FREE RADICAL SCAVENGING ACTIVITY OF SUGAR BEET (*Beta Vulgaris* L.) ROOT EXTRACTS

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ABSTRACT

The ability of *Beta Vulgaris L.* roots to prevent the initiation of free radicals that cause cellular damage was investigated *in vitro*. The sugar beet roots were extracted exhaustively using methanol (SB) or extracted sugar beet pulp the residue left after sugar extraction – using methanol (SBP). In the reducing power assay, the more anti-oxidant compounds convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}). The reducing power capacity of two extracts were found less than that of vitamin C. Total polyphenols capacity (TPC) and total flavonoids capacity (TFC) were evaluated according to Folin-Ciocalteu and aluminium chloride colorimetric assays, respectively. In this study, the SB extract has higher than SBP extract in TPC but lower in TFC. Radical scavenging activities of two extracts were tested against DPPH and SOD. (SB) and (SBP) methanolic showed a very low inhibition (8.17% and 9.75%) comparing to Vitamin C. The maximum inhibition of SOD was 28.65% - 34.97% at 1000 µg/mL for (SB) and (SBP) methanolic extracts, respectively.

1. INTRODUCTION

Free radicals are created when cells use oxygen to generate energy. These by-products are generally reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide that result from the cellular redox process. At low or moderate concentrations, ROS exert beneficial effects on cellular responses and immune function but at high levels, free radicals and oxidants generate oxidative stress, a deleterious process that can damage cell structures, including lipids, proteins, and DNA (**Pham-Huy *et al.,* 2008**). Oxidative stress plays a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular and neurodegenerative diseases, (**Willcox *et al.,* 2004**). The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplements. These antioxidants act as free radical scavengers by preventing and repairing damages caused by ROS, and therefore can enhance the immune defense and lower

the risk of cancer and degenerative diseases (**Pham-Huy *et al.,* 2008**). In recent years, there is an increasing interest in finding antioxidant phytochemicals, because they can inhibit the propagation of free radical reactions, protect the human body from diseases. The most effective components seem to be flavonoids and phenolic compounds of many plant raw materials, particularly in herbs, seeds, and fruits. Their metal-chelating capabilities and radical- scavenging properties have enabled phenolic compounds to be as effective free radical scavengers and inhibitors of lipid peroxidation (**Terao and Piskula, 1997**).

Sugar beet (*Beta vulgaris L*.) is the world's most cultivated crop for sucrose production for human consumption after sugar cane (*Saccharum officinarum* L.). The beet root contains a high level of sucrose, which varies between 12% and 21% on the fresh weight (FW) basis, depending on cultivar and growing condition **(Trebbi, 2005).**

Sugar beet pulp (SBP) is a by-product from sugar industry after extraction and is mainly used for animal feeding **(Michel *et al.,* 1988).** On a dry weight basis, SBP contains 75%–80% polysaccharides, consisting roughly of 22%–24% cellulose, 30% hemicelluloses, mainly arabinans and (arabino) galactans and 25% pectin. Small amounts of fat, protein, ash and lignin contents in SBP are 1.4%, 10.3%, 3.7% and 5.9%, respectively **(Sun and Hughes, 1999).**

We now report the present study on sugar beet root extracts to evaluate the phytochemical and antioxidant effect.

2. MATERIAL AND METHODS

2.1 Sugar beet roots

The roots of sugar beet (*Beta vulgaris L)* were collected from fields of Damietta in February 2014, washed and stored in a refrigerator at 4 ^{o}C till further use within two weeks.

2.2 Extraction

2.2.1 Methanolic Extract of sugar beet (SB)

The sugar beet roots were cut to slices and dried in a single layer at 60 ^{o}C in electric oven. The dried sample were powdered in a laboratory mill, (60 g) of dry sample were mixed with methyl alcohol (1.0 L, 99.9%) in a closed flask and kept for 24 hours. Shaking frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered rapidly taking precautions against the loss of the solvent. The methanolic extract was concentrated to dryness in a rotary evaporator under vacuum and controlled temperature (45 ^{o}C). The extract was stored in a refrigerator at 4 ^{o}C till further use **(Harborne, 1998)**.

2.2.2 Methanolic extract of sugar beet pulp (SBP)

Sugar beet pulp was dried at 60 ^{o}C in electric oven. The dried sample was powdered in a laboratory mill, (60 g) of dried sample was mixed with methyl alcohol (1.0 L, 99.9%) in a closed flask and kept for 24 hours then filtered. The filtrate was concentrated to dryness in a rotary evaporator under vacuum and controlled temperature (45 ^{o}C). The dried extract was stored in a refrigerator at 4^{o}C till further use **(Harborne, 1998)**.

2.3 Phytochemical Screening

Terpenes, tannins, flavonoids, saponins, resins and alkaloids were detected in methanolic extract according to the method described by **Harborne (1998).**

2.4 Determination of total polyphenols capacity (TPC)

Total polyphenols capacity was determined using Folin-Ciocalteu method according to **(Li *et al.,* 2007).** The absorbance was measured at wavelength 750 nm. Total phenol capacity was expressed as milligram gallic acid equivalent (GAE)/g extract.

2.5 Determination of total flavonoids capacity (TFC)

Aluminum chloride colorimetric method was used for quantitative flavonoids determination **(Chang *et al.,* 2002).** The absorbance was measured at wavelength 415 nm. Total flavonoid **capacity** was expressed as milligram quercetin equivalent (QE) / g extract.

2.5 Antioxidant activity

2.5.1 Estimation of reducing power

Reducing power assay was determined as described in the method of **(Ferreira *et al*., 2007).** Ascorbic acid was used as standard reference. Absorbance was measured at wavelength 700 nm against the corresponding blank solution.

2.5.2 DPPH (2, 2 -diphenyl-1-picryl-hydrazyl) free radical scavenging activity

DPPH radical scavenging capacity was determined according to the technique reported by (**Martins *et al.,* 2008**). Sample solution was diluted with dimethyl sulfoxide (DMSO) and in each reaction mixture, the solution was mixed with 2.0 ml of 100 µM DPPH. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 30 min. in dark. Decolorization of DPPH was determined by measuring the absorbance at wavelength 517 nm.

2.5.3 Superoxide Dismutase Activity Assay

Superoxide dismutase activity was assayed by the method of **Dechatelet *et al.* (1974).** The increase in absorbance was measured at wavelength 560 nm during 5 min. The reading was taken in each minute and the change in the mean absorbance/min was then calculated.

3. RESULTS AND DISCUSSION

3.1 Preliminary phytochemical screening of sugar beet extracts.

The preliminary phytochemical screening reveals the presence of terpenes, tannins, flavonoids, saponins and alkaloids, however resins are not found in the sample. The results are shown in **Table 1.**

Table 1: Qualitative analysis of phytochemical constituents of SB and SBP extracts

Constituent	**SB** Level*	**SBP** Level**
Terpenes	+	+
Tannins	+	+
Flavanoids	+	+
Saponins	+	+
Resins	—	—
Alkaloids	+	+

+ = detectable, — = Not detectable.
* SB= Sugar beet extract.
** SBP= Sugar beet pulp extract.

3.2 Quantitative Estimations

Two different extracts were used in this study, since a wide range of extracts hold a better chance for the extraction and isolation of biologically active molecules for general screening of bioactivity.

Data in **Table 2** showed that the lowest amount of total polyphenols capacity (TPC) was 7.32 µg GAE/g extract in SBP extract while the highest was observed in SB extract with a value of 8.45 µg GAE/g extract. Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources, and they have been shown to possess important antioxidant activities (**Nagavani *et al.,* 2010**). The high amount of phenols and flavonoids in extracts may explain their high antioxidative activities. Total flavonoids capacity (TFC) of the extracts were also determined using aluminum chloride assay, a widely adopted method in almost all published works to measure the total flavonoid content of plants. The principle of this colorimetric method is based on the acid-stable complex between aluminum chloride, and the participation of C-4 keto

group and either the C-3 or C-5 hydroxyl group shown in flavones and flavonols of structure below, (**Chang *et al.,* 2002**).

Also from the same table, the SBP extract has the highest amounts of total flavonoid content (TFC) (1.868 mg QE/g extract), while the lowest amount (1.51 mg QE/g extract) was observed in SB methanolic extract. In this study, TPC and TFC can be further tested for methanolic extracts ability to inhibit different free radicals. Total phenolic and flavonoid contents for SB and SBP extracts are demonstrated in **Table 2.**

Table 2: Total polyphenolic and flavonoid capacities for Sugar beet extracts

Extracts	Total Polyphenols Capacity (mg GAE/g)[a]	Total Flavonoids Capacity (mg QE/g)[b]
SB*	8.45	1.51
SBP**	7.32	1.868

[a](mg GAE/ g): mg of gallic acid equivalent per g of dry plant extract.
[b](mg QE/ g): mg of quercetin equivalent per g of dry plant extract.
*** SB= Sugar beet extract.**
**** SBP= Sugar beet pulp extract.**

3.3 Reducing Power Assay

Reducing power assay measures the total reducing capability of antioxidants on the basis of the methanolic extract or vitamin C ability to reduce Fe^{+3} to Fe^{+2} ions. This assay treats the antioxidants in the samples as reductants in a redox-linked colorimetric reaction and is relatively simple and easy procedure to be standardized. One possible disadvantage with this assay is the fact that this assay does not react fast with some antioxidants, such as glutathione. However, It is still suitable for assessment of antioxidant activity of extract because only limited amounts of plant glutathione are absorbed by humans **(Schafer and Buettner, 2001).** The reducing power capacity of SBP methanolic extract as 41.34 µg VC/ml, was higher than that of SBP methanolic extract (40.55 µg VC/ml) at 1000 µg/ml as shown in **(Table 3)**.

Table 3: Reducing power capacity for different SB and SBP methanolic extracts concentrations

Concentration (µg/ml)	Reducing power capacity	
	SB*	SBP**
200	10.95	7.74
400	18.35	16.14
600	25.75	24.54
800	33.15	32.94
1000	40.55	41.34

* SB= Sugar beet extract.

** SBP= Sugar beet pulp extract.

3.4 DPPH assay

There are various methods for determination of antioxidant activities. The measurement of radical scavenging activity of any antioxidant is commonly associated with the using of DPPH method because it is quick, reliable and reproducible method. It is widely used to test the ability of compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidative activity of root extracts. The stable radical DPPH has maximum absorption at wavelength 517 nm and the antioxidant reduces it to the yellow colored diphenyl-picrylhydrazine. Figure (1), as a typical example shows the effect of different concentrations of vitamin C on the electronic absorption spectra of DPPH dissolved in DMSO solvent. The concentration of antioxidant needed to decrease the initial DPPH concentration by 50 % (IC_{50}) is a parameter widely used to measure the antioxidant activity. A lower IC_{50} value indicates a higher antioxidant power. **Figure (2)** shown the changes in DPPH radical scavenging activities according to different concentrations of sugar beet (SB) and sugar beet pulp (SBP) extracts. Both the SB and SBP extracts at concentration 10 mg/mL have the inhibition percentage of 8.17 and 9.75, respectively. These inhibition values show a very weak DPPH radical scavenging. On the other hand, vitamin C gave the inhibition percentage of 50 % (IC_{50}) at 12.34µg/mL.

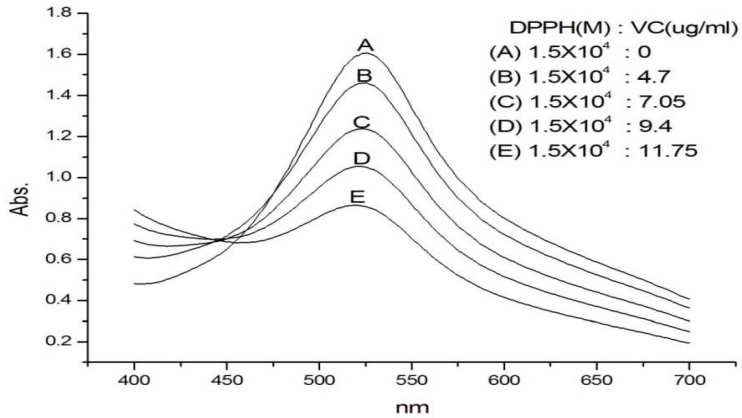

Figure 1: Effect of Vitamin C concentration on the UV-visible spectra of DPPH in DMSO solvent at 25 ^{o}C

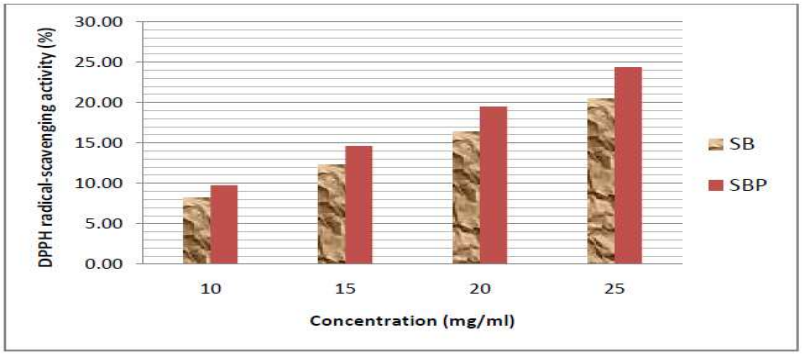

Figure 2: Changes in DPPH radical scavenging activities according to different concentrations of sugar beet (SB) and sugar beet pulp (SBP) extracts

3.5 Superoxide Dismutase Activity Assay

Superoxide anion plays an important role in plant tissues and is involved in the formation of other cell-damaging free radicals (**Duan *et al.*, 2007**). It is an oxygen-centered radical with selective reactivity and precursor to active free radicals as hydrogen peroxide, hydroxyl radical and singlet oxygen that have ability to react with biological macromolecules and thereby inducing tissue damage **(Wickens, 2001).** The inhibition percent of superoxide anion radical generated at 1000 µg/mL concentration of SBP and SB methanolic extracts and the results in **(Table 4)** was found as 34.97% and 28.65% inhibition, respectively.

Table 4: Superoxide dismutase activity for different SB and SBP methanolic extracts concentrations

Concentration (µg/ml)	Inhibition %	
	SB *	SBP **
250	12.30	19.15
500	17.75	24.42
750	23.20	29.70
1000	28.65	34.97

* SB= Sugar beet extract.
** SBP= Sugar beet pulp extract.

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الملخص العربى

القدرة على مسك الشوارد الحرة لمستخلصات من جذور بنجر السكر

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في الدراسة الحالية تم التحقق من مدى قدرة مستخلصات بنجر السكر على مسك الشوادر الحرة . حيث تم استخدام مستخلصين لبنجر السكر (مستخلص ميثانولي لجذور البنجر ، مستخلص ميثانولى للب البنجر) كمضادات اكسدة طبيعية لمسك الشوارد الحرة. يمكن تقدير القوة الإختزالية لمعظم المركبات التى تعمل كمضادات أكسدة والتى يمكنها أن تحول ايونات الحديديك (الثلاثية) إلى ايونات الحديدوز (الثنائية). وقد تم دراسة القدرة الإختزالية للمستخلصين وكانت أقل من القوة الإختزالية لفيتامين (ج). وقد ُقدرت كمية الفينولات الكلية والفلافونيدات في كلا المستخلصين باستخدام محلول الفولين اتشكلت وكلوريد الألومنيوم على الترتيب وتبين ان المستخلص الميثانولى لجذور بنجر السكر يحتوي على تركيز أعلى فى الفينولات 8.45 ملجرام/جرام مقارنة بتركيز قدره 7.32 ملجرام / جرام من المستخلص الميثانولى للب البنجر .فى حين كانت نتائج الفلافونيدات 1.51 و1.868 ملجرام/جرام للمستخلص الميثانولى لبنجر السكر ولب البنجر على الترتيب .

اجريت اختبارات معملية لاثبات قدرة المستخلصين على مسك انواع مختلفة من الشوارد الحرة مثل DPPH و السوبر اكسيد. أظهرت المستخلصات قدرة تثبيط منخفضة مقارنة بفيتامين (ج) وكانت 8.17% و 9.75% فى مستخلص جذور البنجر ومستخلص لب البنجر على التوالى . وكذلك كان أعلى تثبيط للسوبراكسيد 28.65 % - 34.97 % عند استخدام تركيز 1000 µg/mL من مستخلص جذور البنجر ومستخلص لب البنجر على الترتيب .