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Bioactive and Antimicrobial Properties of Oven-Dried Beetroot (Pulp and Peel) Using Different Solvents

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Abstract: Beetroot is a widely consumed crop all over the world and contains plenty of bioactive compounds. In this study, we analyzed the bioactive as well as the antimicrobial properties of the oven-dried beetroot (pulp and peel) using different solvents. The 50% methanolic extract yielded the highest content of total polyphenols and total flavonoids as well as the reducing power of the beetroot. The beetroot peel exhibited a higher content of total polyphenols, total flavonoids, and reducing power activity under all the extraction solvents. Mixture of methanol and water (50% *v*/*v*) extracted the highest chlorogenic acid (78.24 mg/100 g) of the dried beetroot peel, while 1,2-dihydroxybenzene was the most noteworthy phenolic compound (42.52 mg/100 g) in beetroot peel methanolic extract. The 50% methanolic extract of both peel and pulp demonstrated the highest antibacterial and anticandidal potential. These results can be helpful for beetroot consumption.

Keywords: beetroot; flavonoid; oven drying; antioxidant activity

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1. Introduction

Beetroot (*Beta vulgaris* var. rubra L.; BVr) is a member of the Chenopodiaceae family, and it grows in temperate regions. It is responsible for 20% of the world's sugar production [1] and is usually consumed in powdered form, supplemental juice, bread, pickle, as well as pureed, boiled, and even processed as jam [2]. BVr is one of the richest vegetables due to the presence of several phenols such as catechin, vanillic, caffeic epicatechin, p-hydroxybenzoic, p-coumaric, protocatechuic, ferulic, and syringic acids, and other active compounds such as betalains (betacyanins and betaxanhins), folates, flavonoids, and carotenoids [3–6]. The nutritional composition of beetroot and its role in health and diseases has been presented in Tables 1 and 2 [7–15]. These compounds possess many desirable properties such as antioxidant and antimicrobial activity, as well as anti-inflammatory, hypoglycemic, hepatoprotective, hypolipidemic, and antitumor properties [16–20]. Betanin's antioxidant activity is due to the presence of hydroxyl and cyclic amine groups, which are good hydrogen and electron donors, with the capability of stabilizing the reactive species [21]. In cultured endothelial cells, bioactive compounds, such as betanin, support maintaining the redox signaling pathways facilitated by the inflammatory reaction and put forth the anti-proliferative effects on human tumor cell lines [22,23]. Betanin is responsible for most of the total coloring matter found in the beetroot.

Table 1. Nutritional components of beetroot (*Beta vulgaris* L.) [7–9]. g = gram, µg = microgram, mg = milligram.

Nutritional Characteristics of Beetroot			
Nutrients	**Value/100 g of Edible Portion**	**Nutrients**	**Value/100 g of Edible Portion**
Total Carbohydrates	9.96	**Essential and Non-Essential Amino Acids**	
Protein	1.68	**Essential Amino Acids**	
Fat	0.18	Tryptophan	0.019
Total MUFA	0.032	Isoleucine	0.048
Total SFA	0.027	Leucine	0.068
Total PUFA	0.060	Lysine	0.058
Micronutrients		Threonine	0.047
Vitamins		Methionine	0.018
Vitamin A (µg)	2	Phenylalanine	0.046
Thiamine (mg)	0.31	Valine	0.056
Riboflavin (mg)	0.27	Histidine	0.021
Niacin (mg)	0.331	**Non-Essential Amino Acids**	
Pantothenic acid (mg)	0.145	Cystine	0.019
Vitamin B6 (mg)	0.067	Arginine	0.042
Ascorbic acid (mg)	3.6	Alanine	0.060
Folate (µg)	80	Glutamic acid	0.428
Minerals (mg)		Glycine	0.031
Sodium	77	Proline	0.042
Calcium	16	Aspartic acid	0.116
Iron	0.79	Serine	0.059
Phosphorus	38	Tyrosine	0.038
Potassium	305		
Magnesium	23		
Zinc	0.35		
Bioactive Compounds			
Phenolic Acids and Flavonoids	**Value/100 g**	**Phenolic Acids and Flavonoids**	**Value/100 g**
Ferulic acid	0.120 ± 0.005	Caffeic acid	2.40 ± 0.050
Protocatechuic acid	11.53± 0.40	Polyphenols	89.06 ± 4.62
Catechin	0.715 ± 0.018	Flavonoids	79.22 ± 2.04
Gallic acid	0.100 ± 0.003	Betacyanins	0.30 ± 0.01
Betaxanthins	80.06 ± 2.06	Chlorogenic acid	0.34 ± 0.01
Rhamnetin	2.50 ± 0.040	Coumaric acid	0.22 ± 0.10
Quercetin	0.22 ± 0.10	Apigenin	0.10 ± 0.00

Table 2. Role of beetroot (*Beta vulgaris* L) in health-related disease [10–15].

Disease	Model	Treatment	Dosage	Main Outcome	References
Hemoglobin levels	Healthy female volunteers, sample size-19	Beetroot (*Beta vulgaris* L.) for 20 days	8 g	Serum iron levels at all subjects showed up enormous increase.	[10]
LDL-C—Low Density Lipoprotein-Cholesterol	Pilot study controlled and uncontrolled hypertensive patients, sample size-19	Beetroot juice	140 mL (~800 mg NO_3)	↓ Serum LDL-C in uncontrolled patients	[11]
COPD—Chronic Obstructive Pulmonary Disease	Randomized double-blind, placebo-controlled, cross-over single dose, sample size-21	Beetroot juice NO_3-depleted beetroot juice	140 mL (~800 mg NO_3)	↓ Resting DBP	[12]
T2DM—Type 2 Diabetes Mellitus	Randomized double-blind, placebo controlled, cross-over, sample size-27	Daily consumption of beetroot juice NO_3-depleted beetroot juice	250 mL (~500 mg NO_3)	No effect on insulin resistance	[13]
Overweight and obese men	Randomized crossover, sample size-20	Beetroot juice NO_3-depleted beetroot juice	140 mL (~420 mg NO_3)	Improve postprandial endothelial function	[14]
Lung and skin cancer	The animals used were 6-week-old mice	*Beta vulgaris* (beet) root extract on Epstein-Barr virus early antigen (EBV-EA) induction using Raji cells revealed a high order of activity compared to capsanthin, cranberry, red onion skin, and short and long red bell peppers.	0.0025% beetroot extract (2.5 mg/100 mL of water)	Pulmonary tumor indicated that there was a 60% reduction of lung tumors. The combined findings indicated that beetroot was a useful cancer preventive vegetable.	[15]

Vegetable and fruit processing is responsible for the high volume of waste materials and, on a daily basis, waste management is a severe environmental problem. The waste materials procured from the agro-industries have been explored for developing the value-added products that can be utilized as functional foods in addition to the recovery of antioxidants of natural origin [24,25]. The biochemical properties of plant segments that are generally discarded can be equivalent to or even better than the portions consumed [26]. Studies have revealed that the total phenolic content (TPC) reduces in the peel (50%), followed by the crown (37%), and least in the flesh (13%) of fruits and vegetables [4]. In the production of dried vegetables, the drying techniques play a vital role as the drying process may influence the antioxidant and bioactive properties of the vegetables [27]. Several solvent systems have been used for the extraction of polyphenols from plant materials and the extraction yield is dependent on the solvent and method of extraction [28,29], so the solvent choice is very important and often a determining factor on the extraction yields [30]. The aim of this study was to examine the effect of methanolic and aqueous extract on the total polyphenol content, total flavonoid content, antioxidant activity in terms of reducing power, and antimicrobial activity of oven-dried beetroot (peel and pulp).

2. Materials and Methods

2.1. Plant Material

Fresh beetroot (*Beta vulgaris* L.) was purchased from the local vegetable market in Riyadh, Saudi Arabia. Beetroots were washed thoroughly with tap water to remove the soil, and stems were also detached. The outer surface was peeled off, and the pulp was chopped into thin slices to a thickness of 1mm and dried in a hot air oven at 40 °C for 5 days. The dried beetroot slices and peels were ground separately using an electric grinder to obtain the beetroot powder. The fine powder sample (2 g) was extracted with 20 mL of

either absolute methanol, 50% methanol (*v*/*v*), or water for 1 h using a shaker at 30 °C; then, the extract was filtered by Whatman filter No. 2 and the filtrate was stored at 4 °C until further analysis.

2.2. Total Polyphenol Content (TPC)

In this study, the TPC was analyzed according to the method described by Hayat [31]. Firstly, 25 µL of extract was mixed with 1500 µL of water and then 125 µL of undiluted Folin–Ciocalteau reagent was added to the mixture. The mixture was allowed to rest for 1 min. After that, 375 µL of 20% sodium carbonate was added to the mixture. The final volume of the mixture was increased to 2500 µL by adding 475 µL of water. Finally, the mixture was incubated for 30 min at room temperature and the absorbance was noted at 760 nm (Jasco V-630 UV-Vis spectrophotometer, Easton, MD, USA). TPC was calculated as Gallic Acid Equivalent per gram dry weight of the sample (mg GAE/g DW) against a gallic acid standard curve (0.2–1.4 mg/mL).

2.3. Total Flavonoid Content (TFC)

The TFC was analyzed according to the method reported by Hayat [31]. In total, 250 µL of beetroot extract was mixed with 1000 µL of water and then 75 µL each of 5% $NaNO_2$ (*w*/*v*) and 10% $AlCl_3$ (*w*/*v*) was added. The prepared mixture was allowed to stand for 5 min at room temperature. After that, 600 µL of water and 500 µL of 1 M NaOH were added. Blank was prepared without extract. The absorbance was measured at 510 nm (Jasco V-630 UV-Vis spectrophotometer, Easton, MD, USA). TFC was expressed as catechin equivalent per gram dry weight of the sample (mg CE/g DW) against a catechin standard curve prepared at a concentration of 0.05–0.6 mg/mL.

2.4. Reducing Power

The ferric reducing power of the sample was determined according to the method adopted from Hayat [31] with slight modifications. In total, 120 µL of beetroot extract was mixed with 1 mL buffer (0.2 M, pH 6.6) and 1 mL of potassium ferricyanide and then incubated for 20 min at 50 °C. One milliliter of trichloroacetic acid was added after the completion of the incubation time, and then, the mixture was centrifuged at 3000× *g* for 10 min at room temperature. Lastly, an aliquot (1 mL) was acquired from the supernatant, to which 1 mL of water and 200 µL of ferric chloride were added. The absorbance was noted at 700 nm (Jasco V-630 UV-Vis spectrophotometer, Easton, MD, USA). Blank was prepared without extract.

2.5. HPLC Analysis of Phenolic Compounds

Analysis of phenolic compounds (tannic acid, resorcinol, 1,2-dihydroxybenzene, chlorogenic acid, caffeic acid, vanillin, acetyl salicylic acid, 3,5-dinitrosalicylic acid, salicylic acid, and quercetin) in beetroot samples was carried out using HPLC analysis, as described earlier with some modification [32]. The HPLC (Prominence) system, Shimadzu (Kyoto, Japan), was equipped with an LC-20AB binary pump and variable Shimadzu SPD-10A UV detector. The column used was Zorbax SB-C_{18} (250 × 4.6 mm, 5 µm; Agilent, Santa Clara, CA, USA) and the mobile phase was Milli Q water (1% acetic acid, A) and MeOH (B). The binary gradient programme used was 0–10 min 15–30% B; 10–20 min 30–40% B; 20–30 min 40–50% B; 30–41 min 50–60% B; and 41–45 min 15% B. The flow rate was 1.0 mL/min. The injection volume was 10 µL, and the detector was set at 280 nm. Compounds in beetroot samples were identified by comparing their peak retention time with those of standards. All samples were analyzed in duplicate.

2.6. Antimicrobial Activity of Beetroot Extracts

Antimicrobial activity of beet root pulp and peel extracts was assessed against *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Candida albicans* using agar well diffusion assay [33]. Briefly, 0.1 mL of overnight grown cultures

was spread on to Mueller Hinton agar (MHA) plates, agar wells were punched, and the prepared extracts at a concentration of 6 mg/mL were loaded in each well. Solvent (5% DMSO) and Mueller Hinton broth (MHB) were used as negative controls, and antibiotics were used as positive controls. Plates were incubated for 18–24 h at 37 °C and observed for halo zones of inhibition around the well. All the samples were analyzed in triplicate.

2.7. Assessment of Minimum Inhibitory Concentration (MIC)

MIC of the beetroot extracts (methanol and aqueous) was determined using the microbroth dilution method described previously [34].

2.8. Statistical Analysis

All the experiments, unless otherwise stated, were performed in triplicate, and the results were presented as means ± SD (standard deviation). The differences among the treatment groups were analyzed using one way analysis of variance (ANOVA) at a significance level of $p \leq 0.05$. If significant differences were found, a post-hoc analysis using Duncan's multiple range tests was performed at a confidence interval of 95% by SAS (Version 9.2, 2000–2008; SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

3.1. Effect of Extraction Solvents on Total Polyphenol Content

Figure 1 shows the effect of extraction solvents, i.e., 50% methanol (*v*/*v*), methanol, and water, on the total polyphenol content of beetroot peel and pulp. As can be seen, the 50% methanol extract showed the significantly ($p < 0.05$) highest total polyphenol content (TPC), which was followed by water extract and then methanol extract for both pulp and peel. For example, a TPC of 24.04, 7.67, and 14.89 mg GAE/g dry sample was recorded for the 50% methanolic extract, methanolic extract, and water extract of beetroot peel, respectively. The beetroot pulp showed a lower TPC than the peel for all the solvents. For instance, the TPC of the methanolic extract of beetroot peel and pulp was 7.67 and 3.12 mg GAE/g dry sample, respectively. The TPC of the juice of 7 beetroot varieties ranged from 0.885 to 1.29 mg catechin equivalent/mL [35]. Another study reported the TPC of 6 beetroot cultivars from 5.64 to 19.09 mg GAE/g, grown under conventional and organic conditions [36].

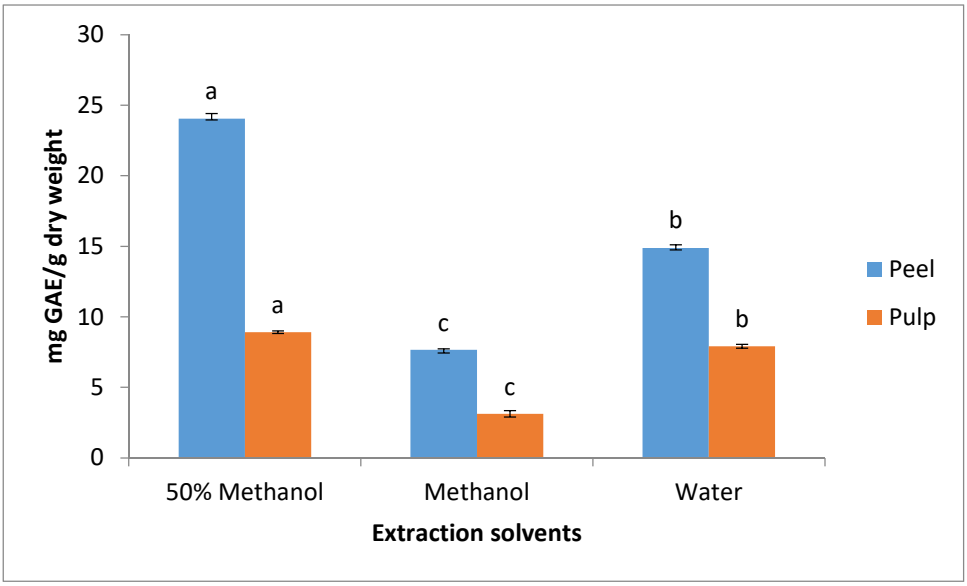

Figure 1. The total polyphenol content of beetroot peel and pulp. The different letters on bars show the significant differences among the extracts for peel and pulp individually ($p < 0.05$).

3.2. Effect of Extraction Solvents on Total Flavonoid Content

The extraction solvents significantly affected the total flavonoid content (TFC) of the beetroot samples. As depicted in Figure 2, the 50% methanolic solvent exhibited the highest extraction of total polyphenols from both the peel and pulp of the beetroot. The 50% methanol, methanol, and water extract gave a TFC of 3.50, 2.49, and 3.01 mg CE/g dry beetroot pulp, respectively. Similarly, like TPC, the TFC was also higher in beetroot peel as compared to the beetroot pulp samples as extracted by all the above-mentioned solvents.

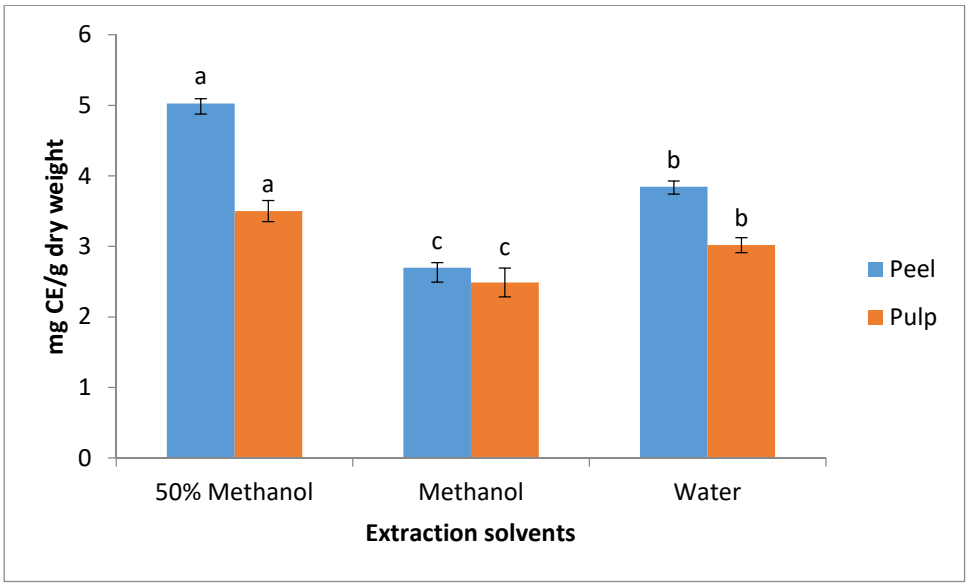

Figure 2. The total flavonoid content of beetroot peel and pulp. The different letters on bars show the significant differences among the extracts for peel and pulp individually ($p < 0.05$).

3.3. Effect of Extraction Solvents on the Reducing Power

The reducing power of beetroot peel and pulp as extracted by different solvents is shown in Figure 3. The reducing power of beetroot peel and pulp extracts extracted with 50% methanol was significantly higher compared to those extracted by methanol and water. The absorbance at 700 nm for beetroot peel extracted by 50% methanol, methanol, and water was recorded as 1.79, 1.47, and 1.60, respectively. A similar trend was also found for the beetroot pulp. The reducing power of beetroot pulp extracts was in the following order: methanol < water < 50% methanol. The beetroot peel exhibited a higher reducing power compared to the beetroot pulp for all the extracts. These results echoed the aforementioned results of TPC and TFC. Another study also reported a high correlation between ferric reducing antioxidant power (FRAP) and TPC of beetroot slices [37]. Our results are in accordance with the findings of Tomasz Sawicki et al. [38], who reported a higher antioxidant activity of beetroot peel compared with the beetroot flesh.

3.4. HPLC Analysis of Phenolic Compounds

The effect of different extracting solvents on the phenolic compounds analyzed by High Performance Liquid Chromatography (HPLC) is shown in Table 3. The mixture of methanol and water (50% *v*/*v*) extracted the highest chlorogenic acid (78.24 mg/100 g) of the dried beetroot peel, whereas 1,2-diphydroxybenzene (1,2-DBH) was the second highest extracted phenolic compound (42.52 mg/100 g). In general, resorcinol and 1,2-DBH were present in almost all the extractions of beetroot samples. Beetroot peel extracted with 50% methanol had the highest total phenolic compounds (106.24 mg/100 g) compared to the beetroot pulp (69.84 mg/100 g), whereas, among all, the methanolic extract of the beetroot pulp showed the lowest content of total phenolic compounds (18.5 mg/100 g). Notably, the chlorogenic acid was found in beetroot peel water extract (1.86 mg/100 g), and in 50%

methanol extract of beetroot peel (78.3 mg/100 g) and pulp (8.97 mg/100 g), while it was not detected in other extracts. Georgiev et al. [39] reported the presence of chlorogenic acid (0.018 mg/g) in the beetroot plant and absence in its hairy root extract, whereas a recent study of Dailey and Vuong [40] stated that a combination of organic solvent with 50% water gave higher yields of phenolic compounds.

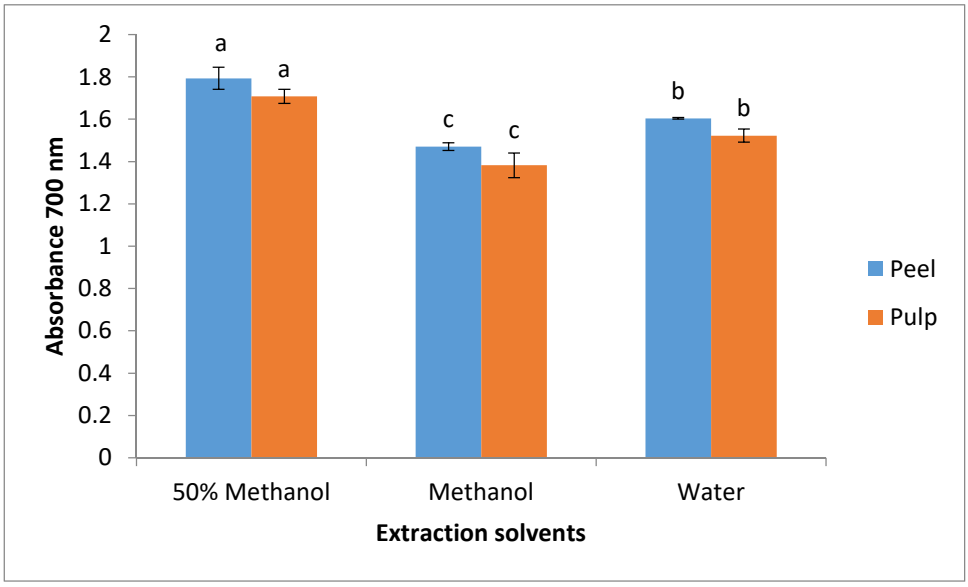

Figure 3. Reducing power of beetroot peel and pulp. The different letters on bars show the significant differences among the extracts for peel and pulp individually ($p < 0.05$).

Table 3. Analysis of phenolic compounds by HPLC (mg/100 g) dry weight. ND = Not detected.

Sample	Extraction Solvent	Resorcinol	1,2-DHB	Chlorogenic Acid	Vanillin
Peel	Methanol	11.69 ± 0.31	42.52 ± 0.16	ND	ND
Peel	Water	39.22 ± 0.35	1.52 ± 0.07	1.86 ± 0.16	ND
Peel	50% Methanol	23.31 ± 0.11	3.50 ± 0.33	78.24 ± 0.19	1.19 ± 0.29
Pulp	Methanol	10.68 ± 0.55	2.94 ± 0.15	ND	4.86 ± 0.23
Pulp	Water	23.41 ± 0.09	5.76 ± 0.31	ND	6.03 ± 0.37
Pulp	50% Methanol	26.36 ± 0.19	27.88 ± 0.17	8.97 ± 0.21	6.61 ± 0.31

3.5. Antibacterial and Antifungal Potential of Beetroot Pulp and Peel

Antibacterial and antifungal potential of the extracts of peel and pulp of beetroot was assessed in the current investigation. All the extracts demonstrated promising antimicrobial activity against the test pathogens, as shown in Figure 4. Methanol-aqueous (50%, *v*/*v*) extracts of both peel and pulp demonstrated the highest antibacterial and anticandidal potential. Pulp (50% methanolic extract) extract showed 16, 15, 21, 18, 16 mm zone of inhibition against *S. aureus*, *L. monocytogenes*, *E. coli*, *P. aeruginosa* and *C. albicans*, respectively. Similarly, beetroot peel (50% methanolic extract) extract exhibited inhibition zones of 15, 15, 17, 18, 15 mm against *S. aureus*, *L. monocytogenes*, *E. coli*, *P. aeruginosa*, and *C. albicans*, respectively. Methanol extracts demonstrated slightly better antimicrobial action compared to the aqueous extracts. An antibiotic (Chloramphenicol) and an antifungal (fluconazole) were used as positive controls for the antimicrobial studies. Chloramphenicol exhibited halo zones ranging from 17 to 25 mm against test bacteria, while fluconazole showed 24 mm zone of inhibition against *C. albicans*. All the extracts were observed to demonstrate higher zones of inhibition against Gram-negative bacteria than the Gram-positive bacteria.

This could be attributed to the difference in the structure of cell walls of the Gram-negative bacteria and the Gram-positive bacteria. The cell wall of the Gram-positive bacteria is made of a thick layer of peptidoglycan with covalently attached teichuronic and teichoic acid, making them less susceptible to the action of the test agent [41,42]. Further, antimicrobial activity was determined in terms of the MIC as depicted in Table 4. Beetroot pulp (methanol and 50% methanolic extract) showed the lowest MIC values of 4 mg/mL against all the test pathogens, while the peel (50% methanolic) extract demonstrated MICs of 8 mg/mL against Gram-positive bacteria (*S. aureus* and *L. monocytogenes*), *C. albicans*, and 4 mg/mL against *E. coli* and *P. aeruginosa*. Not much difference was observed in MIC values of the extracts tested. Similarly, minimum bactericidal concentration (MBC) values ranged from 4 to 16 mg/mL. Our findings are in agreement with the observations made for the methanolic extract of beetroot pomace [43].

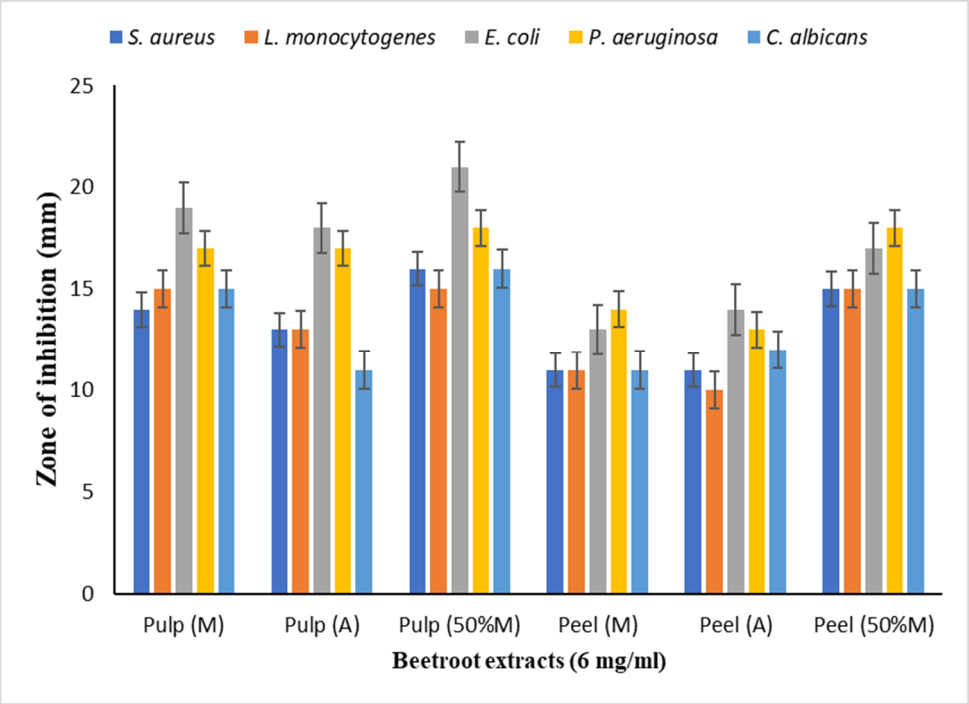

Figure 4. Antimicrobial activity of the beetroot extracts. (M), methanol; (A), water; (50% M), 50% methanol.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of beetroot peel and pulp extracts against test pathogens. MIC and MBC values are given in mg/mL.

Sample	Extraction Solvent	Pathogens									
		S. aureus		*L. monocytogenes*		*E. coli*		*P. aeruginosa*		*C. albicans*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Peel	Methanol	8	16	8	8	4	8	8	8	8	16
Peel	Water	8	16	8	16	8	16	8	8	8	16
Peel	50% Methanol	8	16	8	16	4	8	4	8	8	16
Pulp	Methanol	4	8	4	8	4	8	4	8	4	8
Pulp	Water	8	8	8	16	8	16	8	16	8	16
Pulp	50% Methanol	4	8	4	8	4	8	4	8	4	8

4. Conclusions

The impact of different extraction solvents was evaluated on the bioactive and antimicrobial properties of the peel and pulp. Results showed that all extraction solvents

impacted the properties of beetroot significantly. Among solvents, the 50% methanolic extract showed the highest bioactive characteristics. While the beetroot pulp exhibited lower antioxidant properties as compared to the beetroot peel. Chlorogenic acid was only found in the beetroot peel water extract and the beetroot peel and pulp 50% methanolic extract, while it was not detected in all other extracts. The extracts demonstrated the antimicrobial activity against all the test pathogens; the highest activity was shown by the extract prepared in 50% methanol for both peel and pulp. The higher zones of inhibition were observed against the Gram-negative bacteria than the Gram-positive bacteria. Extracts exhibited MIC ranging from 4 to 8 mg/mL against the bacterial and fungal pathogens. These results might be helpful for the processing and consumption of the beetroot.

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