Assess the antioxidant activity of herbal ice cream prepared by selected medicinal herbs

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Abstract
Herbal ice cream is having number of medicinal properties viz anti-septic, anti-microbial, anti-viral, anti-diabetic, antioxidants and etc. The Asparagus racemosus, Asparagus adscendens R. Punica granatum L. and Dactylorhiza hatagirea are as popular medicine plant to fight many human diseases due to present several antioxidant compounds (glutathione, thioredoxin, lipoic acid, ellagitannin-enriched polyphenol and streptozotocin). The present study is carried out to find the quantitative antioxidant activity of herbal powder inclusion in the ice cream. Ice cream was prepared by this method is subjected to sensory properties of herbal ice cream. The best result of DPPH and FRAP activity of herbal ice cream were found inclusion @ 4% herbal powder of selected ice cream.

Keywords: Antioxidant activity, DPPH, FRAP, anti-diabetic, antimicrobial

1. Introduction
Ice cream is a frozen dairy product made by suitable blending and processing of cream and other milk products, together with sugar and flavour, with or without stabilizer or colour and with the incorporation of air during the freezing process (De Sukumar, 1980). Asparagus adscendens Roxb is a commonly used herbal ingredient for treating various health disorders. Active compounds present in asparagus are well known for their multiple health benefits and increase vigorousness (Thakur and Dixit, 2007) and streptozotocin). The present study is carried out to find the quantitative antioxidant activity of herbal ice cream. Ice cream was prepared by this method is subjected to sensory properties of herbal ice cream. The best result of DPPH and FRAP activity of herbal ice cream were found inclusion @ 4% herbal powder of selected ice cream.

Keywords: Antioxidant activity, DPPH, FRAP, anti-diabetic, antimicrobial

2. Materials and Methods
Ingredients used in ice cream were the collected as whole milk was collected (Brand - amul milk) from the khan choraha, mahewa, Allahabad. Skimmed milk powder brand name ‘Anik Spray’ was obtained from the local market of Allahabad. Cream brand name amul was Obtain from local market of Allahabad. Sugar was collected from the local market of Allahabad. Herbs (asparagus, Green asparagus, Salep orchid, pomegranate) were Obtain from local market of Deoband (Saharanpur). Stabilizer and Emulsifier were obtained from scientific corporation, Allahabad.

2.1 Sample
Ice cream mix prepare by in deference treatment from ingredients is whole milk, sugar, cream, skim milk powder, stabilizer and emulsifier, and herb use in deference level respectively.
Treatment of ice cream control treatment T₀ plain ice cream and T₁ to T₉ experimental treatment which used of deference level of herbs.

### 2.2 Antioxidant activity

#### DPPH ASSAY

Antioxidant activity of herbal ice cream was determined using stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), as described by Brand-Williams et al. (1995) [3].

Antioxidant activity analyzed by Diphenylpicrylhydrazyl (DPPH) radical scavenging activity was determined method is based on the ability of the antioxidant to scavenge the DPPH cation radical. The hydrogen atoms or electrons donation ability of the corresponding extract was measured from the bleaching of purple colored MeOH solution of DPPH. This spectrophotometric assay uses stable radical 1,1-Diphenyl-2-picrylhydrazyl (DPPH) as a reagent. Briefly, 0.1 ml of sample extract or standard was added to 5 ml of DPPH reagent (0.00039 g/m in 1 liter methanol) and vortexed vigorously. The reaction tubes were incubated in dark for 30 min, at room temperature and the discoloration of DPPH was measured against a reagent blank at 517 nm. Percentage inhibition of the discoloration of DPPH by the sample was expressed as Trolox equivalents.

### 2.3 Calculation

- All values obtained are acquired from UV spectrophotometer/colorimeter for assays.
- % Antioxidant activity = \((\text{absorbance at blank}) – \text{(absorbance at test)} / \text{(absorbance at blank)}) \times 100

### 2.4 FRAP

The Ferric reducing antioxidant power (FRAP) test was conducted according to the method Described by Benzie and Strain (1996) [3].

### 2.5 Procedure

Micro liters of sample and 100 μl of standard was taken in different two tubes. 3 ml of FRAP reagent was added. Absorbance at 593 nm was measured at 0 minutes after vortexed. Samples were then placed at 37 °C in water bath and absorption was again measured after 4 minutes. Ascorbic acid standards (100 μM-1000 μM) were to be processed in the same way. The content of above tubes was mixed well. OD of the Standard and Test were measured at Zero minute and again after four minutes at 593 nm.

### 2.6 Calculation

\[
\text{FRAP value of sample (μM)} = (\text{Change in absorbance of sample from 0 to 4 minute} / \text{Change in absorbance of standard from 0 to 4 minutes}) \times \text{FRAP value of standard (1000 μM)}
\]

### 3. Result

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, and ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. These free radicals are the major points in lipid peroxidation. The antioxidants may mediate their effect by directly reacting with ROS, quenching them and/or chelating the catalytic metal ions. Several synthetic antioxidants, e.g., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are commercially available but are quite unsafe and their toxicity is a problem of concern. Natural antioxidants, especially phenolics and flavonoids, are safe and also bioactive which are capable of absorb and neutralize free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Recently focus has been concentrated on identification of plants with antioxidant ability that may be used for human consumption (Raushan Kumar et al., 2013) [8].

The results shown above indicate that the herbal powder based ice cream gives high antioxidant activity, which was confirmed by method used for the antioxidant assay. The herbal ice cream prepared by different concentration of pomegranate powder, Asparagus powder, Salep orchid powder and green Asparagus powder in 1%, 2%, 3% and 4% respectively. The maximum antioxidant activity of herbal ice cream was found 4% level of herbs pomegranate (41.83±0.01%) and followed by green Asparagus (41.83±0.01%) and Asparagus powder (28.37±0.00%). The addition of medicinal herbs @ 3% in ice cream, the antioxidant activity were found pomegranate (38.46±0.03%), green Asparagus (34.18±0.00%), Salep orchid powder (35.10±0.03%) and Asparagus powder (26.92±0.01%). The minimum concentration (i.e. 2% and 1%) of natural herbal powder based ice cream was found minimum antioxidant activity.

### Table 1: Table for antioxidant activity and ferric reducing antioxidant power in herbal ice cream in (%).

<table>
<thead>
<tr>
<th>S. N.</th>
<th>Herb Use In Ice Cream</th>
<th>Treatment Combination</th>
<th>Level of Herb</th>
<th>DPPH (%)</th>
<th>FRAP (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>T₀</td>
<td>0 %</td>
<td>00.00</td>
<td>00.00</td>
</tr>
<tr>
<td>2</td>
<td>Pomegranate</td>
<td>T₁P₁</td>
<td>1%</td>
<td>22.93±0.03</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>T₁P₂</td>
<td>2%</td>
<td>29.33±0.11</td>
<td>0.24±0.02</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>T₁P₃</td>
<td>3%</td>
<td>38.46±0.03</td>
<td>0.28±0.01</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>T₁P₄</td>
<td>4%</td>
<td>41.83±0.01</td>
<td>0.31±0.02</td>
</tr>
<tr>
<td>6</td>
<td>Asparagus</td>
<td>T₂A₁</td>
<td>1%</td>
<td>24.28±0.01</td>
<td>0.22±0.03</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>T₂A₂</td>
<td>2%</td>
<td>26.44±0.00</td>
<td>0.25±0.03</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>T₂A₃</td>
<td>3%</td>
<td>26.92±0.01</td>
<td>0.28±0.03</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>T₂A₄</td>
<td>4%</td>
<td>28.37±0.00</td>
<td>0.31±0.03</td>
</tr>
<tr>
<td>10</td>
<td>Salep Orchid</td>
<td>T₃S₁</td>
<td>1%</td>
<td>25.00±0.03</td>
<td>0.13±0.01</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>T₃S₂</td>
<td>2%</td>
<td>29.81±0.01</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>T₃S₃</td>
<td>3%</td>
<td>32.79±0.02</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>T₃S₄</td>
<td>4%</td>
<td>35.10±0.03</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>14</td>
<td>Green Asparagus</td>
<td>T₄G₁</td>
<td>1%</td>
<td>34.38±0.01</td>
<td>0.19±0.02</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>T₄G₂</td>
<td>2%</td>
<td>33.85±0.01</td>
<td>0.22±0.02</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>T₄G₃</td>
<td>3%</td>
<td>34.18±0.00</td>
<td>0.24±0.03</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>T₄G₄</td>
<td>4%</td>
<td>36.59±0.00</td>
<td>0.28±0.02</td>
</tr>
</tbody>
</table>
Ferric reducing antioxidant power analyzed of different level herbs powder used in herbal ice cream gives high value which was confirmed by method used for the FRAP assay. The maximum Ferric reducing antioxidant power of herbal ice cream were found (0.31±0.03%) in 4% level of pomegranate and Asparagus powder and followed by green Asparagus (0.28±0.02%) and Salep orchid (0.21±0.02%). The addition of medicinal herbs @ 3% in ice cream, the Ferric reducing antioxidant power were found pomegranate (0.28±0.01%), Asparagus powder (0.28±0.03%) green Asparagus (0.24±0.03%), and Salep orchid (0.18±0.02%). The minimum concentration (i.e. 2% and 1%) of natural herbal powder based ice cream was found minimum antioxidant power.

4. Discussion
Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. Antioxidant compounds such as polyphenols may be more efficient reducing agents for ferric iron but some may not scavenge DPPH free radicals as efficiently due to Odukoya OA, et al., (2007) [7]. Pulido, et al., (2000) [7] reported that, in general, the ferric ion reducing ability of antioxidants correlates with the results from other methods used to estimate antioxidant capacity. Reducing DPPH radicals were also able to reduce ferric ions. Arnous, et al. (2000) [11] reported a strong correlation between DPPH free radical scavenging ability and ferric ion reducing ability in wines. In the present study herbal ice cream showed the ability to reduce free radicals which may stop the free radicals initiation or retard free radical reaction in the propagation of the oxidative mechanisms.

5. Conclusion
The antioxidant activity of herbal ice cream prepared by different level of selected medicinal herbs can be determined accurately, conveniently, and rapidly using DPPH testing. The results of the present study revealed that the inclusion of herbs powder in the ice cream significantly altered the antioxidant properties of the ice cream samples. Among the different inclusion 4% levels of herbs had the maximum % of DPPH and FRAP activity. Hence, it may be recommended that the herbs could be added at 4 % in the preparation of herbal ice cream.

6. Reference
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10. Thakur and Dixit. Evaluation of antioxidant activity and ameliorative effect Dactylorhiza hatagirea on sexual dysfunction in hyperglycemic male rats Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar (M.P.) 470003, India, 2007.