agar with the help of a spreader. Sterile paper disc (8 mm in diameter) were infused with 100 μL of WC extract concentration (33 mg mL^{-1}) and aseptically put on the agar surface. Spread plates were then kept at ambient temperature for 30 min to allow diffusion of antibiotics prior to incubation at 37 °C for 12–16 h. Antibiotic, ampicillin with a concentration of 30 μg mL^{-1}, and DMSO were used as a positive and negative control, respectively. Microorganism growth inhibition was measured as the diameter of the zone of inhibition surrounding the disc (mm). Three measurements were taken from three different directions to measure the average diameter of the zone of inhibition. The observation was taken after 12 h of incubation for the zone of inhibition (including the diameter of the disk). The presence of inhibition zones was measured, documented, and taken a demonstration for antimicrobial activity, and values < 8 mm were considered as not active against the test microorganisms. All tests were performed in triplicate.

3.5.3. Thermostability of the antimicrobial compound. The thermostability of the vegetable extracts was evaluated using the method of our previous study.[45] Briefly, 1 mL of the chloroform extract of WC was incubated at 95 °C for different times (5, 45, and 90 min). The extracts were preserved at 4 °C for further investigation for the potential of antimicrobial properties. The antimicrobial activity of the heat-treated extracts was evaluated by the disc diffusion method as described above in Section 2.5.2.

3.5.4. Determination of MIC. Minimum inhibitory concentration (MIC) values were determined by the growth curve assay. The growth curve assay for each extract was carried out by serial dilution method, starting from a concentration of 3.30 to 33 mg mL^{-1}, inoculated with 100 μL of a bacterial suspension at a density of 10^2 CFU mL^{-1} and incubated at 37 °C for 8 h. The growth of the bacteria was observed at turbidity determined by the spectrophotometer at the optical density (OD_{600} nm).

3.5.5. Cytotoxicity assay. The cytotoxic effect of WCCE was on *C. elegans* was determined using chemotaxis assay by following the previously reported methodology.[45] Chemotaxis index (CI) was measured using the following formula:

$$\text{Chemotaxis index(CI)} = \frac{\text{number of worms in WCCE} - \text{number of worms in OP50}}{\text{total number of worms applied}}$$

The CI was expressed in percentage, which refers to the number of worms in the extract by the total number of worms used.

3.6. Application of extract on raw beef meat

3.6.1. Experimental design. Fresh boneless and skinless lean beef (1 kg) was purchased from a local market and immediately transferred to the laboratory in an insulated box containing ice and kept at 4 °C until use. Meat samples were then cut to 50 g portions using sterilized scissors and divided into three groups including the control group. Each group received 5 pieces of meat for further microbial and qualitative analysis for 16 days of shelf-life study. The WCCE was incorporated at various levels; WCCE-A (0.5% v/v) and WCCE-B (1.0% v/v) were dissolved in DMSO. Meat samples were dipped in the corresponding solution for 20 min. After dipping, the meat samples were allowed to drain for 1 h (approximately) under a biological safety cabinet and the samples were packed in low-density polyethylene bags (Whirl-Pak, Nasco, Fort Atkinson, WI, USA). The packed treated samples along with control were kept at 4 °C for further microbiological and chemical analysis. The analysis was performed at 4 day intervals for quality assessment of samples for 16 days of shelf-life study.

3.6.2. Microbial analysis. Microbial quality of the beef was determined at days 0, 4, 8, 12, and 16 of storage at 4 °C. Beef sample approximately 10 g was aseptically transferred to a sterile stomacher bag (Whirl-Pak) and homogenized with 90 mL of sterile buffered peptone water (BPW: 0.1%) using a bag mixer (BagMixer® 400, Interscience Co., Saint Nom la Bretéche, France) for 2.5 min. For microbial computation, 0.1 mL samples from a suitable serial dilution (1 : 10 diluent, 0.1% BPW) of beef homogenate were spread on agar plates. Total viable counts (TVC) were computed using PCA plates after incubation of 48 h at 37 °C. Yeast and molds were computed using DRBC after incubation of 3–5 days at 25 °C. Lastly, psychrotrophic bacteria were determined on PCA and incubated at 7 °C for 10 days. All the culture plates were observed visually for typical colony counts and results were expressed as log CFU g^{-1}.

3.6.3. Physicochemical analysis

3.6.3.1. Determination of pH value. The pH values of the beef were recorded in triplicate by using a pH meter (Metler-Toledo SB 8001; Metler-Toledo, Greifensee, Switzerland) equipped with a glass electrode, which was directly placed into the samples. Briefly, approximately 10 g of beef sample was homogenized with 90 mL of distilled water for 1 min in the homogenizer (Ultra-turrax, T25-S1, IKA, Staufen, Germany) and the homogenate was subjected immediately to pH determination.

3.6.3.2. Color measurements. Changes in color values of beef meat were monitored using the Minlota colorimeter (CR300; Minolta Co., Osaka, Japan) of the CIELAB system with a D65 illuminant and 90° angle throughout the storage period. The color parameters were described as lightness (L^*), redness (a^*), and yellowness (b^*) color space values. For each sample, color readings were measured three times in different positions and directions to avoid any orientation effects of the muscle fiber and were utilized as an estimate of meat discoloration.[46]

3.6.3.3. Texture profile analysis (TPA). The TPA test of beef samples was performed at room temperature using an instrumental texture analyzer (Brookfield; AMETEK GmbH, Lorch, Germany) by the procedure described previously.[33] The texture parameters (hardness, springiness, chewiness, gumminess, and cohesiveness) were derived from the force–time curve by using the software.

3.6.3.4. Lipid oxidation. Lipid oxidation was assessed using the indicative method of thiobarbituric acid reactive substances (TBARS) according to[5]. Approximately 5 g of meat was weighed and homogenized with 15 mL of deionized water and 1 mL of homogenate transferred into a separate tube. Briefly, meat homogenate (sample + 7.2% of butylated hydroxytoluene (BHT) + 15 mm thiobarbituric acid (TBA) combined with 15% of trichloroacetic acid (TCA)) were heated in boiling water for 15 min, cooled down for 10 min and absorbance was measured at 531 nm using a spectrophotometer. The results were expressed as μmol kg^{-1} per unit of the sample using 1,1,3,3-tetraethoxypropane (TEP) standard curve.

3.6.3.5. Moisture analysis. The moisture content of the beef sample was determined by using. The 3 g piece of beef was placed in for 20 min at 140 °C. The percentage decrease in weight was expressed as moisture content.[47] The measurement was replicated trice and the average was taken as the moisture content of the product.

3.7. Statistical analysis

All experiments were carried out in replicates and the average zone of inhibition and standard deviation were calculated. A comparative analysis of means was performed using the analysis of variance (ANOVA) and significant differences between the means were determined by Duncan's multiple comparison test (p-value). An ANOVA test was performed in SPSS.

4. Conclusions

The study was conducted to explore the possibilities of utilization of WC in the preservation of raw beef during refrigerated

storage. In summary, WC extract act as a promising source of antioxidant and antimicrobial against a broad spectrum of foodborne pathogens. Data suggests that WCCE containing bioactive heat-stable compounds. They could be a potential source of inhibitory substances against some foodborne pathogens as well as antioxidant agents. Based on molecular docking, this compound showed that it acts on multi targets and serves as an antibacterial agent. The research indicated that the majority of Brassica vegetables are a source of bioactive compounds with antioxidant and antimicrobial and other functions. Taken together, especially the results of MICs, the chloroform extract had better antimicrobial activities among all extracts. This is probably because the WCCE may include some active components. However, it is critical to note that, if active components are isolated and purified, their antimicrobial activities could become stronger. Also, the WCCE has the potential to become a good alternative to synthetic preservative. Comparison of control and WCCE treated beef meat samples during storage at 4 °C for 16 days showed that the addition of the extract was effective as an antibacterial agent for improving the properties of the samples from a quality viewpoint. It can be concluded that 1% was the optimum concentration of the investigated extract, effective in reducing TVC, psychrotrophic bacteria, and yeast and molds. Based on microbiological, color, and texture analysis studies, a shelf life extension of 8 days was obtained in 1% of WCCE treated beef meat, indicating 4 days longer than that of controls. The results of this work proposed that WC is a good source of bioactive components and has great prospects to prolong the shelf life of beef meat, for their antimicrobial ability towards a wide range of microorganisms, the economically sustainable extraction, and health benefits. Taken together, such natural extracts might be used as a multifunctional preservative in the meat industry.

Conflicts of interest

The authors declare no conflict of interest.

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References

1 M. Efenberger-Szmechtyk, A. Nowak and A. Czyzowska, *Crit. Rev. Food Sci. Nutr.*, 2020, 1–30.
2 S.-J. Kim, A. R. Cho and J. Han, *Food Control*, 2013, **29**, 112–120.
3 V. Sirocchi, F. Devlieghere, N. Peelman, G. Sagratini, F. Maggi, S. Vittori and P. Ragaert, *Food Chem.*, 2017, **221**, 1069–1076.
4 I. Khan, C. N. Tango and D.-H. Oh, *Int. J. Food Sci. Technol.*, 2017, **52**, 1111–1121.
5 B. Bazargani-Gilani, J. Aliakbarlu and H. Tajik, *Innovative Food Sci. Emerging Technol.*, 2015, **29**, 280–287.
6 N. B. Khadidja Mazari, C. Bekhechi and X. Fernandez, *J. Med. Plants Res.*, 2010, **4**, 959–964.
7 A. A. Mostafa, A. A. Al-Askar, K. S. Almaary, T. M. Dawoud, E. N. Sholkamy and M. M. Bakri, *Saudi J. Biol. Sci.*, 2018, **25**, 361–366.
8 M. Aziz and S. Karboune, *Crit. Rev. Food Sci. Nutr.*, 2018, **58**, 486–511.
9 R. Al Akeel, A. Mateen, K. Janardhan and V. Gupta, *Saudi J. Biol. Sci.*, 2017, **24**, 11–14.
10 R. D. Pacheco-Cano, R. Salcedo-Hernandez, J. E. Lopez-Meza, D. K. Bideshi and J. E. Barboza-Corona, *J. Appl. Microbiol.*, 2018, **124**, 126–135.
11 A. K. Pandey, P. Kumar, P. Singh, N. N. Tripathi and V. K. Bajpai, *Front. Microbiol.*, 2017, **7**, 2161.
12 F. Shahidi and P. Ambigaipalan, *J. Funct. Foods*, 2015, **18**, 820–897.
13 L. Bouarab Chibane, P. Degraeve, H. Ferhout, J. Bouajila and N. Oulahal, *J. Sci. Food Agric.*, 2019, **99**, 1457–1474.
14 S. Ahmed, M. Ahmad, B. L. Swami and S. Ikram, *J. Adv. Res.*, 2016, **7**, 17–28.
15 M. Daglia, *Curr. Opin. Biotechnol.*, 2012, **23**, 174–181.
16 F. Cabello-Hurtado, M. Gicquel and M.-A. Esnault, *Food Chem.*, 2012, **132**, 1003–1009.
17 M. E. Cartea, M. Francisco, P. Soengas and P. Velasco, *Molecules*, 2011, **16**, 251–280.
18 D. Šamec and B. Salopek-Sondi, in *Nonvitamin and Nonmineral Nutritional Supplements*, ed. S. M. Nabavi and A. S. Silva, Academic Press, 2019, pp. 195–202, DOI: 10.1016/B978-0-12-812491-8.00027-8.
19 G.-U. Seong, I.-W. Hwang and S.-K. Chung, *Food Chem.*, 2016, **199**, 612–618.
20 A. K. Verma, V. Pathak, V. P. Singh and P. Umaraw, *J. Appl. Anim. Res.*, 2016, **44**, 409–414.
21 M. Sanz-Puig, M. C. Pina-Pérez, D. Rodrigo and A. Martínez-López, *Food Control*, 2015, **50**, 435–440.
22 D. Šamec, J. Piljac-Žegarac, M. Bogović, K. Habjanič and J. Grúz, *Sci. Hortic.*, 2011, **128**, 78–83.
23 H. M. Santos Júnior, V. A. C. Campos, D. S. Alves, A. J. Cavalheiro, L. P. Souza, D. M. S. Botelho, S. M. Chalfoun and D. F. Oliveira, *Crop Prot.*, 2014, **62**, 107–114.
24 C. Cafarchia, N. De Laurentis, M. Milillo, V. Losacco and V. Puccini, *Parassitologia*, 1999, **41**, 587–590.
25 S.-H. Hu, J.-C. Wang, H.-F. Kung, J.-T. Wang, W.-L. Lee and Y.-H. Yang, *Kaohsiung J. Med. Sci.*, 2004, **20**, 591–599.
26 I. Amin and W. Y. Lee, *J. Sci. Food Agric.*, 2005, **85**, 2314–2320.
27 W. Shi, Y. Wang, J. Li, H. Zhang and L. Ding, *Food Chem.*, 2007, **102**, 664–668.
28 A. K. Jaiswal, N. Abu-Ghannam and S. Gupta, *Int. J. Food Sci. Technol.*, 2012, **47**, 223–231.
29 L. W. Yee, E. H. K. Ikram, A. M. Mhd Jalil and A. Ismail, *Malays. J. Nutr.*, 2007, **13**, 71–80.
30 R. Amorati and L. Valgimigli, *J. Agric. Food Chem.*, 2018, **66**, 3324–3329.

31 P. Kuppusamy, M. M. Yusoff, N. R. Parine and N. Govindan, *Saudi J. Biol. Sci.*, 2015, **22**, 293–301.
32 M. S. Fernandez-Panchon, D. Villano, A. M. Troncoso and M. C. Garcia-Parrilla, *Crit. Rev. Food Sci. Nutr.*, 2008, **48**, 649–671.
33 M. Rubab, R. Chellia, K. Saravanakumar, S. Mandava, I. Khan, C. N. Tango, M. S. Hussain, E. B.-M. Daliri, S.-H. Kim, S. R. Ramakrishnan, M.-H. Wang, J. Lee, J.-H. Kwon, S. Chandrashekar and D.-H. Oh, *PLoS One*, 2018, **13**, e0203306.
34 T. Hintz, K. K. Matthews and R. Di, *BioMed Res. Int.*, 2015, **2015**, 246264.
35 S.-J. Lee, H. J. Kim, S. H. Cheong, Y.-S. Kim, S.-E. Kim, J.-W. Hwang, J.-S. Lee, S.-H. Moon, B.-T. Jeon and P.-J. Park, *Process Biochem.*, 2015, **50**, 2099–2104.
36 B. Abdolghafour and A. Saghir, *Scholars J. Agric. Vet. Sci.*, 2014, **1**, 201–210.
37 S. Biswas, A. Chakraborty, G. Patra and A. Dhargupta, *Int. J. Livest. Prod.*, 2011, **2**, 001–006.
38 S. Mexis, E. Chouliara and M. Kontominas, *Innovative Food Sci. Emerging Technol.*, 2009, **10**, 572–579.
39 L. A. Ortega-Ramirez, I. Rodriguez-Garcia, J. M. Leyva, M. R. Cruz-Valenzuela, B. A. Silva-Espinoza, G. A. Gonzalez-Aguilar, M. W. Siddiqui and J. F. Ayala-Zavala, *J. Food Sci.*, 2014, **79**, R129–R137.
40 E. J. Brown, H. Khodr, C. R. Hider and C. A. Rice-Evans, *Biochem. J.*, 1998, **330**, 1173–1178.
41 E. Lee and D. Ahn, *J. Food Sci.*, 2003, **68**, 1631–1638.
42 S. Karlović, D. Ježek, M. Blažić, B. Tripalo, M. Brnčić, T. Bosiljkov and M. Šimunek, *Croat. J. Food Sci. Technol.*, 2009, **1**, 1–6.
43 T. Margraf, A. R. Karnopp, N. D. Rosso and D. Granato, *J. Food Sci.*, 2015, **80**, C2397–C2403.
44 G. da Silva Dannenberg, G. D. Funck, F. J. Mattei, W. P. da Silva and Â. M. Fiorentini, *Innovative Food Sci. Emerging Technol.*, 2016, **36**, 120–127.
45 M. Rubab, R. Chelliah, K. Saravanakumar, K. Barathikannan, M.-H. Wang and D.-H. Oh, *J. Food Process. Preserv.*, 2019, **43**, e14240.
46 C. Gajana, T. Nkukwana, U. Marume and V. Muchenje, *Meat Sci.*, 2013, **95**, 520–525.
47 G. W. Latimer, *Official methods of analysis of AOAC International*, AOAC international, 2012.
48 N. Zekeya, M. Chacha, F. Shahada and A. Kidukuli, *J. Pharmacogn. Phytochem.*, 2014, **3**, 246–252.
49 L. W. Foo, E. Salleh and S. N. Hana, *Chem. Eng. Trans.*, 2017, **56**, 109–114.
50 E. T. Ibibia, K. N. Olabisi and O. S. Oluwagbemiga, *Gas*, 2016, **9**(4), 179–182.
51 M. S. Khyade and M. B. Waman, *Pharmacogn. J.*, 2017, **9**(2), 213–220.