A Study on the Antioxidant and Anticancer Activity of red beet pigment of betalains from *Beta vulgaris*

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**Abstract:** In this present investigation, the effect of pH on changes in antioxidant activity and the content of betalain pigments were evaluated during the heating of a beetroot extracts. With pH ranging from 5 to 10 during the heat treatment, the content of red pigments was reduced depending on the pH level of the sample. The greatest losses were recorded at pH of 10. Simultaneously, the heated betalains preparation solution was shown to exhibit a higher antioxidant capacity at pH of 6.0 (66.0%) than at pH of 5.0 (63.3%). Furthermore, anticancer activity was evaluated against HepG2 cell and highest inhibitory was observed with 300µg dried pigment. From the current study, it can be clearly noted that the betalains is a promising source of natural antioxidant and anticancer agent and definitely provides an alternative towards synthetic antioxidant because of its beneficial components.

1. **INTRODUCTION**

Natural products or their derivatives serve as approximately 60 percentages of all chemotherapeutic agents approved by the Food and Drug Administration (FDA). Even though, the search for medicinal plants with therapeutic properties have aggregative in recent days since chemotherapeutic agents are limited by a rise of drug resistance, high cost and severe side effects. Health concerns are on the rise today, nontoxic materials are taken into attention in various industrial fields.

Numerous studies have evaluated the therapeutic effects of extracts from medicinal plants and their derivatives. Natural pigments, derived from plants are biodegradable, renewable, environmentally friendly and known for their use in textile dyeing, food ingredients, cosmetics and pharmaceutics (1). Pigments are secondary metabolities that provide different colors to plant leaves, flowers, fruits, stem and roots, and other vegetative tissues, which serve as bioactive agents for cancer, antioxidant anti-inflammatory, anti-analgesic (2).

In recent years vegetable *Beta vulgaris* has attracted much debate as a health-promoting functional food. The number of reports represents that beetroot as medicinal therapy and use as a natural medicine date back to Roman times (3). Beetroot is a rich source of phytochemical compounds, which consists of ascorbic acid, phenolic acid carotenoids, and flavonoids. Among the vegetables, beetroot had the highest bioactive pigments known as Betalains, which was categorized as either betacyanin pigments that are red-violet in colour or betaxanthin pigment that is yellow orange in colour (4).

The beetroot as a consequential source of polyphenols in addition to Betalains pigments, which are compounds that have to a great antioxidant effect and radical scavenging capacity. A number of investigations have pronounced Betalains to have high antioxidant and anti-inflammatory capabilities (5, 6, & 7). Although there may be no compilation of the research on dietary protection of Betalains and biological activity and also there is few literature only available. Therefore, there is an urgent need for streamlining the biological activity of Betalains. Taking into consideration the above facts, an attempt has been made to evaluate antioxidant, anticancer activities of Betalains.

2. **METHODOLOGY**

**Plant material**

The fresh red beet plant were obtained from the local vegetable market in Namakkal and stored at 4°C. The experiments were generally performed immediately after procurement.

**Separation of pigment (Betalains) from Beetroot**

About 20 g of red beet was mixed in a blender with 100 ml of ethanol (acidified with 2% citric acid) for 15 min at room temperature and left for 24 hours. The extract was filtered and concentrated under vacuum by a rotary vacuum evaporator at 40°C as reported by Francis (8) with some modification. From this stock solution, 1ml was diluted with 1ml of water (1:1); this solution was used for determination betalain content.

**Determination of betalains content**

The Betalains content was determined spectrophotometrically at 536 nm and 486 nm, using a UV-Vis spectrometer, as a sum of betacyanins (BC) and betaxanthins (BX) contents, calculated as follows:

\[
\text{betacyanins (betaxanthins) content (in mg/L)} = \frac{A \times DF \times MX \times 1000}{e \times IX \times W_d} 
\]

Total Betalaines (mg/g) = BC + BX (9)

**Antioxidant activity of Betalains**

The antioxidant activity was a carryout with non enzymatic method. The DPPH was used to determine the antioxidant according to Lee et al., (10) method with some modification and ascorbic acid was used as standard. Extracts each of 0.1 ml were vortexed for 30 sec with 3.9 ml of DPPH solution and left to react for 30 min (dissolve 22mg of DPPH in 50ml of methanol and from this stock 6ml was mixed with 100 of methanol), after which the absorbance at 515 nm was recorded. A control with no
Anticancer activity of Betalains

The cytotoxic effect of Betalains pigment was measured on HepG2 cells using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay. The 100 μL of cells (1X10^6 cells) were added in each well of culture plate. After 24hrs incubation period, add 100 μL of various concentrations of extracts (20, 40, 60, 80, 100, 150, 200, 250 and 300μg) and incubated for 48hrs. Then, the freshly prepared MTT solution (10 μL of 5 mg/mL) was added to treated cells and incubated at 37°C for 4-6 h. The medium was removed, and 200 μL DMSO was added to each well to dissolve the MTT metabolic product. The absorbance measurement was recorded at 570 nm with a spectrophotometer. The values were expressed as mean ± standard deviation (SD). The relative viability of treated cells was expressed in percentage of control untreated cells and was calculated manually using the below formula:

\[
\% \text{ cytoviability} = \frac{A_{\text{S60 of treated cells}}}{A_{\text{S60 of control cells}}} \times 100%.
\]

### RESULTS AND DISCUSSION

Recently, natural pigments have been appreciated and accepted all over the world and they have made an impact on both global health and international trade. Among the various plant pigments, beetroot containing Betalains as one of the major pigment. In recently, Betalains have shown promising bioactive properties, previous reports were exhibiting a strong free radical scavenging capacity of Betalains purified from beetroot. Stintzing and Carle were evaluated that Betalains had anticancer properties. According to previous studies, most of the biological activities listed were documented with number of extraction methods for the separation of Betalains, although, very limited study about the effect of pH and temperature on the separation of Betalains.

In this current study, Betalains was separated from ethanol extract and estimate the concentration of pigment. The total Betalains concentration was determined by the spectrophotometric method. The present study was carried out to determine the stability of Betalains pigments separated from beetroot in different pH media. The results obtained in Table 1 exhibited that there is a relationship between color changes and pH variation. The fall of red pigments in the Betalains preparation solution increased along with rising pH levels. Among the various pH, the pigment was stable in ranges from pH 4 to 6. This was similar to an earlier study of Herbach et al., They were also determined the stability of red pigments in pH 3-7.

### Table 1

<table>
<thead>
<tr>
<th>S. No</th>
<th>pH</th>
<th>Betalains mg/100gm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
</tr>
<tr>
<td>1.</td>
<td>2</td>
<td>129.1</td>
</tr>
<tr>
<td>2.</td>
<td>3</td>
<td>156.3</td>
</tr>
<tr>
<td>3.</td>
<td>4</td>
<td>165.8</td>
</tr>
<tr>
<td>4.</td>
<td>5</td>
<td>183.5</td>
</tr>
<tr>
<td>5.</td>
<td>6</td>
<td>158.2</td>
</tr>
<tr>
<td>6.</td>
<td>7</td>
<td>131.5</td>
</tr>
</tbody>
</table>

In the present study, various temperatures also utilized for the optimization process. The reduction of red pigments raised by the heating of the Betalains preparation solution was the most occurred at the 35°C (Table 2). The increasing degradation value was observed when increasing temperature (Table 2). This phenomenon was similar to previous studies of Anton et al., In recently Afnan et al. was studied the stability of Betalains with in between 25 - 60°C. In an analysis of pigment degradation, temperature and pH were important parameters. The red pigment of Betalains stability is influenced by different internal factors such as, pigment content, pH, moisture content and external factors such as temperature, light, oxygen, which were helping to improve optimum pigment and color retention in foods."
TABLE 2
EFFECT OF TEMPERATURE ON THE SEPARATION OF BETALAINS

<table>
<thead>
<tr>
<th>S. No</th>
<th>Temperature</th>
<th>Betalains mg/100gm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ethanol</td>
</tr>
<tr>
<td>1.</td>
<td>25°C</td>
<td>143.4</td>
</tr>
<tr>
<td>2.</td>
<td>35°C</td>
<td>167.5</td>
</tr>
<tr>
<td>3.</td>
<td>45°C</td>
<td>138.1</td>
</tr>
<tr>
<td>4.</td>
<td>55°C</td>
<td>101.8</td>
</tr>
</tbody>
</table>

Other than their use as natural and harmless pigments in the food industries, Betalains are used for medicinal purpose. Kavitha et al. (19), Attia et al (16), Katarzyna et al. (20), were determined the antioxidant activity of Betalains. Furthermore, Strack et al. (21) showed that Betalains had antiviral and antimicrobial effects. In addition Betalains was active against lung and skin cancer, which was documented by Kapadia et al. (22). According to the backdrop, present work was attempted to determine the antioxidant and anticancer activity of Betalains.

According to optimization results, Betalains was separated with best parameters of pH and Temperature, which was subjected to antioxidant activity with DPPH method (Fig.1). The graphical representation shows the increase in DPPH activity with a response to the increase in drug concentration. The highest antioxidant activity was observed while observed in 5mg concentration of Betalains (66.6±0.946%). This result was in agreement with the result of Kavitha et al., (19), they found that Betalains had an antioxidant activity with DPPH. The IC50 value of Betalains and ascorbic acid was found to be 4mg/ml and 3mg/ml respectively (Fig.1). In the present study, percentage of antioxidant activity was low compared than ascorbic acid (80.6±1.25% at 5mg/ml), which was contrary to the previous study of Stintzing et al., (13), they found that antioxidant activity of betalains are higher than ascorbic acid.

Recent investigation with different cancer cell lines has shown a high chemopreventive potential of Betalains. Fig.2 recorded the anticancer efficiency of Betalains against HepG2 cell lines. Results were reported as the percent growth of the treated cells when compared to the untreated control cells. It could also specify an indication of possible cytotoxic properties of the tested Betalains. The highest cytotoxicity was found in 300 and 200 µg/ml concentrations with 85.3 and 74.5 percent of cell growth inhibition (Fig. 2). It was found that the percentage of growth inhibition to be reduced with increasing of test compounds, and IC50 value of this assay was 162.7µg/ml. A review by Ravi (2) was a report that Betalains had anticancer activity against HEp-2 and MCF-7 cells.

Fig. 1 Antioxidant activity of betalains

Fig. 2 Anticancer activity of betalains against HepG2 cell line
4. CONCLUSION

It can be concluded that the pigment of betalains was selected in the present study having importance in traditional medicine can be considered as a source for the isolation, identification, and development of novel and effective antioxidant and anticancer agents. It is recommended that further studies be conducted to identify and characterize the specific bioactive compounds responsible for the observed antioxidant and antitumor activity and their exact mechanism(s) of action.

REFERENCE