Novel health promoting role for papaya fruit extracts: *Biochemical, Molecular and Clinical evidence*

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ANDI Center for Biomedical and Biomaterials Research
University of Mauritius
Themes under the NRC Program

(A) CANCER CHEMOPREVENTION
Cancer chemopreventive actions of Natural products: an insight into their molecular mechanisms

(B) FUNCTIONAL FOODS & NUTRACEUTICAL RESEARCH
Physiological, Biochemical, Clinical, Molecular & Cellular effects of Functional foods/dietary factors

(C) MARINE PHARMACEUTICALS
Biomedical Evaluation of selected Mauritian marine macroalgae, sponges & soft corals in relation to their bioactive constituents
Knowledge of bioactive constituents, their clinical effects and molecular action mechanisms are relevant to maximize health benefits.

Mortality due to NCDs in Mauritius:
- Cardiovascular diseases: 31%
- Diabetes Mellitus: 26%
- Cancers: 12%
- Other NCDs: 11%
- Communicable, Maternal, Nutritional: 7%
- Injuries: 6%
- Respiratory diseases: 5%
Papaya: the miracle fruit?

**Pulp**
Minerals, Vitamins C, lycopene, β-carotene, β-cryptoxanthin
Caffeic acid, gallic acid, protocatechuic acids, caffeoyl hexose deoxyhexoside

**Seed**
Glucosinolates, oleic acid, palmitic acid, β-cryptoxanthin, tannins, alkaloids, phenols

**Peel**
Ferulic acid, caffeoic acid, rutin, quercetin, coumaric acid, kaempferol, isohamnetin

**Fermented Papaya Preparation (FPP)**

Fermentation of ripe papaya pulp gives rise to novel oligosaccharides and increased amino acid levels that exert antioxidant properties

Varieties in Mauritius: Solo, Waimanalo, Ecsotika, Wilcox, Taniung and Rodrigues

- The polyphenolic profile of papaya fruit depends greatly on several factors
  - e.g. *Stage of maturity, temperature, sunlight exposure, attack by insects/infections & quality of soil*

  **Polyphenols work in synergy to contribute to the overall antioxidant potential of papaya**

- The exact profile of FPP is the center of on-going investigations
Fermented Papaya Preparation (FPP) as a novel functional food: What makes it so interesting to study?

- Made from ripe papaya pulp that is fermented by yeast
- Extensively documented for its antioxidant & immune boosting properties
- Bio-fermentation process has rendered the nutritional composition highly complex
- Properties due to formation of novel oligosaccarides and amino acids that are antioxidant in nature

<table>
<thead>
<tr>
<th>General Compounds</th>
<th>Amino Acids</th>
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<tbody>
<tr>
<td>Carbohydrate</td>
<td>Arginine</td>
</tr>
<tr>
<td>Protein</td>
<td>Lysine</td>
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<tr>
<td>Fat</td>
<td>Histidine</td>
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<tr>
<td>Dietary fiber</td>
<td>Phenylalanine</td>
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<tr>
<td>Moisture</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Energy</td>
<td>Leucine</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Folic acid B9</td>
<td>Methionine</td>
</tr>
<tr>
<td>Niacin</td>
<td>Valine</td>
</tr>
<tr>
<td>Sodium</td>
<td>Cysteine</td>
</tr>
<tr>
<td>Iron</td>
<td>Alanine</td>
</tr>
<tr>
<td>Calcium</td>
<td>Glutamic acid</td>
</tr>
<tr>
<td>Potassium</td>
<td>Serine</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Theronine</td>
</tr>
<tr>
<td>Copper</td>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Zinc</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Glycine</td>
<td>Proline</td>
</tr>
</tbody>
</table>
How can papaya be beneficial for health?

Excessive free radicals can attack cellular lipids, proteins and damage DNA causing oxidative stress.

**Free Radicals**
- Superoxide, hydroxyl, nitric oxide, peroxyl, alkoxyl, thyl, sulphonyl, thyl peroxyl, hydrogen peroxide, singlet oxygen, hypochlorous acid, peroxynitrite, organic hydroperoxide

**Oxidative Stress**
- Neurodegenerative diseases
- Diabetes
- Cancer
- Cardiovascular complications
- Cardiac & cerebral ischemia
- Pulmonary & rheumatic diseases

Overproduction of free radicals and oxidative destruction of antioxidant enzymes can seriously burden the defense system causing oxidative stress build up in tissues of major organs.

**Natural defense system**
- Superoxide dismutase, catalase, glutathione, glutathione peroxidase/reductase, transferase, glutaredoxin, thioredoxin, thioredoxin peroxidase/reductase, peroxiredoxin, sulfiredoxin

**Papaya**

- Prevents excess free radicals, restoring internal redox balance hence reducing oxidative stress build up within tissues of major organs
- Boosts the natural defense system by influencing the transcription and translation of genes encoding for enzymatic and non-enzymatic antioxidants
- Promotes good health and better quality of life
Evaluation of the effects of *Carica papaya* fruit extracts on biomarkers of oxidative stress and inflammation
AOA screening of papaya fruit

<table>
<thead>
<tr>
<th></th>
<th>Total Phenol Content (g GAE/100g DW)</th>
<th>Total Flavonoid Content (g QE/100g DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ripe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp</td>
<td>2.32</td>
<td>0.0077</td>
</tr>
<tr>
<td>Peel</td>
<td>3.45</td>
<td>0.034</td>
</tr>
<tr>
<td>Seed</td>
<td>2.18</td>
<td>0.0094</td>
</tr>
<tr>
<td>Unripe</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulp</td>
<td>1.64</td>
<td>0.0029</td>
</tr>
<tr>
<td>Peel</td>
<td>3.66</td>
<td>0.0517</td>
</tr>
<tr>
<td>Seed</td>
<td>3.97</td>
<td>0.0121</td>
</tr>
<tr>
<td>FPP</td>
<td>0.360</td>
<td>0.0066</td>
</tr>
</tbody>
</table>

In general, papaya peel exerted a better antioxidant activity which was related to its polyphenolic composition.
Adipocytes, upon exposure to oxidative/metabolic stress, release Monocyte Chemoattractant Protein-1 (MCP-1). MCP-1 stimulates macrophages to migrate from bone marrow and infiltrate into adipose tissue.

Oxidative/metabolic stress signals will activate these macrophages and cause them to release copious amounts of pro-inflammatory cytokines into the environment. It is these cytokines that can trigger onset of inflammation and cancer development.
Cell Viability under oxidative stress

Ripe Peel + 77.6%
Ripe Seed -26.3%
Unripe Peel + 62.4%
Unripe Seed -39.5%

ROS Accumulation

Ripe Peel - 74.9%
Ripe Seed +12.2%
Unripe Seed -37.7%
Unripe Peel - 74.1%
Pro-inflammatory cytokines

**TNF-α**
- ↓ 59.6% by Unripe Pulp

**IL-6**
- ↓ 41.1% by ripe Seed

**MCP-1**
- ↓ 93.8% by Ripe Seed
Activity of endogenous antioxidant enzymes

**SOD:**
- ↑ 9.7% by Ripe Peel

**CAT:**
- ↓ 36.1% by Unripe Pulp

**GPX:**
- ↑ 12.6% by Ripe Peel

In general, (ripe) **peel and pulp** exerted better antioxidant enzyme-boosting effects at a dose of **20mg DW/ml**
Inhibitory effects of *Carica papaya* fruit extracts against common oral bacteria: *its implication in oral health improvement of diabetics*
Why are diabetics susceptible to dental caries & oral health complications?

- Reduced nutritional supply to gums
- Slowed healing process of injured gums
- Reduced saliva production (dry mouth)
- High sugar content in saliva
- Increased plaque build up in mouth
  - = Dental Plaque & Caries, Bad breath
- Imbalance between production of inflammatory cytokines & growth factors affects healing
  - = Gingivitis, Persistent mouth ulcers
- High blood sugar
- Impaired immune system
- Low blood supply
- Reduced nutritional supply to gums
- Slowed healing process of injured gums
  - = Sore, Bleeding gums
- Reduced collagen production
- Weakened support between gums & teeth
  - = Periodontitis, Brittle teeth, Premature loss of teeth
Simulatory models of dental plaque & caries formation (mimics hydrophobic nature of teeth & gum surface)

<table>
<thead>
<tr>
<th></th>
<th><em>S. mutans</em></th>
<th><em>S. mitis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhibition of Growth</strong></td>
<td>Ripe Seed ↓ 58.5%</td>
<td>Ripe Seed ↓ 87.7%</td>
</tr>
<tr>
<td><strong>Reduction of Biofilm formation</strong></td>
<td>Ripe Seed ↓ 71.7%</td>
<td>Unripe Pulp ↓ 88.7%</td>
</tr>
<tr>
<td><strong>Inhibition of Acid Production</strong></td>
<td>Ripe Seed ↓ 11.8%</td>
<td>Ripe Peel ↓ 35.9%</td>
</tr>
<tr>
<td><strong>Reduced Hydrophobicity</strong></td>
<td>Ripe Pulp ↓ 131.9%</td>
<td>Ripe Pulp ↓ 112.8%</td>
</tr>
</tbody>
</table>
Protective effect of FPP on N-methyl N-nitrosourea-induced hepatocarcinogenesis in balb/c mice
Diets high in nitrosatable foods cause cancer

- high levels of nitrates used in food preservation are **carcinogenic**

- 300 carcinogenic nitrogenous compounds identified in several commercial foods:

  - *Cigarette smoke, Beer & wine, Cheese, luncheon & sausage meats, Canned foods, Chinese-style salted fish, Soy sauce, Pickled vegetables, fish sauce*

- Examples of foods **naturally** high in nitrates:
  
  *Fish, oysters, mussels, crab, lobster, Chinese cabbage, some leafy vegetables*

A possible reason why Asians have high rates of stomach & mouth cancers?

Example: **N-methyl-N-nitrosurea (MNU)**

- MNU causes several cancers in animal models including monkeys, targets the **liver** in mice

  ![Diagram of DNA base pairs](image)

  - Methyl group transferred to nucleobases

  - Leads to **AT:GC** mutations
Balb/c Mice

administered orally twice/day for 12-weeks

**Study Design**

- **FPP**
- **MNU**
- **CONTROL**

Dose administered (mg/kg BW)
- FPP: 300, 500, 700, 1000
- MNU: 50
- CONTROL: PBS

**Analysis**

- HEMATOLOGICAL ASSAYS & BIOMARKER ANALYSIS
- HISTOLOGICAL STUDY OF LIVER CELLS
- DNA ISOLATION & RAMAN ANALYSIS
A dose of 700 mg FPP/kg was optimum for:
- Boosting the antioxidant status (↑65.3%)
- Increase CAT activity (↑87.8%)

A dose of 500mg FPP/kg was optimum for:
- Increase SOD activity (↑18.8%)
- Increase GPX activity (↑7.8%)
- Decrease MDA lipid peroxidation (↓46.9%)

exposure to MNU induced liver tumor formation in Balb/c, FPP supplementation could reduce these effects of MNU
Clinical effects of a short-term supplementation of FPP on biomarkers of oxidative stress in a multi-ethnic pre-diabetic population
# 127 subjects recruited

<table>
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<tr>
<th>FPP (n=49)</th>
<th>Control (n=78)</th>
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<tbody>
<tr>
<td>2 sachets (6 grams) / day before mealtimes for 14 weeks</td>
<td>2-3 glass of water/day before mealtimes for 14 weeks</td>
</tr>
<tr>
<td>“wash-out” period of 2 weeks</td>
<td></td>
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<tr>
<td>44 completed study</td>
<td>57 completed study</td>
</tr>
<tr>
<td>5 discontinued</td>
<td>21 discontinued</td>
</tr>
</tbody>
</table>

**Inclusion Criteria for Participation:**

- blood glucose range 110-126 mg/dL
- age range 35 – 65 years
- non-smoker (or stopped since 6 months)
- daily alcoholic intake < 2 drinks per day
- post-menopausal women not on hormone replacement treatment
- non-hypertensive
- not taking anti-hypertension/anti-diabetes drugs
Blood & urine samples were analyzed using a fully automated clinical chemistry analyzer AU480 Beckman Coulter Inc.

<table>
<thead>
<tr>
<th>Biomarkers of Oxidative Stress &amp; Type 2 Diabetes Mellitus tested for…</th>
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<tbody>
<tr>
<td><strong>Blood Sugar Profile</strong></td>
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<tr>
<td><strong>Lipid Profile</strong></td>
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<td></td>
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<td></td>
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<tr>
<td><strong>Immune Defense System</strong></td>
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<tr>
<td><strong>Liver Functioning</strong></td>
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<tr>
<td><strong>Kidney Functioning</strong></td>
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<td></td>
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<tr>
<td><strong>Systemic Inflammation</strong></td>
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<tr>
<td><strong>Iron Accumulation</strong></td>
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</tbody>
</table>
Effects of a short term supplementation of a fermented papaya preparation on biomarkers of diabetes mellitus in a randomized Mauritian population

Jhoti Somanah a, Okezie I. Aruoma b,*, Teeluck K. Gunness c, Sudhir Kowelssur d, Venkatesh Dambala e, Fatima Murad f, Kreshna Googoolye g, Diana Daus h, Joseph Indelicato h, Emmanuel Bourdon i, Theeshan Bahorun a,*,

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Male (at week 14)</th>
<th>Female (at week 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial blood pressure</td>
<td>1%</td>
<td>3.1%</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>6.5%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Uric acid</td>
<td>1.9%</td>
<td>7.3%</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>13.3%</td>
<td>55.3%</td>
</tr>
<tr>
<td>Total Antioxidant Status</td>
<td>4.9%</td>
<td>5.7%</td>
</tr>
<tr>
<td>AST &amp; ALT</td>
<td>3.6%</td>
<td>12.6%</td>
</tr>
<tr>
<td>Microalbumin to Urinary Creatinine ratio</td>
<td>21.8%</td>
<td>84.1%</td>
</tr>
</tbody>
</table>
Compared to the control group, FPP increased the antioxidant status and reduced the susceptibility of human red blood cells to undergo hemolysis:

- **Week 14**
  - Males: ↓4.4%
  - Females: ↓23.3%

- **Wash-out**
  - Males: ↓2.8%
  - Females: ↓39.1%

Compared to baseline values, FPP consumption reduced the formation of protein carbonyls by:

- **Week 14**
  - Males: ↓1.9%
  - Females: ↓9.7%

- **Wash-out**
  - Males: ↓5.8%
  - Females: ↓11.9%
Summary

Papaya fruit extracts:
- very good free radical scavenging activities in both cell-free and human cell systems (RBCs, SW872), attributed to its polyphenolic content
- boost antioxidant status through the up-regulation of endogenous antioxidant enzymes (SOD, GPx)
- down-regulate the over-secretion of pro-inflammatory cytokines (TNF, IL-6, MCP-1)
- Papaya seed has potential to be studied as anti-cancer agent

Papaya fruit extracts:
- FPP is a very good free radical scavenger.
- Consuming 2 sachets per day can reduce oxidative stress in major organs and in red blood cells
- Consuming FPP on a daily basis can greatly reduce the risk of type 2 diabetes
- FPP causes no side effects
Biopharmaceutical unit: structure and Organisation

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MPhil student

Arvind Gopeechund
MPhil student

Rima Beesoo
MPhil student

Functional Foods & Diabetes

Cancer Biology and Chemoprevention

Marine Pharmaceuticals
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Ministry of Health and Quality of Life
Apollo Bramwell Hospital
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Touro College of Pharmacy (USA)
Osato Research International (Japan)
CSJM Kanpur University (India)
Banarasi Hindu University (India)