ANTIOXIDANT ACTIVITY OF ETHANOLIC LEAVES EXTRACTS OF *BETA VULGARIS*

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Abstract

The many number of medicinal plants are used in the cellular and metabolic disease treatment such as diabetes, obesity, poly cystic ovarian disorder and cancer etc. There are some speculations that the generation of free radicals inside the body in some physiological conditions is resulted in the cellular changes and development of degenerative disease and this could be neutralized by the antioxidants from different medicinal plants. Several studies have shown that plant derived antioxidant neutraceuticals scavenge free radicals and modulate oxidative stress-related degenerative effects. The beet, *Beta vulgaris* is a plant in the Chenopodiaceae family. It is best known in its numerous cultivated varieties, the most well-known of which is the purple root vegetable known as beetroot or table garden beet. In the present study, the *In vitro* antioxidant activity was carried out by the inhibitory activity of against the DPPH, H₂O₂ Scavenging Assay and Reducing power Assay. The inhibition of these compounds may increase the anti-oxidant capability.

Key words: Antioxidant neutraceuticals, *Beta vulgaris*.

Introduction

Traditional medicine from plant extracts has proved to be clinically effective and relatively less toxic than the existing drugs [1]. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure [2]. Phytochemicals (secondary metabolites) are bioactive chemicals of plant origin. They are naturally synthesized in all parts of the plant body: bark, leaves, stems, roots, flowers, fruits, seeds, and so on [3]. They have been recognized as the basis for traditional herbal medicine practiced in the past and now [4]. All plant parts are usually screened for phytochemicals that may be present; the presence of a phytochemical of interest may lead to its further isolation, purification, and characterization. Then it can be used as the basis for a new pharmaceutical product. Medicines derived from plant extract are being used to treat a wide variety of clinical disease [5]. Traditionally, natural products has established store house of numerous bioactive compounds, which provide an endless source of medicine. Crude herbs have long been the basis of many traditional medicines worldwide. The leaves of *Beta vulgaris* contain powerful antioxidants. The beet, *Beta vulgaris* is a plant in the Chenopodiaceae family. It is best known in its numerous cultivated varieties, the most well-known of which is the purple root vegetable known as beetroot or table garden beet. Beets have been used in traditional medicine for hundreds of years to treat constipation, gut and joint pain, dandruff [6]. Modern pharmacology shows that red beet extracts exhibit antihypertensive and hypoglycaemic activity as well as excellent antioxidant activity. The promising results of their phytochemicals in health protection suggest the opportunity for their use in functional foods [7].

Material and methods

*In Vitro* antioxidant activity(8,9 and 10)

Scavenging activity of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical (10)
The ability to scavenging the stable free radical, DPPH was measured as a decrease in absorbance at 517 nm by the method. About 0.1 ml of DPPH-methanol solution (0.135 mM) was mixed with 1.0 ml of different concentrations of various extracts of Beta vulgaris leaf extract. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Rutin and Butylated hydroxyl toluene (BHT) were used as standard drugs. The percentage of free radical scavenging was calculated according to the following equation:

\[
\% \text{scavenging} = 100 - \frac{(\text{Abs sample} - \text{Abs blank})}{\text{Abs Control}} \times 100.
\]

Scavenging of hydrogen peroxide

The ability of the ethanolic leaf extract of Beta vulgaris to scavenge H$_2$O$_2$ was determined.

A solution of H$_2$O$_2$ (40 mM) was prepared in phosphate buffer (pH 7.4). H$_2$O$_2$ concentration was determined spectrophotometrically from absorption at 230 nm in a spectrophotometer (SL 159, UV-Visible Spec, Elico, India). Extracts (200, 400, 600, 800 and 1000 µg) in distilled water were added to a H$_2$O$_2$ solution (0.6 mL, 40 mM). Absorbance of H$_2$O$_2$ at 230 nm was determined after ten minute against a blank solution containing phosphate buffer without H$_2$O$_2$. The percentage of scavenging of H$_2$O$_2$ of Beta vulgaris and standard was calculated using the following equation:

\[
\% \text{Scavenging} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100
\]

Reducing Power

The reducing power of ethanolic leaf extract of Beta vulgaris was determined by the method of Oyaizu (1986). Substances which have reduction potential react with potassium ferricyanide to form potassium ferrocyanide, which then reacts with ferric chloride to form ferric-ferrous complex that has an absorption maximum at 700 nm. An increase in the reduction of ferric to ferrous ion increases the absorbance indicating the reducing ability of ethanolic leaf extract of Beta vulgaris.

Procedure

Varying concentrations of ethanolic leaf extract of plant in double distilled water was mixed with 2.5 mL of phosphate buffer and 2.5 mL of potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, after which, 1.5 mL of TCA was added and centrifuged at 3000xg for 10 min. From all the tubes, 0.5 mL of supernatant was mixed with 1 mL of distilled water and 0.5 mL of ferric chloride. The absorbance was measured at 700 nm in a spectrophotometer. The increased absorbance of the reaction mixture indicated increasing reducing power. Incubation with water in place of additives was used as the blank.

Result and Discussion

Reducing assay

Table 1: Anti-Oxidant Activity of leaf extract of Beta vulgaris by using Reducing assay

<table>
<thead>
<tr>
<th>TEST</th>
<th>CONCENTRATION OF PLANT EXTRACT (mg/ml)</th>
<th>% OF INHIBITION</th>
<th>Ascorbic acid</th>
</tr>
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<tbody>
<tr>
<td>Reducing assay</td>
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<td></td>
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</tr>
<tr>
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<td>60</td>
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<tr>
<td>80</td>
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<td>75</td>
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</tr>
<tr>
<td>100</td>
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<td>80</td>
<td>80</td>
</tr>
<tr>
<td>IC$_{50}$ Value</td>
<td></td>
<td>28.8</td>
<td>22.6</td>
</tr>
</tbody>
</table>
Fig 1: Anti-Oxidant Activity of leaf extract of *Beta vulgaris* by using Reducing assay

### Table 2: Anti-Oxidant Activity of leaf extract of *Beta vulgaris* by using DPPH assay

<table>
<thead>
<tr>
<th>TEST</th>
<th>CONCENTRATION OF PLANT EXTRACT (mg/ml)</th>
<th>% OF INHIBITION</th>
<th>Ascorbic acid</th>
</tr>
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<tr>
<td>DPPH assay</td>
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<td></td>
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<tr>
<td>IC50 Value</td>
<td>60</td>
<td></td>
<td>8.5</td>
</tr>
</tbody>
</table>

Anti-Oxidant Activity of Ascorbic acid by using Reducing assay

\[ y = 0.375x + 41.5 \]

\[ R^2 = 0.9868 \]

Anti-Oxidant Activity of Plant extract by using DPPH assay

\[ y = 0.45x + 37 \]

\[ R^2 = 0.9878 \]
Fig 2: Anti-Oxidant Activity of leaf extract of *Beta vulgaris* by using DPPH assay

Table: 3 Anti-oxidant activity of leaf extract *Beta vulgaris* by using H2O2 assay

<table>
<thead>
<tr>
<th>Test</th>
<th>Concentration of plant formulation</th>
<th>% of inhibition for plant Extract</th>
<th>% of inhibition for Ascorbic acid</th>
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<tr>
<td>H2O2 assay</td>
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<td></td>
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</table>

IC 50 Value

38

20

Figure: 3 - Anti-oxidant activity of *Beta vulgaris* by using H2O2 assay
In Reducing assay the percentage of inhibition of 45, 55, 65, 75, 80 at 20, 40, 60, 80, 100 mg/mL concentration respectively and the IC50 value was for Beta vulgaris was found to be 28.8 mg/mL while for standard drug it was found to be 22.6 mg/mL. The results of reducing assay scavenging activity of this study were similar to the results of the in vitro anti-oxidant activity of Acacia fistula (16) (Table 1 and figure 1).

Some of the phytochemical constituents of the Beta vulgaris ion extract may be responsible for the anti-oxidant activity as demonstrated in the present study. Flavonoids or bioflavonoids are a ubiquitous group of polyphenolic substances which are present in most plants, concentrated in seeds, fruit skin or peel, bark, and flowers. Numerous studies have shown that flavonoids possess potent-anti-oxidant activities capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxyl radicals [17] documented the pharmacological activities (anti-inflammatory, anti-viral, anti-bacterial, anti-ulcer, anti-osteoporotic, anti-allergic, and anti-hepatotoxic actions) of flavonoids for their potent anti-oxidant activity (18).

In the present study, the percentage of scavenging effect on the DPPH• radical was concomitantly increased with the increase in the concentration of both standard and Beta vulgaris from 20 to 80 mg/mL. The percentage of inhibition was from 45, 55, 65, 75, 80 at 20 mg/mL to 100 mg/mL for Beta vulgaris and the IC50 value for Beta vulgaris was 60 mg/mL while for standard it was found to be 8.5 mg/mL (Table 2 & Fig 2).

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of the seed extract. Scavenging of DPPH radical is related to the inhibition of lipid peroxidation (11). DPPH is usually used as a substance to evaluate the anti-oxidant activity (12).

The results of the present findings indicated that the DPPH easily accepted the electrons or hydrogen radical from anti-oxidant compounds. When the DPPH had gained the hydrogen atom from the anti-oxidant compounds the colour will be changed. In the present study, the intensity of the colour is directly proportional to the inhibitory activity of the anti-oxidant compound in Beta vulgaris ion. It shows the inhibitory activity is due to the maximum hydrogen donating ability of Beta vulgaris ion. Based on this result the maximum inhibitory activity is noticed in the aqueous extract at 80 mg/mL.

In H2O2 assay the percentage of inhibiton of 34, 55, 66 and 76 at 20, 40, 60 and 80 mg/mL concentration respectively. The IC50 value for Beta vulgaris ion was 38 mg/mL while for standard drug it was found to be 20 mg/mL. The result of H2O2 scavenging activity of this study is similar to the results of the in vitro anti-oxidant activity of Cinnamomum verum (13). (Table 3 and figure 3).

The present study proves the inhibition of hydroxyl radical production from H2O2 in a dose dependent manner. H2O2 can easily penetrate the cell membranes. These molecules will be converted into hydroxyl radicals and damage the cell. The compounds which donated the electrons to H2O2 are called anti-oxidants. The donating electron reacts with H2O2 and neutralizes it, by converting them into water.

Anti-oxidants are important in the prevention of human diseases. Compounds with anti-oxidants activity may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents, and quenchers of single-oxygen formation or reactive oxygen species, thereby protecting the body from degenerative diseases such as cancer. The reactive oxygen species (ROS) are harmful by products generated during normal cellular metabolism or from toxic insult. They lead to a state of oxidative stress that contributed to the pathogenesis of a number of human diseases by damaging lipids, proteins and DNA (14).

Conclusion
From this study it is observed that all the plants posses marked antioxidant effect. The results obtained showed that this plant Beta vulgaris is very important from medicinal point of view, and it
needs further phytochemical exploitation to isolate phytochemical constituents showing antioxidant activity.

**Reference**