Variation of Antioxidant Properties among Madan (*Syzygium cumini*) Trees in Belihuloya Region, Sri Lanka: Potential for Improvement for Community Use

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BELIHULOYA, SRI LANKA
Climatic condition of Belihuloya region

Climatically transitional area

- **Coordinates** 6°43´5´´N  
  80°46´2´´E
- **Temperature**
  - High 28-31°C
  - Low 18-21 °C
- **Wind** 13-16km/h
- **Day light** 12.5hrs (April-August)
  ranged from 11.5-12.5hrs
- **Elevation** 616m (2021ft above sea level)
Madan (Syzygium cumini)
Taxonomy

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Myrtales
Family : Myrtaceae
Genus : Syzygium
Species : Syzygium cumini (L.) Skeels

(Source: USDA - NRCS, 2011)
• Multipurpose tree

  • Food crop (Fruit), medicinal plant, timber crop, tree for forestry etc.
Effective medicine in Ayurvedic medicine against

- Diabetics,
- Heart and liver diseases
- Anticancer
- Anti-hyperlypidemic activities
- Hypoglycemic,
- Antibacterial
- Anti-HIV activity
- Antidiarrheal effects and
- Antioxidant activity.
Antioxidants

- Molecules which are capable of slowing or preventing the oxidation of other molecules, thereby protecting cells from damages caused by exposure to reactive species

- Protective agents against non-communicable diseases
  - Cancer
  - Alzheimer’s disease
  - Parkinson’s disease
  - Down’s syndrome
  - Diabetes
  - Asthma
Oxidative stress

Free radicals

Antioxidant

Oxidative damage

Lipids Proteins Nucleic acids
Objectives

• To evaluate the variation of antioxidant properties of fruits among naturally available S. cumini trees in Belihuloya region
Materials and methods

- Sample collection
- Sample preparation
- Preparation of crude extracts
- Preparation of stock solutions
- In vitro antioxidant assays
- Calculation of antioxidant activity
- Data analysis
Sample collection

- Healthy disease free fruits from nine trees
- Air dried
- Sealed
- Labeled
  
  (Sc-1, Sc-2, Sc-3, Sc-5, Sc-7, Sc-9, Sc-10, Sc-11, Sc-22)
- Store (-80 °C)
Seed

Pericarp
Sample preparation

99.9% Ethanol / Methanol (50ml)

(32 °C, 30 min)
Preparation of crude extracts

(32 °C, Rotavapor)
- Preparation of stock solutions

DMSO (1ml) → (3-5 min) → 10 mg/ml 

- Ethanol Pericarp (EP)
- Ethanol seed (ES)
- Methanol pericarp (MP)
- Methanol Seed (MS)

Dilution series
### Invitro antioxidant assays

<table>
<thead>
<tr>
<th>Invitro antioxidant assay</th>
<th>Standard method</th>
<th>Absorbance(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Polyphenolic Content (TPC) assay</td>
<td>Folin-Ciocalteu method (Singleton <em>et al.</em>, 1999)</td>
<td>765</td>
</tr>
<tr>
<td>Total Flavonoid Content (TFC) assay</td>
<td>Aluminium Chloride method (Siddharaju and Becker, 2003)</td>
<td>415</td>
</tr>
<tr>
<td>Free radical scavenging activity</td>
<td>2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS⁺) free radical scavenging assay (Re <em>et al.</em>, 1999)</td>
<td>734</td>
</tr>
</tbody>
</table>
Spectrophotometric analysis

**Reaction Mixture**

125 µl of *extracts* + 1 ml of *Folin-Ciocalteu reagent* + 625 µl of *distilled water* + 750 µl of *10% sodium carbonate*

Absorbance at 765 nm in spectrometer

20 µl of *extracts* + 80 µl of *methanol* + 100 µl of 2% *Aluminium chloride* in methanol

Absorbance at 415 nm after ten minutes of incubation

50 µl of *extracts* + 110 µl of *50 mM PBS* + 40 µl of *ABTS*⁺ (incubation 25± °C for 10 minutes)

Absorbance was recorded at 734 nm.
- **Data analysis**

  - Analysis of variance by GLM procedure in Statistical Analysis Software (SAS)v 9.0
  - Means were compared using Duncan's’ multiple Range test
  - Microsoft Excel was use for graphical analysis of data
Results and Discussion

*Syzygium cumini* fruit extracts

![Graph showing extractable crude dry weight (g/5g of dry fruit) for different parts of the fruit and extracting solvents: Ethanol pericarp, Methanpl pericarp, Ethanol seed, and Methanol seed. The graph indicates varying levels of extractable dry weight across the different parts and extracting solvents.]
- Dry weight of ethanol and methanol extracts of *Syzygium cumini* fruit

![Graph showing dry weight of ethanol and methanol extracts of *Syzygium cumini* fruit. The graph displays values with the same letter as not significantly different (p<0.05).]
Total Phenolic Content (TPC) of *Syzygium cumini* trees

\[ y = 2.9169x - 0.052 \]

\[ R^2 = 0.9954 \]

*Values presented are means with 3 independent replicates*
Total phenolic content of *Syzygium cumini* pericarp and seed extracts

values with same letter are not significantly different (p<0.05)
- Total phenolic content of *Syzygium cumini* pericarp extracts and solvent used to extract

(values with same letter are not significantly different (p<0.05))
- Total phenolic content of *Syzygium cumini* seed extracts and solvent used to extract.

Values with same letter are not significantly different (p<0.05)
Total Flavonoid Content (TPC) of *Syzygium cumini* trees

\[ y = 2.152x - 0.0173 \]

\[ R^2 = 0.9968 \]

(Values presented are means with 3 independent replicates)
- Total flavonoid content of *Syzygium cumini* pericarp and seed extracts

Values with the same letter are not significantly different (p<0.05)
- Total flavonoid content of *S. cumini* pericarp extracts and solvent used to extract

![Graph](image)

Values with same letter are not significantly different ($p<0.05$)
- Total flavonoid content of *S. cumini* seed extracts and solvent used to extract

(values with same letter are not significantly different (p<0.05))
ABTS$^+$ radical scavenging activity as Inhibitory concentration 50%

\[
y = 10048x + 21.769 \\
R^2 = 0.9874
\]

(Values presented are means with standard error of 3 independent replicates)
- Inhibitory concentration 50% (IC$_{50}$) value of *Syzygium cumini* methanol pericarp and seed extracts

```
<table>
<thead>
<tr>
<th></th>
<th>Pericarp</th>
<th>Seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ at 734nm (mg/ml)</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>30,0</td>
<td>0,0</td>
</tr>
</tbody>
</table>
```

Values with the same letter are not significantly different (p<0.05)
Inhibitory concentration 50% (IC$_{50}$) of *S. cumini* methanol extracts of seed and pericarp

Values with same letter are not significantly different (p<0.05)
Conclusion

• The best solvent for the extraction of antioxidant compounds is methanol. *S. cumini* seed contains the highest antioxidant compounds compared to pericarp.

• It is evident from that *S. cumini* trees possess diverse antioxidant actions among the population as determined by ABTS radical scavenging assay.

• Phenolic and flavonoid compounds are highly diversified among the *S. cumini* trees.
Suggestions

• Further studies are needed in different maturity stages of fruits and different agro ecological zones
Thank you
Free radicals

A free radical can be defined as any molecular species that contains an unpaired electron in an atomic orbital.

React with proteins, lipids, carbohydrates and DNA, and alter their function.

Attack the nearest stable molecules, stealing its electron, when the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction, finally resulting in the description of a living cell.
### Reactive oxygen species

<table>
<thead>
<tr>
<th>Radicals</th>
<th>Non radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide radical (O$_2$•⁻)</td>
<td>Hydrogen peroxide (H$_2$O$_2$)</td>
</tr>
<tr>
<td>Hydroxyl radical (OH•⁺)</td>
<td>Hypochlorous acid (HOCl)</td>
</tr>
<tr>
<td>Peroxyl radical (RO$_2$•⁻)</td>
<td>Hypobromous acid (HOBr)</td>
</tr>
<tr>
<td>Alkoxy radical (RO•⁻)</td>
<td>Ozone (O$_3$)</td>
</tr>
<tr>
<td>Hydroperoxyl radical (HO$_2$•⁻)</td>
<td>Singlet oxygen</td>
</tr>
</tbody>
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### Reactive nitrogen species

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<td>Nitric oxide (NO•⁻)</td>
<td>Nitrous acid (HNO$_2$)</td>
</tr>
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<td>Nitrogen dioxide (NO$_2$•⁻)</td>
<td>Nitrosyl cation (NO)</td>
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<td>Nitric oxide (NO•⁻)</td>
<td>Nitrosoyl anion (NO)</td>
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<tr>
<td>Nitrogen dioxide (NO$_2$•⁻)</td>
<td>Dinitrogen tetroxide (N$_2$O$_4$)</td>
</tr>
<tr>
<td>Nitric oxide (NO•⁻)</td>
<td>Dinitrogen trioxide (N$_2$O$_3$)</td>
</tr>
<tr>
<td>Nitrogen dioxide (NO$_2$•⁻)</td>
<td>Peroxynitrite (ONOO)</td>
</tr>
<tr>
<td>Nitric oxide (NO•⁻)</td>
<td>Peroxynitrous acid (ONOOH)</td>
</tr>
<tr>
<td>Nitrogen dioxide (NO$_2$•⁻)</td>
<td>Alkyl peroxynitrites (ROONO)</td>
</tr>
<tr>
<td>Nitric oxide (NO•⁻)</td>
<td>Nitryl chloride (NO$_2$Cl)</td>
</tr>
</tbody>
</table>
Beer-Lambert Law

- Monochromatic light of initial intensity $I_0$ passes through a solution in a transparent vessel, some of the light is absorbed so that the intensity of the transmitted light $I$ is less than $I_0$. There is some loss of light intensity from scattering by particles in the solution and reflection at the interfaces, but mainly from absorption by the solution.

- The relationship between $I$ and $I_0$ depends on the path length of the absorbing medium $l$ and the concentration of the absorbing solution, $C$. These factors are related in the laws of Lambert and Beer.
Comparison of present and previous study of antioxidant properties of Madan in Sri Lanka

<table>
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<th>Previous study (mg equivalent/ g extract)</th>
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<tr>
<td>TPC</td>
<td>27.65-149.33</td>
<td>56.77</td>
</tr>
<tr>
<td>TFC</td>
<td>4.21-24.098</td>
<td>3.22</td>
</tr>
<tr>
<td>IC_{50}</td>
<td>9.88-47.85 (mg/ml)</td>
<td>103.42 (mg/ml)</td>
</tr>
</tbody>
</table>

(L.M.P.R.Elamaldeniya, 2015)