DETERMINATION OF ANTIOXIDANT ACTIVITY OF SOUTH INDIAN TROPICAL FRUITS ANANAS COMOSUS (PINEAPPLE) AND LEMON (CITRUS LIMON) BY PRUSSIAN-BLUE METHOD

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Abstract:
Pineapple and lemon plant samples was extracted with 10ml of 100% methanol and left it overnight, Next day filtered with Whitman filter paper and make up the volume up to 25ml with 100 ethanol. The anti-oxidant activity was done by Prussian-blue method. A comparison of two fruits confirms that pineapple shows lowest antioxidant activity than lemon.

Keywords: Pineapple, lemon, antioxidant activity, Prussian blue method

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INTRODUCTION:
Antioxidants work by donating an electron to free radicals to convert them to harmless molecules. This protects cells from oxidative damages that lead to aging and various diseases. Manorama Singh studied the most popular and abundant antioxidant vitamins are ascorbic acid, tocopherol and beta carotene [1]. Medical studies show that antioxidants help in preventing certain diseases such as arteriosclerosis. Masaaki Terashima et.al evaluates the antioxidant activities of water soluble ingredients of foods [2-5]. Protective effects of antioxidants against hypochlorite radicals or hydroxyl radicals have been studied by comparing changes in absorbance of myoglobin at 409nm. Marino B. Arnao Studied the antioxidant activity of biological material ABTS and DPPH radical. Kitt and Weiler K studies the antioxidants in food materials [6-8]. The reports show that oxidative stress is closely related to aging process [9-11]. Madsen H L and Bertelsen G. Analysed the oxidation lipids in food stuffs results in the development of off flavor, rendering the product unacceptable for human consumption. Karadeniz Feryal et.al Studied the antioxidant activities of fruits apple quince, grape, pear and pomegranate, potato, onion, spring onion, red reddish and red cabbage were determined. Naik Seema Met al studied that the non-edible portion of custard apple were extracted with solvents of varying polarity and evaluated for their antioxidants activity using different chemical reagents. Ramakrishna B V et al Reports the antioxidant activity of rosella ( Hibiscus sabdariffa) using alpha –alpha diphenyl beta picrylhydrazyl( DPPH) and beta carotene [12].

MATERIAL AND METHODS:
All solvents and chemicals used were analytical /BDR grade. DPPH was obtained from March, Mumbai, India. Fruits were purchased from local market in Nagpur Maharashtra India. 
Uv-Vis. Spectrophotometer
Uv- visual measurement were performed on a UV-Visible 1700 spectrophotometer SHIMADZU
Physico Chemical analysis
Sampling
Two fruits of each treatment were used for all analysis
Acidity
The acidity was measured by titration with 0.1N NaOH to pH 8.1 and expressed as malic acid. Acidity was expressed as % (g/100g) pH
10 g of samples were homogenized for pH measurements. A digital Ph meter was employed at 25°C. 
Total soluble solids:
The content of total soluble solids was determined using samples of fruits pulp with a hand refractometer, at room temperature (range from 18-23°C). 
Ratio (Solids/acidity)
The ratio was calculated using the relation between the total soluble solids by acidity

Tannin
Tannin content was determined according to Hurwitz.

Determination of total phenolic contents
This is a nondestructive rapid and sensitive which can detect a very small quantity following three different methods were used for the determination of phenolic phenolic content which was calculated from the standard graph.

Prussian-blue method
Plant material
Healthy and fresh grapes and pineapple were selected and purchased from the total market of Hyderabad.

Preparation of Samples
Fruits were cleaned under running tap water excessive water was drained off. The fruits were cut into small pieces and subjected to size reduction using kitchen blender with a kitchen mixer to get a thick paste, and kept at 20°C for further analysis.

Preparation of extract:
250mg of plant samples was extracted with 10ml of 100% methanol and left it overnight, Next day filtered with Whitman filter paper and make up the volume up to 25ml with 100% ethanol.

RESULT AND DISCUSSION:
All results were obtained from a minimum of four independent experiments and the relevant means were calculated. Date was expressed on a dry weight basis.

Prussians -blue method
Table 2 shows the phenolics compound present is 86.96 % 141.98% for grapes and pineapple respectively. By this method pineapple shows the highest polyphenolic content as compared to grapes. This test is based on the reduction of tannin and other polyphenolics of ferric ion to ferrous ion , followed by the formation of ferricyanide ferrous iron complex. The colour product known Prussian blue absorbs maximum at 725nm. Increase amount of tannin results in the production of increasing amount of the blue pigment, which absorbs the red end of the spectrum
Table 1: Physicochemical analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lemon</th>
<th>Pineapple</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidity</td>
<td>3.77</td>
<td>2.56%</td>
</tr>
<tr>
<td>pH</td>
<td>4.2</td>
<td>3.20-4.00</td>
</tr>
<tr>
<td>Total soluble solids</td>
<td>1.82</td>
<td>2.36</td>
</tr>
<tr>
<td>Ratio (solid/acidity)</td>
<td>63.38</td>
<td>18.62</td>
</tr>
<tr>
<td>Tannin %</td>
<td>4.419</td>
<td>2.77</td>
</tr>
</tbody>
</table>

Table 2: Prussians-blue method

<table>
<thead>
<tr>
<th>Fruits</th>
<th>OD</th>
<th>% Dry Matter</th>
<th>OD × 78.26 × dilution factor</th>
<th>% Tannin Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>grapes</td>
<td>0.030</td>
<td>82.4</td>
<td>0.072 × 78.26 × 50/82.4</td>
<td>1.424</td>
</tr>
<tr>
<td>pineapple</td>
<td>0.052</td>
<td>89.4</td>
<td>0.052 × 78.26 × 50/82.4</td>
<td>2.276</td>
</tr>
</tbody>
</table>

Factor = 78.26
Dilution factor (DF): ration of final volume/ aliquot volume
Final volume = aliquot + dilute
When DF= 25/0.5= 50
The two fruits have different tannin content. The low value of tannin present in pineapple fruit indicates better antioxidant activity.

CONCLUSION:
- The value of the polyphenolic content is high.
- The results are a direct measure of soluble polyphenolic content
- The test is so sensitive that no interfering color is present at the dilutions used
- The antioxidant activity of fruits varies considerably according to the type of fruit
- The formation of Prussian blue complex offers sensitive, versatile method for spectrophotometric determination of total polyphenols.

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