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Research Article
Enhancement of Antioxidant Activity, Phenolic Contents and Protective Effects of *Beta vulgaris* Root Extract Against DNA Damage by Fermentation using Lactic Acid Bacteria

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Abstract
Background and Objective: Beetroot is a vegetable and its juice was used in folk medicine to treat constipation, dandruff, gut and joint pain. It also has been used as antihypertensive, hypoglycaemic and showed antioxidant activity. This study aimed to investigate the effect of fermentation of red beetroot on the total phenolic content, antioxidant activity and DNA damage protection. **Materials and Methods:** The red beetroot (*Beta vulgaris*) root extract was fermented using different strains of lactic acid bacteria (*Lactobacillus plantarum* P108 and *Lactobacillus acidophilus* P110). DPPH, (ABTS) cation and superoxide anion assays were used in assessing the antioxidative potential of fermented beetroot. Total phenolics content of fermented *B. vulgaris* was estimated using Folin Ciocalteu reagent. Protection against DNA damage induced by the bleomycin iron complex was also studied. **Results:** The results showed that, both methanolic and aqueous extracts of the fermented *B. vulgaris* root had significantly higher ($p<0.05$) total phenolic content compared to the unfermented extract. Both methanolic and aqueous extracts of the fermented *B. vulgaris* root had significantly higher ($p<0.05$) ($DPPH^{\cdot}$, $ABTS^{+}$ and superoxide anion) radical scavenging activity compared to the unfermented extract. Also, the fermented extract exhibited greater protection against oxidative DNA damage induced by bleomycin than the unfermented extract. **Conclusion:** The present study concluded that fermented *B. vulgaris* root might be used as a functional food and in pharmaceutical industries.

Key words: *Beta vulgaris*, antioxidant, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, bleomycin, polyphenol, fermentation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reactive Oxygen Species (ROS) includes various chemical species such as oxygen radicals (superoxide anions, OH^-) and oxygen derivatives such as; H_2O_2. They are produced during the eukaryotic cells metabolism, which involve mitochondrial electron transport, microsomal P450 and other processes[1]. Oxidative stress is generated by a disruption in balance between ROS and antioxidants. The ROS facilitated oxidative damage to macromolecules such as; lipids, proteins and DNA. They are considered one of the major causes of major diseases such as; cancer, cardiovascular diseases, arthritis and degeneration process of aging[2]. There are many endogenous antioxidant enzymes, such as; glutathione peroxidase, catalase and superoxide dismutase, which are involved in scavenging of free radicals and sustaining optimal cellular functions[3]. However, they may not be adequate to overcome the increased oxidative stress. Different synthetic antioxidants are widely used and in spite of their favorable effect, it was discovered that some of them are toxic and even carcinogenic. Consequently natural antioxidants have become important recently due to their economical cost and large availability as raw material[4,5].

The red beetroot (*Beta vulgaris*) belongs to the Chenopodiaceae family[6]. Beetroot is a vegetable and its juice was used in folk medicine to treat constipation, dandruff, gut and joint pain[7,8]. Recently red beet extract is used as antihypertensive and it showed also considerable hypoglycaemic and antioxidant activity[9]. Red beetroot contains N-containing water soluble plant pigments called betalains which are aromatic indole derivatives synthesized from tyrosine, they involve red-violet betacyanins and yellow betaxanthins. Betalains are characteristic for the plant order Caryophyllales (Centrospermae)[10]. Red beetroot also contains a large amount of phenolics, catechin, epicatechin and phenolic acids such as; caffeic, syringi, protocatechuic, p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids[11,12].

Phenolics are considered the most common antioxidants in the human diet[13]. Phenolics and betalains have a potent antioxidant activity because of the presence of hydroxyl substitution and aromatic ring in their structure which enables them to become free radicals scavenger[14,15]. Polyphenols are recognised to cure cancer, cardiovascular diseases and neurodegenerative diseases[16]. Betalains have been proved to have high bioavailability and to have high gastrointestinal tract stability in its antioxidant activity which improves its use as a functional food[17]. Cho *et al.*[18] confirmed that injection of mice subjected to beta ray radiation injected by beetroot extract minimized DNA damage of splenocytes, improved differentiation of hematopoietic stem cells, enhanced hematocrit and blood hemoglobin and improved the survival rate of lethally exposed mice. Betanin, the major betacyanin pigment of beetroot, inhibited cyclooxygenase (COX) enzymes and scavenged oxidants produced by neutrophils, during the inflammation and so have a potent anti-inflammatory activity[19].

New valuable properties of the products are added by biochemical modification by the microorganisms[20]. Recently, the antioxidant activity of many plants were enhanced by fermentation using probiotics[21]. Fermentation of vegetables and fruits beverages are used medicinally to treat lactose intolerance[22]. Fermented beverages are used also to treat colon cancer due to decrease of level of carcinogen by probiotics[23]. Lactic Acid Bacteria (LAB) are regarded as one of the most common probiotics which are non-pathogenic bacteria which are widely used in industrial applications[24]. *Lactobacillus acidophilus* is mostly used in dairy manufacture[25]. *Lactobacillus plantarum* is another type of lactic acid bacteria which has flavor improving properties and also has the advantage of tolerance of acids and bile salt[26].

In this study, the effect of fermentation of red beetroot on the total phenolic content, antioxidant activity and DNA damage protection was investigated. The data concerning effect of fermentation using lactic acid bacteria on the antioxidant activity of *B. vulgaris* root extract is not abundant. The work aimed to obtain fermented red beetroot with added-value which can be applied as a potential functional food.

MATERIALS AND METHODS

Study area: This study was carried out in the lab of the Department of Industrial Biotechnology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt, from March-August, 2018.

Plant material and extract preparation: The plant material was fresh beet root (*Beta vulgaris*), which is called red beet. It was cultivated in Egypt and was purchased from local market. The red beet roots were washed with tap water, sliced and then extracted. Maceration was used for extraction of beetroot by soaking of beetroot in distilled water and methanol as organic solvent (200 g L^{-1}) in closed container which was shaken periodically. After the extraction process is complete, the plant material is separated by filtration and all extracts were concentrated using a rotary vacuum evaporator at 40°C until the solvent had been completely evaporated.

Microorganism: The probiotic mother culture containing *Lactobacillus acidophilus* P110 and *Lactobacillus plantrum* P108 were isolated and tested for its probiotic's properties by Mahrous *et al.*[27]. They were added to sterile (De Man Rogosa and Sharpe) MRS broth then anaerobically incubated using BBL gas packs at 37°C for 18 h. Strains were stored at -80°C in MRS broth supplemented with 25% (v/v) glycerol. For routine analysis, the strains were subcultured twice in MRS broth at 37°C for 24 h. The inoculum of each bacteria was adjusted to ½ McFarland (1/2 MC = 1×CFU mL^{-1}) in order to neglect the variation of bacterial count. (½ McFarland was prepared by mixing 0.05 mL of 1.175% $BaCl_2$ with 9.95 mL of 1% sulfuric acid to obtain turbidity equal to 1.5×10^8 which have Optical Density (OD) measured at 600 nm.

Inoculum preparation and *Beta vulgaris* fermentation: Beetroot aqueous extract (mg mL^{-1}) was pasteurized at 80°C for 15 min. Then it was cooled to room temperature. The lactic acid bacteria culture was centrifuged at 6000 rpm for 15 min. The supernatant was discarded and the pellet was washed with 0.9% saline solution. About 10% (v/v) co-culture of *L. acidophilus* P110 and *L. plantarum* P108 was inoculated in 500 mL of pasteurized extract and fermented at 37°C for 24 h. After 24 h of fermentation, the extract samples were stored at refrigeration temperature[28].

Total phenolics determination: The method is based on the ability of phenols to react with Folin-Ciocalteu reagent and form chromogens, which can be recorded spectrophotometrically. Dried samples and standards were prepared in 60:40 acidified methanol/water (0.3% HCl). About 100 µL of acidified samples were added to 2.0 mL of 2% Na_2CO_3. After 2 min, 100 µL of 50% Folin-Ciocalteu reagent were added and allowed to stand at room temperature for 30 min with periodical shaking. Absorbance was measured at 750 nm on a Unico 1200 spectrophotometer, USA. The standard was gallic acid prepared in concentrations of 1.9-1000 µg mL^{-1}. The total phenolic concentrations in fermented and unfermented *B. vulgaris* root extract were evaluated as microgram of gallic acid equivalent (GAE) per/milliliter extract[29].

Antioxidant determination

DPPH radical scavenging activity: About 3.9 mL of methanol solution of DPPH radical in the concentration of (0.0634 mM) and 1 mL of fermented and unfermented *B. vulgaris* root extract 62.5, 125, 250, 500 and 1000 µg mL^{-1} were mixed and put in cuvettes. The mixture was shaken energetically and then left at room temperature for 30 min. The absorbance was measured at 517 nm. The positive control was ascorbic acid. The experiment was repeated three times and the results are presented as the mean±standard error[30].

This antioxidant activity was given as DPPH scavenging (%) and calculated as:

$$\text{DPPH scavenging (\%)} = \frac{\text{Control absorbance-Extract absorbance}}{\text{Control absorbance}}\times100$$

DPPH scavenging activity (%) was plotted versus the different treatments of beetroot extracts.

ABTS cation radical scavenging activity: The ABTS cation radical ($ABTS^{\cdot+}$) scavenging activity of the sample was analyzed using the method reported by Liu *et al.*[31]. $ABTS^{\cdot+}$ was generated by the reaction of a 7 mM aqueous solution of ABTS with 2.45 mM aqueous solution of $K_2S_2O_8$ in the dark at room temperature for 16 h. The $ABTS^{\cdot+}$ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm and equilibrated at 30°C. About 1 mL of fermented and unfermented beetroot extracts (100, 200, 400, 800 and 1200 µg mL^{-1}), was mixed with 4 mL of ethanolic solution of $ABTS^{\cdot+}$ and the absorbance was read at 734 nm using a spectrophotometer after 6 min.

The capability to scavenge the $ABTS^{\cdot+}$ was calculated using the following equation:

$$\text{ABTS radical cation scavenging activity (\%)} = \frac{A_{control}-A_{sample}}{A_{control}}\times100$$

where, $A_{control}$ is the absorbance of the blank without extract and A_{sample} is the absorbance in the presence of the extract.

Superoxide anion radical scavenging activity: A mixture containing 0.1 mL of tested extracts (0.4, 0.8, 1.2, 1.6 and 2 mg mL^{-1}), 1 mL Nitroblue Tetrazolium (NBT) solution (52 µM in 0.1 M phosphate buffer, pH 7.4) and 1 mL Nicotinamide Adenine Dinucleotide (NADH) solution (156 µM in 0.1 M phosphate buffer, pH 7.4) was prepared. After that, 100 µL of Phenazine Methosulfate (PMS) solution (20 µM in 0.1 M phosphate buffer, pH 7.4) was added. Then, the mixture was incubated at the room temperature for 5 min and the absorbance was recorded spectrophotometrically at 560 nm against the blank sample (phosphate buffer). Ascorbic acid represented the positive control[31]:

$$\text{Superoxide radical scavenging activity (\%)} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

where, A_{blank} is the absorbance of the blank without extract and A_{sample} is the absorbance in the presence of the extract.

Bleomycin-dependent DNA damage assay: About 0.5 mL of calf thymus DNA (1 mg mL^{-1}) was added to 0.05 mL of bleomycin sulphate (1 mg mL^{-1}), 0.1 mL of $MgCl_2$ (50 mM), 0.1 mL of fermented and unfermented beetroot extracts, 0.05 mL of HCl (10 mM), pyrogen-free water (0.1 mL) and mixed. After that 0.1 mL of ascorbic acid solution was added. The reaction mixture was mixed and then incubated at 37°C for 2 h with shaking. After incubation, 1 mL of 0.1 MEDTA is added to stop the reaction. DNA damage was assessed by adding 1 mL 1% (w/v) Thiobarbituric Acid (TBA) and 1 mL of 25% (v/v) hydrochloric acid followed by heating in a water bath maintained at 100°C for 15 min. The chromogenic formed was extracted into 1-butanol and the absorbance was measured[32] at 532 nm.

Statistical analysis: All data was presented as mean±standard deviations (mean±SD) of three parallel measurements. The statistical analysis of the data was performed using SPSS software packages version 15 for Windows® (SPSS Inc, Chicago, IL, USA). The one-way analysis of variance (ANOVA) and the significance of differences between sample means were calculated by Duncan's multiple range test. The $p \leq 0.05$ were regarded as significant.

RESULTS

Determination of total polyphenol content (TPC): In case of aqueous extract, the highest phenolic content (51.63 µg GAE mg^{-1}) was obtained after fermentation of *Beta vulgaris* roots with *Lactobacills acidophilus*, followed by 48.67 µg GAE mg^{-1} which was obtained with *B. vulgaris* roots fermented with *Lactobacillus plantarum* and finally the phenolic content of the non-fermented *B. vulgaris* roots (20.56 µg GAE mg^{-1}) as presented in Fig. 1.

Regarding the total phenolic content of methanolic extract of *B. vulgaris,* as shown in Fig. 2. The highest phenolic content (60.51 µg GEA mg^{-1}) was obtained after fermentation of *B. vulgaris* roots with *L. acidophilus*, followed by *B. vulgaris* roots fermented with *L. plantarum* (56.81 µg GEA mg^{-1}) and finally the phenolic content of the non-fermented *B. vulgaris* roots (25 µg GEA mg^{-1}).

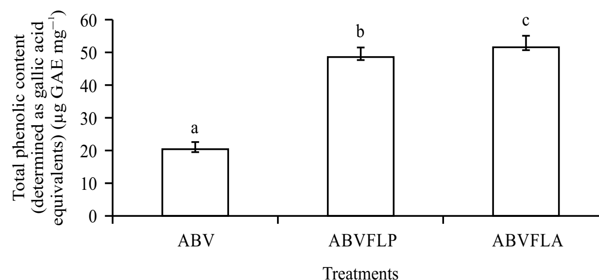

Fig. 1: Total Phenolic Content (TPC) of aqueous extracts of fermented and unfermented *Beta vulgaris* roots

ABV: Aqueous extract of *Beta vulgaris* roots, ABVFLA: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, ABVFLP: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*. [a-c]Means with different superscript are significantly different ($p<0.05$)

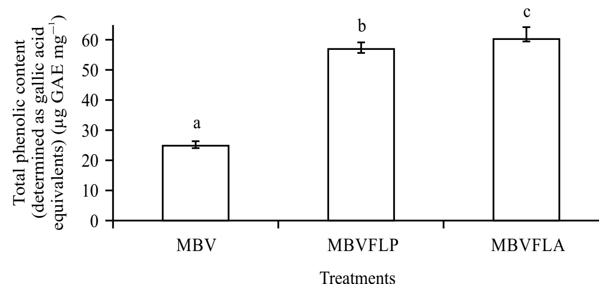

Fig. 2: Total Phenolic Content (TPC) of methanolic extracts of fermented and unfermented *Beta vulgaris* roots

MBV: Methanolic extract of *Beta vulgaris* roots, MBVFLA: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, MBVFLP: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*. [abc]Means with different superscript are significantly different ($p<0.05$)

Determination of antioxidant activity

DPPH radical scavenging activity assay: In case of aqueous extract, as can be seen in Table 1, there is significant increase in free radical scavenging activity (%) ($p<0.05$) compared to the unfermented extract. The highest free radical scavenging activity (52.77±7.66%) was obtained with *B. vulgaris* roots fermented with *L. acidophilus*, followed by 50.53±7.33% in case of *B. vulgaris* roots fermented with *L. plantarum* and finally that of the non-fermented *B. vulgaris* root (31.98±4.64%). The same results were obtained on using methanol as the extraction solvent, where there is also significant increase in free radical scavenging activity (%) ($p<0.05$) compared to the unfermented extract. The highest free radical scavenging activity (55.96±8.12%) was obtained with *B. vulgaris* roots fermented with *L. acidophilus*, followed by 54.37±7.89% in case of *B. vulgaris* roots fermented with *L. plantarum* and finally that of the non-fermented *B. vulgaris* root (42.38±5.60%).

Table 1: Radical scavenging activity of different concentrations of aqueous and methanolic extracts of unfermented and fermented *Beta vulgaris*

Extract concentration ($\mu g\ mL^{-1}$)	DPPH radical scavenging activity (%) of extracts						Concentration (mean±SE)
	ABV	ABVFLA	ABVFLP	MBV	MBVFLA	MBVFLP	
1000	54.80±0.05[a]	90.42±0.05[a]	86.58±0.06[a]	70.50±0.06[a]	95.90±0.05[a]	93.16±0.07[a]	81.89±4.56[a]
500	47.00±0.05[b]	77.55±0.05[b]	74.26±0.05[b]	59.80±0.06[b]	82.25±0.05[b]	79.90±0.05[b]	70.13±5.54[b]
250	35.00±0.04[c]	57.75±0.05[c]	55.30±0.05[c]	46.20±0.05[c]	61.25±0.04[c]	59.50±0.05[c]	52.50±5.27[c]
125	19.70±0.05[d]	32.50±0.06[d]	31.12±0.04[d]	27.30±0.05[d]	34.47±0.04[d]	33.49±0.05[d]	29.76±5.46[d]
62.5	03.40±0.05[e]	05.61±0.04[e]	05.37±0.04[e]	08.10±0.04[e]	05.95±0.03[e]	05.78±0.04[e]	05.70±5.73[e]
Group (mean±SE)	31.98±4.64[a]	52.77±7.66[d]	50.53±7.33[c]	42.38±5.60[b]	55.96±8.12[e]	54.37±7.89[de]	

[a-e]Means followed by the same letter(s) within a column are not significantly different (p<0.05) according to Duncan's multiple range test, ABV: Aqueous extract of *Beta vulgaris* roots, ABVFLA: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, ABVFLP: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*, MBV: Methanolic extract of *Beta vulgaris* roots, MBVFLA: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, MBVFLP: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*

Table 2: ABTS radical cation scavenging activity (%) of different concentrations of aqueous and methanolic extracts of unfermented and fermented *Beta vulgaris* roots

Concentration ($\mu g\ mL^{-1}$)	Antioxidant activity of different concentrations of unfermented and fermented *Beta vulgaris* extracts						Concentration (mean±SE)
	ABV	ABVFLA	ABVFLP	MBV	MBVFLA	MBVFLP	
1200	42.30±0.06[a]	70.01±0.02[a]	67.20±0.08[a]	53.40±0.03[a]	89.90±0.09[a]	78.30±0.06[a]	66.85±15.58[a]
800	34.90±0.05[b]	63.70±0.02[b]	55.81±0.05[b]	45.60±0.05[b]	84.30±0.04[b]	71.02±0.03[b]	59.22±16.21[b]
400	23.70±0.01[c]	51.20±0.03[c]	38.90±0.04[c]	34.20±0.05[c]	72.30±0.04[c]	58.00±0.03[c]	46.38±16.01[c]
200	06.10±0.04[d]	26.10±0.06[d]	10.20±0.04[d]	17.80±0.02[d]	51.20±0.05[d]	39.10±0.05[d]	25.08±15.89[d]
100	00.00[e]	07.50±0.04[e]	02.90±0.01[e]	03.50±0.01[e]	27.80±0.03[e]	18.60±0.02[e]	10.05±09.91[e]
Group (mean±SE)	21.40±16.22[f]	43.70±23.53[c]	35.00±25.02[d]	30.90±18.20[e]	65.01±22.89[a]	53.00±21.75[b]	

[a-f]Means followed by the same letter(s) within a column are not significantly different (p<0.05) according to Duncan's multiple range test, ABV: Aqueous extract of *Beta vulgaris* roots, ABVFLA: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, ABVFLP: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*, MBV: Methanolic extract of *Beta vulgaris* roots, MBVFLA: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, MBVFLP: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*

In case of aqueous extract, the best free radical scavenging activity was obtained with *B. vulgaris* roots fermented with *L. acidophilus* was 356 $\mu g\ mL^{-1}$, followed by the *B. vulgaris* roots fermented with *L. plantarum* 383 $\mu g\ mL^{-1}$. In comparison with unfermented extract 777 $\mu g\ mL^{-1}$, there was a statistically significant difference ($p<0.05$). In case of methanolic extract, value of IC_{50} for *B. vulgaris* roots fermented with *L. acidophilus* was 318 $\mu g\ mL^{-1}$ and for *B. vulgaris* roots fermented with *L. plantarum* was 334 $\mu g\ mL^{-1}$. In comparison with unfermented extract (519 $\mu g\ mL^{-1}$), there was a statistically significant difference ($p<0.05$).

Also, the results indicate that the scavenging potency increase by increasing the *B. vulgaris* root extract concentration. Based on statistical analysis, the highest scavenging activity (81.89±4.56%) was observed using the concentration of 1000 $\mu g\ mL^{-1}$ that was followed by 500 $\mu g\ mL^{-1}$ (70.13±5.54%) and 250 $\mu g\ mL^{-1}$ (52.5±5.27%), respectively. On the other hand, the concentration of 62.5 $\mu g\ mL^{-1}$ gave the lowest scavenging potency with an average of 05.70±5.73% ($p<0.05$).

ABTS radical cation scavenging assay: In case of aqueous extract, as can be seen in Table 2, there is significant increase in free radical scavenging activity (%) ($p<0.05$) compared to the unfermented extract. The highest free radical scavenging activity (43.70±23.53%) was obtained with *B. vulgaris* roots fermented with *L. acidophilus*, followed by 35.00±25.02% in case of *B. vulgaris* roots fermented with *L. plantarum* and finally that of the non-fermented *B. vulgaris* root (21.40±16.22%).

The same results were obtained on using methanol as the extraction solvent, where there is also significant increase in free radical scavenging activity (%) ($p<0.05$) compared to the unfermented extract. The highest free radical scavenging activity (65.01±22.89%) was obtained with *B. vulgaris* roots fermented with *L. acidophilus*, followed by 53.00±21.75% in case of *B. vulgaris* roots fermented with *L. plantarum* and finally that of the non-fermented *B. vulgaris* root (30.90±18.20%).

Regarding IC_{50}, In case of aqueous extract, the lowest IC_{50} was obtained with *B. vulgaris* roots fermented with *L. acidophilus* was 0.663 $mg\ mL^{-1}$, followed by the *B. vulgaris* roots fermented with *L. plantarum* (0.803 $mg\ mL^{-1}$). They were significantly ($p<0.05$) lower than that obtained with unfermented extract (1.292 $mg\ mL^{-1}$), there was a statistically significant difference ($p<0.05$). In case of methanolic extract, value of IC_{50} for *B. vulgaris* roots fermented with *L. acidophilus* was 0.241 $mg\ mL^{-1}$ and for *B. vulgaris* roots fermented with *L. plantarum* 0.48583 $mg\ mL^{-1}$. In comparison with unfermented extract (1.015 $mg\ mL^{-1}$), there was a statistically significant difference ($p<0.05$).

Table 3: Superoxide anion radical scavenging activity of different concentrations of aqueous and methanolic extracts of unfermented and fermented *Beta vulgaris*

Extract	Antioxidant activity of different concentrations of unfermented and fermented *Beta vulgaris* extracts						Concentration
(mg mL^{-1})	ABV	ABVFLA	ABVFLP	MBV	MBVFLA	MBVFLP	(mean±SE)
2.0	36.00±0.03^{a}	59.40±0.05^{a}	56.88±0.06^{a}	53.40±0.03^{a}	66.60±0.08^{a}	63.10±0.07^{a}	55.90±9.85^{a}
1.6	31.20±0.05^{b}	51.49±0.04^{b}	49.29±0.05^{b}	50.50±0.06^{b}	57.72±0.05^{b}	54.60±0.05^{b}	49.13±8.49^{b}
1.2	27.40±0.04^{c}	42.21±0.05^{c}	43.29±0.05^{c}	43.80±0.05^{c}	50.90±0.02^{c}	47.95±0.06^{c}	42.59±7.42^{c}
0.8	18.60±0.05^{d}	30.69±0.06^{d}	29.38±0.03^{d}	30.20±0.05^{d}	34.41±0.04^{d}	32.55±0.05^{d}	29.30±5.06^{d}
0.4	03.30±0.02^{e}	05.44±0.04^{e}	05.21±0.04^{e}	03.80±0.04^{e}	06.10±0.03^{e}	05.77±0.04^{e}	04.94±1.03^{e}
Group (mean±SE)	23.30±11.51^{d}	37.85±18.82^{c}	36.81±18.19^{c}	36.34±18.13^{c}	43.15±21.31^{a}	40.79±20.17^{b}	

Radical scavenging activity given as percentage inhibition, the percentage inhibition of the standard ascorbic acid was 100%, values are means of three replicates and the relative standard deviations 1%, [a-e]Means followed by the same letter(s) within a column are not significantly different (p<0.05) according to Duncan's multiple range test, ABV: Aqueous extract of *Beta vulgaris* roots, ABVFLA: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, ABVFLP: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*, MBV: Methanolic extract of *Beta vulgaris* roots, MBVFLA: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, MBVFLP: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*

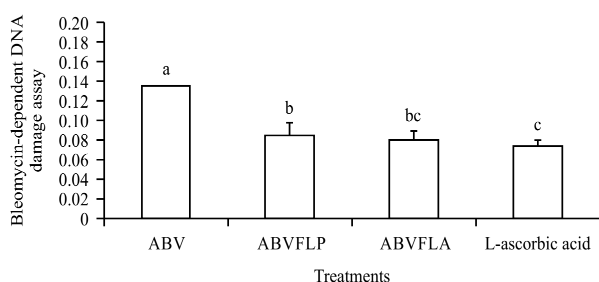

Fig. 3: Bleomycin-dependent DNA damage assay of aqueous extracts of fermented and unfermented *Beta vulgaris*
Positive control was L-ascorbic acid, (0.24 mM), ABV: Aqueous extract of *Beta vulgaris* roots, ABVFLA: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, ABVFLP: Aqueous extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*. [a-c]Means with different superscript are significantly different (p<0.05)

It can be also concluded that the scavenging potency increase by increasing the *B. vulgaris* root extract concentration. Based on statistical analysis, the highest scavenging activity (66.85%) was observed using the concentration of 1200 µg mL^{-1} that was followed by 800 µg mL^{-1} (59.22%) and 400 µg mL^{-1} (46.83%), respectively. On the other hand, the concentration of 100 µg mL^{-1} gave the lowest scavenging potency (10.05%) (p<0.05).

Superoxide anion scavenging assay: In case of aqueous extract, as can be seen in Table 3, there is significant increase in free radical scavenging activity (%) (p<0.05) compared to the unfermented extract. The highest free radical scavenging activity (37.85±18.82%) was obtained with *B. vulgaris* roots fermented with *L. acidophilus* with IC_{50} = 1.586 mg mL^{-1}, followed by 36.81±18.19 with IC_{50} = 1.672 mg mL^{-1} in case of *B. vulgaris* roots fermented with *L. plantarum* and finally that of the non-fermented *B. vulgaris* root (23.3±11.51%) with IC_{50} = 2.637 mg mL^{-1}.

The same results were obtained on using methanol as the extraction solvent, where there was also significant increase in free radical scavenging activity (%) (p<0.05) compared to the unfermented extract. The highest free radical scavenging activity (43.15±21.31%) was obtained with *B. vulgaris* roots fermented with *L. acidophilus* and IC_{50} = 1.393 mg mL^{-1}, followed by 40.79±20.17% in case of *B. vulgaris* roots fermented with *L. plantarum* with IC_{50} = 1.477 mg mL^{-1} of and finally that of the non-fermented *B. vulgaris* root (36.34±18.13%) with IC_{50} = 1.707 mg mL^{-1}.

Also, the results indicate that the scavenging potency increase by increasing the *B. vulgaris* root extract concentration. Based on statistical analysis, the highest scavenging percentage (55.9±9.85%) was observed using the concentration of 2 mg mL^{-1} that was followed by 1.6 mg mL^{-1} (49.13±8.49%) and 1.2 mg mL^{-1} (42.59±7.42%), respectively. On the other hand, the concentration of 0.4 mg mL^{-1} gave the lowest scavenging potency with an average of 4.94±1.03%, (p<0.05).

Bleomycin-dependet DNA damage assay: To show the mechanism of action of our different fermented and unfermented samples, their protective activity against DNA damage induced by the bleomycin iron complex were studied. The results in Fig. 3 showed that aqueous extracts of *B. vulgaris* root fermented with *L. acidophilus* have higher protection against DNA damage induced by the bleomycin iron complex than that fermented with *L. plantarum* and both of them have significant higher protection (p<0.05) than that of unfermented *B. vulgaris* root. The same results obtained with methanolic extract as presented in Fig. 4. Thus, all the tested compounds diminish the chromogen formation between the damage DNA and TBA with different activity.

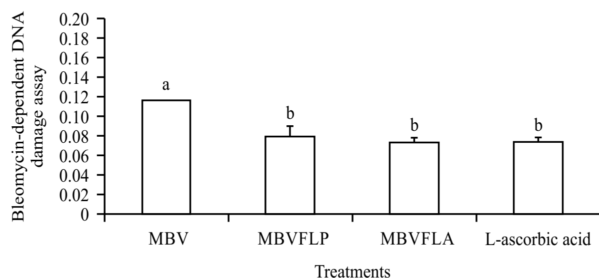

Fig. 4: Bleomycin-dependent DNA damage assay of methanolic extracts of fermented and unfermented *Beta vulgaris*

MBV: Methanolic extract of *Beta vulgaris* roots, MBVFLA: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus acidophilus*, MBVFLP: Methanolic extract of *Beta vulgaris* roots fermented with *Lactobacillus plantarum*. [a-c]Means with different superscript are significantly different (p<0.05)

DISCUSSION

This study confirmed that fermentation of beetroot extract using lactic acid bacteria enhanced total phenolic content, antioxidant activity and protection against bleomycin dependant DNA damage. Results showed that fermentation of red beetroot enables it as a potent source of antioxidants and can be used as a value-added ingredient for functional foods and in pharmaceutical industry.

In the present study, the fermented beetroot extract showed significantly higher Total Phenolic Contents (TPC) (p<0.05) than that of unfermented beet extract regardless of the solvents used during extraction. Xiao *et al.*[33] also reported significant increase in TPC after microbial fermentation in legume products. Higher total phenolic content in fermented *Beta vulgaris* might be due to liberation of free phenolic molecules by the proteolytic enzymes produced by the fermenting microbial flora[20,34].

The total phenolic content of fermented methanolic extract of *B. vulgaris* was higher than that of the aqueous extract. The higher total phenolic content of methanolic extract of *B. vulgaris* may be due to the presence of hydroxyl groups in methanol which is polar organic solvent which leads to higher solubility of phenolic compounds[35]. The higher phenolic content can also be explained by the lower polarity of methanol which means that it is more efficient in cell walls degradation, which cause polyphenols to be released from cells[36].

In determination of antioxidant activity using DPPH radical scavenging activity, the methanolic extract showed more potent *in vitro* antioxidant activity, with higher percentage inhibition, than the aqueous extract. The high scavenging activity obtained with methanolic extract are in accordance with results obtained by Kweon *et al.*[37] who reported that organic solvents have higher DPPH scavenging activity than the aqueous extract of bamboo leaf. The higher antioxidant activity of the methanolic extract compared to the aqueous one is due to the higher solubility of phenolic compounds in organic solvent[38]. Also, the results indicated that the scavenging potency increase by increasing the *B. vulgaris* root extract concentration. The same finding was supported by Al-Saman *et al.*[39] who found that increasing concentration of methanolic extract of kumquat increase antioxidant activity.

These results indicated that antioxidative potential and total phenolics content are correlated. That agree with Pyo *et al.*[40], Kosanic *et al.*[41] and Sowndhararajan and Kang[42] who reported that there was linear correlation between the TPC and the scavenging of DPPH radical in numerous vegetables, fruits and beverages. The antioxidant activity of phenolic compounds depends on the presence of phenolic hydrogens and on the stability of the resulting phenoxy radicals formed by hydrogen donation[43].

In determination of antioxidant activity using ABTS cation radical scavenging activity, there is significant increase in ABTS radical cation scavenging activity (%) (p<0.05) of the fermented *B. vulgaris* root extract compared to the unfermented extract. Kim *et al.*[44] reported that the increase in ABTS radical scavenging ability resulted from the increase in the total phenolic content after fermentation which are more effective in termination of free radical reactions.

In determination of antioxidant activity using superoxide anion radical scavenging activity, there is significant increase in free radical scavenging activity (%) (p<0.05) compared to the unfermented extract. Magnani *et al.*[45] stated that phenolic compounds have antioxidant activity and can quench superoxide anions. These results were similar to Lee *et al.*[46] who also reported that microbial fermentation enhanced the superoxide scavenging activity of soy products due to the formation of aglycones from glycosides of total phenolic and flavonoid during *L. plantarum* P108 and *L. acidophilus* P110 fermentation.

The results also showed that the methanolic extract of fermented *B. vulgaris* root was higher than that of aqueous extract of fermented *B. vulgaris* root. The high scavenging activity obtained with methanolic extract are in accordance with results obtained by Thavamoney *et al.*[47] who reported that organic solvents have higher super oxide anion scavenging activity than the aqueous extract of *Dacryodes rostrate* fruit.

The results also showed that fermented *B. vulgaris* root extract have higher protection against DNA damage induced by the bleomycin iron complex than that of unfermented *B. vulgaris* root. Cho *et al.*[18] demonstrated that beetroot significantly reduced DNA strand breaks in splenocytes exposed to irradiation and stimulated cell proliferation. The protective effects of phenolic compounds against cyto and geno-toxicity of BLM can be also explained by several mechanism of such as DNA repair. Phenolic compounds are good electron or H atom donors; therefore, they may repair some oxidative DNA damage[48]. However, the mechanisms underlying this DNA repair remain unclear and further studies must be carried to know the mechanism of action.

CONCLUSION

Natural antioxidants have become important recently due to their economical cost and large availability as raw material. From this study, can be concluded that the fermented *Beta vulgaris* root extract using lactic acid bacteria possess significant amounts of phenolic compounds and betalains which leads to potent significant radical scavenging activity. The red beetroot extract also has significant protective activity against oxidative DNA damage. The results showed that red beetroot, an inedible waste product in juice manufacture, might be a potent source of antioxidants and has a potential as a added-value ingredient for functional foods which should be used commercially. Its application in pharmaceutical industry is also possible. In future studies, it would be desirable to do more *in vivo* study on the fermented extract.

SIGNIFICANCE STATEMENT

This study revealed that fermentation of beetroot extract using lactic acid bacteria improved total phenolic content, antioxidant activity and protection against bleomycin dependant DNA damage. This study will help the researchers to uncover the critical areas of fermented products that many researchers were not able to explore. Thus a new theory on functional food and drug production may be arrived at.

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