Assessment of Protective Antioxidant Mechanisms in some Ethno-Medicinally Important wild Edible Fruits of Odisha, India

Uday Chand Basak*, Ajay K Mahapatra and Satarupa Mishra
Regional Plant Resource Centre, R and D Institute of Forest and Environment Department, Govt. of Odisha, Bhubaneswar, India

Abstract
Fruits and vegetables have now been documented as nutraceuticals or functional foods useful for health and medical benefits including prevention and treatment of diseases. With the background of ethno medicinal evidences and the view to utilize the wild fruits resources of Odisha, India as functional foods enriched with antioxidants, 4 wild edible fruits were studied for in vitro radical scavenging activity and antioxidant enzymes such as Peroxidase, Catalase and Superoxide dismutase (SOD) following standard methods. It was found that the fruit with highest DPPH scavenging activity is Antidesma ghaesembilla (1020.6 AEAC mg/100 g dwt) and the lowest recorded in Morinda tinctoria (235 AEAC mg/100 g dwt). The highest FRAP value was recorded in Antidesma ghaesembilla (2114 µM AEAC/g dwt) and the fruit with lowest FRAP value was Careya arborea (538 µM AEAC/g dwt) Antidesma ghaesembilla showed the highest Peroxidase value of 1.2 OD/min/g tissue wt while the lowest was found in Morinda tinctoria (0.054 OD/min/g tissue wt). Catalase was found in high amounts in Antidesma ghaesembilla (5.4×10^4 IEU/g fresh tissue), the lowest value was observed in Dillenia pentagyna (1.2×10^4 IEU/g fresh tissue). Similarly for superoxide dismutase (SOD), the highest value was recorded in Morinda tinctoria (4.43 Δ OD/min/mg protein) and lowest in Careya arborea (1.12 Δ OD/min/mg protein). Current research reveals that these wild edible fruits, especially Antidesma ghaesembilla are rich source of antioxidants and can further be subjected to identification of individual compounds responsible for such high antioxidant activity.

Keywords: Antioxidants; Catalase; Enzymes; Fruits; Peroxidase; Superoxide dismutase

Introduction
It is now well accepted fact that fruits and vegetables are nutraceuticals or functional foods [1]. Nutraceuticals are food or food products that are reported to provide health and medical benefits, including the prevention and treatment of disease. Besides the commercially grown and popularly consumed fruits, wild edible fruits can also be considered part of the continuum since they may be potential source of nutrients and antioxidants.

Antioxidant is a substance that has the ability to delay the oxidation of a substrate by inhibiting the initiation or propagation of oxidising chain reactions caused by free radicals [2]. It plays important roles to prevent fats and oils from becoming rancid and protects human body from detrimental effects of free radicals [2]. Each cell in the body has adequate protective mechanisms against any harmful effects of free radicals i.e. superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding are buffering systems in every cell. α-Tocopherol (vitamin E) is an essential nutrient which functions as a chain-breaking antioxidant which prevents the propagation of free radical reactions in all cell membranes in the human body. Ascorbic acid (vitamin C) is also part of the normal protecting mechanism. Other non-enzymatic antioxidants include carotenoids, flavonoids and related polyphenols, α-lipoic acid, glutathione etc.

There are several methods to investigate the in vitro antioxidant potential of biological samples. These methods differ in the way the free radicals are scavenged by the sample molecules. In this study we have adopted the DPPH (2, 2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric reducing antioxidant capacity) assay to evaluate the total antioxidant potential of the fruit pulp extracts. Similarly for the enzyme assays Peroxidase assay was carried out following Luhova et al. [3], Catalase following Beauchamp et al. [4].

The present research paper explores 4 ethno-medicinally important wild edible fruits namely Antidesma ghaesembilla, Careya arborea, Dillenia pentagyna and Morinda tinctoria for their in vitro free radical scavenging potential and antioxidant enzymatic activity.

Materials and Methods
Four wild edible fruits namely Antidesma gaesembilla, Careya arborea, Dillenia pentagyna and Morinda tinctoria were shortlisted and selected owing to their ethno-medicinal importance. Healthy and infection-free ripe wild fruits were collected from Similipal Biosphere Reserve (District Mayurbhanj), Odisha, India. Voucher specimens were identified in the institutional herbarium. A general account of the selected fruit plants and their uses is presented in Table 1.

Methods
Sample preparation for anti-oxidant activity: The fruits were cleaned and dried at 40°C (not exceeding 50°C) following the suggestion by Khamsah et al. [5]. Then, the dried fruits were ground into fine powder using mortar and pestle. 1 g dried powder of each fruit was weighed and transferred into a beaker. 20 mL of solvent (i.e.

*Corresponding author: Uday Chand Basak Regional Plant Resource Centre, R andD Institute of Forest and Environment Department, Govt. of Odisha, Nayapalli, Bhubaneswar-751015, Odisha, India, Tel: 09437481352; E-mail: uc_basak07@yahoo.co.in.

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FRAP method: The total antioxidant activity was measured using Ferric reducing antioxidant power (FRAP) assay [7]. FRAP assay was determined based on the reduction of Fe$^{3+}$TPTZ to a blue coloured Fe$^{2+}$TPTZ. The FRAP reagent was prepared by mixing 300 mM acetate buffer and the residual H$_2$O$_2$ was titrated against 0.01 N KMnO$_4$ until a faint purple color persisted for at least 15 sec. A control was run at the same time in which the enzyme activity was stopped at “zero” time.

Results and Discussion

Antioxidant capacity

The antioxidant capacity measured by DPPH and FRAP assay demonstrates that the fruit pulp extracts have different free radical scavenging ability for the above methods. This finding corroborates with the findings of Pellegrini et al. [10]. The findings are presented in Table 2 and Figure 1.

DPPH radical scavenging assay

The DPPH test is the oldest indirect method for determining the antioxidant activity which is based on the ability of the stable free radical 2, 2-diphenyl-1-picrylhydrazyl to react with hydrogen donors including phenols [11]. The antioxidant capacity of 4 ethno medicinally important wild edible fruits evaluated by DPPH assay and expressed as AEAC or ascorbic acid equivalent antioxidant capacity, ranged between 235 and 1020.6 AEAC mg/100 g. The DPPH assay depicts that the fruit with highest DPPH scavenging activity is Antidesma ghaesembilla (2114 µM AEAC/g 100 g) and the lowest recorded in Morinda tinctoria (235 µM AEAC/g 100 g).

FRAP antioxidant assay

The FRAP assay measures the ability of antioxidant to reduce Fe$^{3+}$ to Fe$^{2+}$. FRAP values were expressed as µM AEAC/g dw. The highest FRAP value was recorded in Antidesma ghaesembilla (2114 µM AEAC/g dw) followed by Dillenia pentagyna (1099 µM AEAC/g dw). The fruit with lowest FRAP value was Careya arborea (538 µM AEAC/g dw). Values are mean ± SD (n=3).

Table 2: DPPH and FRAP assay of 4 ethno medicinally important wild edible fruits of Odisha.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Fruit Species</th>
<th>Local Name</th>
<th>Ethno medicinal Uses</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Antidesma ghaesembilla</td>
<td>Nuniari</td>
<td>Fruit contains high amount of vitamin C.</td>
<td>[12]</td>
</tr>
<tr>
<td>2</td>
<td>Morinda tinctoria</td>
<td>Achhu</td>
<td>There is greater demand for fruit extract of Morinda species in treatment for arthritis, cancer, gastric ulcer and other heart diseases.</td>
<td>[13]</td>
</tr>
<tr>
<td>3</td>
<td>Dillenia pentagyna</td>
<td>Rai</td>
<td>Tribal folks use various parts of the plant for the treatment of ailments and diseases like delivery (stem), bone fracture (bark), body pain (root), piles (leaf), diabetes (bark), diarrhoea and dysentery (bark).</td>
<td>[14]</td>
</tr>
<tr>
<td>4</td>
<td>Careya arborea</td>
<td>Kumbhi</td>
<td>The fruit extract is used as decoction to promote digestion.</td>
<td>[15]</td>
</tr>
</tbody>
</table>

Table 1: General account of 4 selected wild edible fruits and their ethno medicinal importance.

<table>
<thead>
<tr>
<th>Fruit Species</th>
<th>Sample</th>
<th>DPPH AEAC mg/100 g dry weight</th>
<th>FRAP µM AEAC/g dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antidesma ghaesembilla</td>
<td>1020.66 ± 3.21</td>
<td>2114 ± 1.00</td>
<td></td>
</tr>
<tr>
<td>Careya arborea</td>
<td>850.66 ± 3.00</td>
<td>538 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Dillenia pentagyna</td>
<td>550 ± 3.60</td>
<td>1099 ± 4.00</td>
<td></td>
</tr>
<tr>
<td>Morinda tinctoria</td>
<td>235 ± 13.20</td>
<td>724.5 ± 3.70</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SD (n=3).

Figure 1: FRAP assay of 4 ethno medicinally important wild edible fruits of Odisha.
Antioxidant enzyme assay

The findings of Peroxidase, Catalase and superoxide dismutase assay were revealed in Table 3 and Figures 2-4.

Peroxidase assay

Antidesma ghaesembilla showed the highest Peroxidase value of 1.12 OD/min/g tissue weight while the lowest was found in Morinda tinctoria (0.054 OD/min/g tissue weight). Careya arborea recorded 0.09 while Dillenia pentagyna showed 0.76 OD/min/g tissue weight of Peroxidase activity.

Catalase assay

Catalase helps in breaking down hydrogen peroxide into water and oxygen. Highest Catalase activity was found to be again in Antidesma ghaesembilla 5.4×10^4 IEU followed by Careya arborea 3.1×10^4 IEU and subsequently Morinda tinctoria 2.7×10^4 IEU. The lowest value was observed in Dillenia pentagyna 1.2×10^4 IEU.

Superoxide Dismutase assay (SOD)

SOD ranged between 1.12 and 4.43 Δ OD/min/mg protein. The highest being Morinda tinctoria (4.43 Δ OD/min/mg protein) followed by Antidesma ghaesembilla 2.66 Δ OD/min/mg protein and thereby lowest in Careya arborea (1.12 Δ OD/min/mg protein).

This study establishes that all the 4 wild edible fruits can serve as supplements of natural antioxidants. But notably Antidesma ghaesembilla is the fruit that is not only relishing in taste but is a powerhouse of antioxidants.

Conclusion

Four wild edible fruits namely Antidesma ghaesembilla, Careya arborea, Dillenia pentagyna and Morinda tinctoria were analyzed for their total antioxidant capacity through in vitro radical scavenging assays such as DPPH and FRAP and antioxidant enzyme content for three enzymes namely Peroxidase, Catalase and superoxide dismutase (SOD). The result so obtained clearly signifies that these fruits are rich source of antioxidants (both enzymatic and non-enzymatic). Since antioxidants play crucial role in prevention of many degenerative diseases, these fruits have the scope to be included in functional foods that provide natural antioxidant supplement.

Acknowledgements

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References


