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ORIGINAL RESEARCH

A Study of the effect of Lactic acid bacteria on antioxidant content of beetroot (*Beta vulgaris*)

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ABSTRACT
The antioxidant capacity of different fermented *Beta vulgaris* root extracts and unfermented *Beta vulgaris* root extracts were checked in order to find out their nutraceutical potential. Unfermented *Beta vulgaris* (UBV) extracts were prepared using three different solvents (distilled water, methanol and petroleum ether). Fermented *Beta vulgaris* was prepared using two different types of lactobacillus species (*Lactobacillus plantarum P108* and *Lactobacillus acidophilus P110)*. The effects of fermentation on *Beta vulgaris* in terms of total phenolic content (TPC), antioxidant activities and DNA damage protection were investigated. The antioxidant activities of fermented *Beta vulgaris* (FBV) and unfermented *Beta vulgaris* (UBV) were determined by different standard methods. We evaluated the antioxidant activity of the aqueous and methanolic extract of the roots of Beta vulgaris by 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation scavenging assay, Superoxide anion scavenging assay (SASA). The results showed that, FBV extracts compared to UBV extracts possessed more TPC, and had higher ABTS radical scavenging activity, higher radical scavenging activity using DPPH method and SASA method. Also, FBV exhibited greater protection against oxidative DNA damage induced by Bleomycin. Additionally, FBV using *Lactobacillus acidophilus P110* had higher TPC, antioxidant activities than fermented *Beta vulgaris* using *Lactobacillus Planatrum P108.* Although, the methanolic extract was more efficient than aqueous extract from UBV and FBV .The petroleum ether was not efficient and strong in the extraction process. The results suggested that FBV with enhanced antioxidant capacity could provide a functional *Beta vulgaris* to contribute to the health and nutritional status improvement of consumers, demonstrating the potential for industrial applications.

KEY WORDS: *Beta vulgaris -antioxidant-Lactobacillus acidophilus, Lactobacillus plantarum.-fermentation*

INTRODUCTION

There is currently an upsurge of interest in phytochemicals as a modern source of natural antioxidants to be utilized in nourishments and pharmaceutical preparations to replace synthetic antioxidants, which are being restricted due to due to their potential wellbeing dangers and toxicity (Moussa *et al.*, 2011) Phenolic compounds are omnipresent in the plant kingdom and they have been reported to possess many biological effects (Kujala, 2002).

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage (Nimse and Pal, 2015)

The red beetroot (Beta vulgaris) has been cultivated for many hundreds of years in all temperate climates. Beetroot is used as a vegetable, and its juice and extracts also as traditional medicine, food colorant and additive to cosmetics (Georgiev *et al.*, 2010).

Nowadays it is well known that phenolic compounds are highly responsible of the health effects derived from consumption of plant origin food. They play a key role as antioxidants due to the presence of hydroxyl substituents and their aromatic structure, which enables them to scavenge free radicals (Khan and Giridhar, 2015)

The antioxidant activity of beet extracts, especially beet peel extracts, reported recently increase the interest in beetroot compounds (Vinson *et al*., 1998).

Reports on BRJ fermentation either spontaneously or by using lactic cultures are very scarce (Halász & Zalán, 2009). Lactic acid bacteria (LAB) have a long history of safe exploitation by humans, being used for centuries in food production and preservation and as probiotic agents to promote human health (Cano-Garrido, Seras-Franzoso and Garcia-Fruitós, 2015).

Probiotics have been defined as "live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance". Among these microorganisms, lactic acid bacteria are regarded as a major group of probiotic bacteria. They are non-pathogenic, technologically suitable for industrial processes (Mourad, 2006) .

Lactobacillus acidophilus is a common commercial bacterial strain used in the manufacturing of dairy products (Li *et al.*, 2016). *Lactobacillus plantarum* is a resourceful bacteria of LAB group which has been bestowed with potential virtues to be used as a successful probiotic, efficiency to withstand in harsh conditions, acid and bile stability, its effect on flavor, texture, and other sensory attribute of the final product with antioxidant potential as the most important idiosyncrasy (Geeta and Yadav, 2017).

Biochemical modification by microorganism contributes directly to many advantageous properties of products (Singh *et al.,* 2010). In recent years, fermentation was observed to serve as an efficient approach to enhance the antioxidant properties (Xiao *et al.*, 2015). The aim of this study to determine the antioxidant capacity of different fermented *Beta vulgaris* root extracts and unfermented *Beta vulgaris* root extracts to check out their nutraceutical potential. Unfermented *Beta vulgaris* (UBV) extracts were prepared using three different solvents (distilled water, methanol, and petroleum ether).

RESULTS AND DISCUSSION

Extraction of *Beta vulgaris* root result

The aqueous extract of *Beta vulgaris* root gave the highest yield (200 gm of BV 22.3 gm., than the methanolic extract (200 gm of 18.7 gm BV gm).

Petroleum ether is one of the least polar solvents that may cause that it was not efficient and strong in the extraction process.The result suggests that the major phytochemicals in *Beta vulgaris* root are mostly high in polarity and soluble in water. The basis of selection of solvent is based on their polarity. An extraction of *Beta vulgaris* root resulted that highest extract yield obtained from water compared to methanol. This result was in agreement of that of (Reichardt and Welton, 2011) who reported that water more polar than methanol.

Colorimetric Determination of Total Phenols results

The Total phenolic of *Beta vulgaris* root extract is presented in figure 1 and figure 2. The maceration with methanol gave the highest yield, Methanol could dissolve the phenolic compounds that were polar to nonpolar. It caused methanol have the highest yield. (Wahyudi, Ratnadewi and Siswoyo, 2016)

It was known that phenolic compounds, especially flavonoid compounds are responsible for effective free radical scavenging.(Moussa *et al.*, 2011) Phenolic compounds have redox properties, which allow them to act as antioxidants , as their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity (Baba and Malik, 2015).

A high correlation was observed between the total phenolic content and $ABTS^{+}$ scavenging activity, DPPH radical scavenging activity and superoxide anion reducing power of the extract. These data are in accordance with others, which have shown that a high total phenolic content increases antioxidant activity, and that there is a linear correlation between phenolic content and antioxidant activity (Holasova *et al.*, 2002).

These results indicate that the higher antioxidant activity of chard extract may be associated with its total phenolic content.

Higher TPC in fermented *beta vulgaris* observed in our results might be due to formation or mobilization of free phenolic and flavonoid molecules during LAB fermentation. As compared to the aqueous unfermented extract, which showed an absorbance of 0.066, 24 hr fermented sample with *L plantarum* showed absorbance of 0.104, while 24 h fermented sample with *L acidophilus P110* showed an absorbance of 0.108, implying that there is an increase in the total polyphenolic content after fermentation.

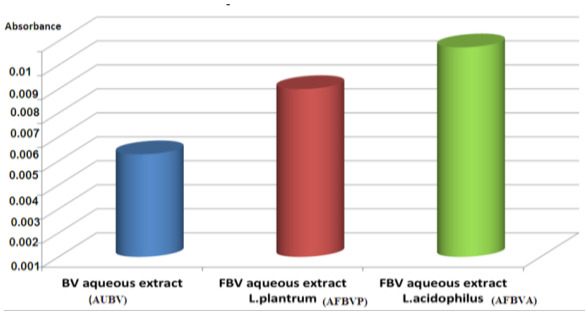

Figure 1: Total phenolic contents of AUBV: Aqueous unfermented Beta vulgaris, AFBVP: Aqueous unfermented Beta vulgaris by *L.Plantarum P108*, AFBVA: Aqueous fermented Beta vulgaris by *L.acidophilus P110.*

Comparing with the standard graph, the concentration of polyphenols in AUBV < AFBVP < AFBVA, respectively.

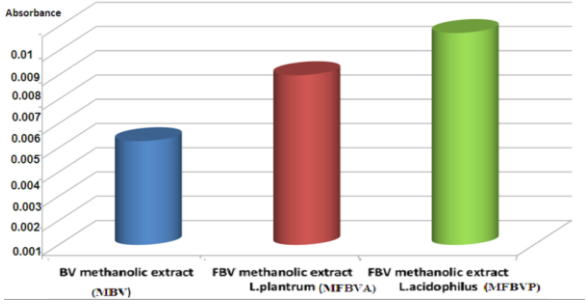

Figure 2: Total phenolic contents of MUBV: Methanolic unfermented Beta vulgaris, MFBVP: Methanolic unfermented Beta vulgaris by *L.Plantarum*,P 108 MFBVA: Methanolic fermented Beta vulgaris by *L.acidophilus* P110.

With concern to the methanolic extract , the methanolic unfermented extract , which showed an absorbance of 0.072, 24 h fermented sample with *L.plantarum* P 108 showed absorbance of 0.115, while 24 h fermented sample with *L.acidophilus* P110 showed an absorbance of 0.12, ensuring that there is an increase in the total polyphenolic content after fermentation.

Comparing with the standard graph, the concentration of polyphenols in MBV < MFBVA < MFBVP, respectively.

Beet roots have recently been attracting more scientific attention from researchers, since beetroots are an excellent source of polyphenols which are proven to cure neurodegenerative conditions, cure human cardiovascular diseases and cure cancer (Scalbert, Johnson and Saltmarsh, 2005).

In the present study, beetroot juice fermented with LAB showed a significant increase ($P<0.05$) in the concentration of polyphenols, when compared to unfermented beet juice. The standard was gallic acid prepared in concentrations of 1000 µg/ml to 1.9 µg/ml. The phenolic concentrations were determined by comparison with the standard calibration curve.

DPPH radical scavenging activity assay

All the extracts were significantly ($p < 0.05$) able to scavenge DPPH radical in a dose-dependent manner. The Comparison of antioxidant activity between each aqueous and methanolic extract of *Beta vulgaris* root showed that the MFBVA, AFBVA showed significantly ($P<0.05$) higher radical scavenging activity while AUBV, MUBV showed the lowest activity.

The reason for the high activity of fermented aqueous and methanolic; possibly the extract contained additional phenolic anthocyanins such as betacyanin (Oki *et al.*, 2002) . (Pyo *et al.*, 2004) reported that anthocyanins and polyphenols play an important role in the DPPH radical scavenging activity of *Beta vulgaris*. There was a good linear correlation between the TP content and the scavenging of DPPH radical in each extract.

These results indicated that the radical scavenging capacity of each extract might be mostly related to their concentration of phenolic hydroxyl group. The antiradical activity of phenolic compounds depends on their molecular structure, that is, on the availability of phenolic hydrogens and on the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation (Rice-Evans, Miller and

Paganga, 1996) A linear correlation between radical scavenging activity and polyphenolic concentration has been reported in an extensive range of vegetables, fruits and beverages (María I. Gil *et al.*, 2000) (Namuli *et al.*, 2011) Similar trends of antioxidant activity were observed using either the DPPH or ABTS.

These results suggested that the *Beta vulgaris* root extracts might react with free radicals, particularly peroxy radicals, which are the major propagators of the autoxidation of fat, thereby terminating the chain reaction (Frankel, 1991) Using the DPPH radical scavenging method, the antioxidant activity of aqueous extracts from Beta vulgaris root in the order AUBV<AFBVP< AFBVA. The same results reported with methanolic extracts MUBV<MFBVP< MFBVA.

CONCLUSION

These results of the study suggest that the extracts obtained from red beet root possess considerable amounts of phenolic compounds and betalains, based on a significant radical scavenging activity towards stable DPPH. The correlation coefficients exhibited a positive relationship between the antiradical activities of red beet root and the contents of total phenolics, anthocyanin, and betaxanthins.

Our results showed that red beet root, an inedible waste product in juice manufacture, might be a potent source of antioxidants, and has a potential as a value-added ingredient for functional foods should to be used commercially In future studies, it would be desirable to employ such experimental conditions that can more specifically reflect the in vivo antioxidant activities of the extracts obtained.

MATERIALS AND METHODS

Material

Plant

Fresh red beet root (*Beta vulgaris*), (Mansoura Carfour market), Egypt.

Microorganisms

The lactic acid bacteria strain *L. plantarum* P108, *L.acidophilus* P110 were identified by Mahrous et al.(2010) (Industrial Biotechnology Department, Genetic Engineering & Biotechnology Research Institute).

Methods

Plant material and preparation of extract

The plant material was represented by fresh beet root (*Beta vulgaris*), also known as red beet. The species was cultivated in Egypt, and was purchased from Carfour market in Mansoura.

Multiple solvents have been commonly used to extract phytochemicals, Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute of interest. The different extracts was prepared according to (GOWRI, 2010) with slight modifications.

For the study, The red beet roots were washed with running tap water to get rid of any impurities adhered to the surface of the vegetable ,then cutted , sliced and then used for extraction. Maceration for the extraction is achieved by steeping *Beta vulgaris* root slices in distilled water and organic solvents (e.g., methanol and petroleum ether) in a closed container that is stirred frequently to increase the rate of extraction from the plant matrix. After the extraction is complete, the plant material is separated from the liquid by filtration.

Extraction of *Beta vulgaris* pigments from red beet

About 200 g of red beet was mixed with 1 liter of distilled water four successive times at room temperature. The solvents after extraction were rotary-evaporated to get a semi solid residue (22.3 gm.). Another 200 g of red beet was mixed with 1 liter of methanol four successive times at room temperature. The solvents after extraction were rotary-evaporated to get a semi solid residue (18.7 gm.). Another 200 g of red beet was mixed with 1 liter of Petroleum ether four successive times at room temperature; the solvent was not efficient and strong in the extraction process. The extracts were transferred to beakers and stored at 4 ºC until analysis.

Preparation of Microorganism

The probiotic mother culture containing *Lactobacillus acidophilus* P110 isolated and tested for its probiotics properties by (Mahrous *et al.,* 2010) was added to sterile MRS broth then anaerobically incubated using BBL gas packs at 37°C for 16 hours. Strain was stored at – 80°C in MRS broth supplemented with 25% (v/v) glycerol.

For routine analysis, the strains were subculture twice in MRS broth at 37°C for 24 h.

Inoculum preparation and *Beta vulgaris* fermentation

According to (Vaithilingam *et al.*, 2016) , Freshly prepared beetroot juice was pasteurized at 80˚C for 15 min, for eliminating the unwanted microorganisms. Following pasteurization, the juice was cooled to room temperature for microbial inoculation. The LAB culture was centrifuged at 6,000 rpm for 15 min. The supernatant was discarded and the pellet was washed with 0.9% sodium chloride solution. 8-10% (v/v) co-culture of *L. acidophilus P110* and *L. plantarum P105* was inoculated in 500 ml of pasteurized juice and fermented at 37˚C for 24 h.

Storage of the Fermented Juice

After 24 h of fermentation, the juice samples were stored at refrigeration temperature and determined for antioxidant activity.

Total phenolics assay

Total concentration of phenolics in the crude extract was determined by a modification of the method of (Bray and Thorpe, 1954) (Taga, Miller and Pratt, 1984). Dried samples and standards were prepared in 60:40 acidified methanol/water (0.3% HC1). Test solutions (samples or standards) of 100 µL were added to 2.0 mL of 2% Na_2CO_3. After 2 min, 100 µL of 50% Folin-Ciocalteau reagent were added and allowed to stand at room temperature for 30 min. Absorbance was measured at 750 nm on a Unico 1200 spectrophotometer, USA. The blank consisted of all reagents and solvents without test compounds or standard. The standard was gallic acid prepared in concentrations of 1.9 µg/mL to 1000 µg/mL. The phenolic concentrations were determined by comparison with the standard calibration curve.

Antioxidant properties

Radical scavenging activity using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method

According to (Villaño *et al.*, 2007) , A methanolic solution (25 mgL^{-1}) of the radical $DPPH^{\bullet}$ was prepared daily and protected from light. Absorbance was recorded to check the stability of the radical throughout the time of analysis. Five different concentrations of $DPPH^{\bullet}$ radical, comprising from 1000 to 62.5 µg/ml, were also prepared every day and a linear relationship between radical concentration and absorbance was established.

The effect of phenolic compounds on the $DPPH^{\bullet}$ absorbance was estimated by using the procedure described by (Larrauri and Saura-calixto, 1998). 1 mL of different phenol concentrations dissolved in ethanol in water were added to 3.9 mL of DPPH• methanolic solution. Absorbance at 515 nm was recorded at different time intervals until the reaction reached equilibrium. The initial absorbance was close to 0.700 in all cases. The blank reference cuvette contained methanol. All measurements were performed in duplicate. Five different concentrations of each phenolic compound studied have been assayed in order to check the linearity of response and to establish the antioxidant activity values in the adequate linear range.

The ability of the sample to scavenge DPPH radical was determined from:

DPPH scavenging effect= (Control A− Sample A) / Control A ×100

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