In Vitro Antioxidant and α-Amylase Inhibition Activities of Spiced Red Chili Paste (Datta) from South Ethiopia

Conference place: Desalegn Hotel
By: Dr. Engida Dessalegn (engidae@yahoo.com)
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Introduction

• Spices & herbs are most ancient and traditional products in Ethiopia.
• Ethiopia is among the largest consumer in Africa
• The major uses:
  - preparation of spiced ground red pepper (Berbere).
  - preparation of spiced chili paste (Datta).
  - flavor milk, bread, butter, meat, soups, and different vegetables.
  - as medicines.

• Rich sources of secondary metabolites (large family of phytochemicals).
Introduction…

• Spiced chili paste (Datta) is a traditional food in the southern part of Ethiopia, consumed mainly with raw meat and known to improve appetite.

• In general, the ingredients used in the paste are ginger, garlic, basil (leaf), korarima (seed), coriander (leaf and fruit) and chili pepper (red or green).

• The ratio and types of spices and herbs used may differ from home to home or region to region (Siripongvutikorn et al., 2008) that may also affect antioxidant activity.

• The product is commonly available in local market and supermarkets.
Introduction…

• What are free radicals?
• Oxidants (ROS & RNS)
• Highly reactive compounds created in the body or introduced from the environment. (radiation, smoking etc)
• Oxidation and damage of "cellular molecules" such as proteins, lipids and DNA.
• If not eliminated, may lead to chronic diseases: Alzheimer’s, cancer, atherosclerosis, diabetes mellitus, hypertension and aging.
Introduction…

• What are antioxidants?
• A substance that inhibits oxidation or reactions promoted by free radicals.
• Protect organs from excess ROS/RNS (protecting from oxidative damage).
• Synthetic and natural antioxidants
Introduction…

• **Synthetic antioxidant**
  butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as food additives.

  associated with liver damage and carcinogenesis.

• **Natural antioxidants**
  • Sources: vegetables, fruits, spices, herbs, cereals etc.)
  • **Why natural antioxidants?**
  • variety of structures and chemical interactions.
  • numerous biological activities they can perform.
    (antimicrobial, anticancer, antidiabetic, etc)

• Save
Introduction…

- Phytochemicals are Bioactive, naturally occurring plant products.
- Phenolic compounds, large family of phytochemicals (flavonoids, phenolic acids, tannins etc.).
- Present in foods of plant origin and medicinal plants.
- Diets rich in phenolic compounds have many health benefits in relation to antioxidant activity: antimicrobial, antidiabetic, anticancer etc.
- Phenolic compounds scavenge free radicals originating from different oxygen and nitrogen species (ROS/RNS).
Introduction...

- superoxide anion (O2•−), hydroxyl radical (HO•) peroxyl radical (ROO•), nitric oxide (NO•), hydrogen peroxide (H2O2), and peroxynitrite (ONOO•−).

\[
\text{OH} + \text{ROO} \cdot \rightarrow \text{O} + \text{Phenol} \rightarrow \text{Phenoxide radical} + \text{RCOOH}
\]
Introduction…

- Type 2 diabetes mellitus is the most encountered form of diabetes, accounting for more than 80% of the total cases of diabetes.
- Prolonged exposure to elevated glucose induces the production of free radicals, particularly reactive oxygen species (ROS), through glucose auto-oxidation and protein glycosylation.
- The non-enzymatic glycation of proteins (Maillard reaction) is a process closely linked to oxidative stress and is associated with the formation of complex compounds (AGE).
Both synthetic compounds and natural products have been evaluated as inhibitors against the formation of AGEs.

Many synthetic inhibitors were withdrawn from clinical trials due to relatively low efficacies and unsatisfactory safety.

Most people have limited resources and do not have access to modern treatment.

Plants have long been used for the treatment of diabetes, particularly in developing countries.

α-Amylase
  – Catalyzes digestion of starch and oligosaccharides
  – **Inhibitors of digestive enzymes** slow the absorption of carbohydrates.
  – Phenolic compounds inhibit the activity of alpha-amylase
Objectives

• Determine the TPC and TFC contents.
• Evaluate *in vitro* antioxidant activity.
• Determine the *in vitro* alpha-amylase inhibition activity
• Evaluate the relationship between TPC/TFC and antioxidant and alpha amylase inhibition activity to establish phenolic constituents contributions.
Methodology

- Sample collection & preparation of Datta paste

- Datta paste
- Coriander (fruit)
- Chili pepper
- Ginger
- Garlic
- Basil (leaf)
- Korarima (Seed)
- Coriander (leaf)
Methodology…

• Purchased from local market in Hawassa town, South Ethiopia, in October, 2012.
• All samples sorted and washed thoroughly to remove dust and dirt.
• 100 g of chili pepper and 10 g from each spice and herbs were pounded together and then the resulting paste was salted.
• Paste was freeze-dried (Model 2085C0000, Kinetics Thermal Systems, Stone Ridge, NY, USA) and then ground to fine powder using electric grinder (FM100 model, China).
• Sample was stored at −20 °C until used for the in vitro assays.
Methodology...

• Extraction of Datta paste
• Freeze dried sample soaked in solvents- pet. ether, water, acetone, methanol, and methanol (80%) for 6 h at room temperature, in a mechanical shaker.
• Filtered and evaporated to dryness with a rotary evaporator.
• stored in a sealed plastic bottles at -20 °C
Methodology...

- **TPC**
- Folin-ciocalteu method
- The absorbance of the resulting blue color was measured at 765 nm with a UV-visible spectrophotometer
- Expressed as mg GAE/g of dried extract.

Fig 2. Gallic acid calibration curve

\[ y = 0.02x + 0.09, \quad R^2 = 0.99 \]
Methodology...

**TFC**

- Flavonoid-aluminum complex
- Absorbance at 415 nm were taken after 1 h of incubation
- TFC Expressed as mg QE/g of dried extract

**Fig 3 Quercetin calibration curve**

\[ y = 0.024x + 0.11, \quad r^2 = 0.98 \]
Methodology...

- **Antioxidant activity**
  1. **DPPH scavenging**
  - 1mL of the extract (10-1000µg/ml)
  - DPPH radical dissolved in methanol
  - The absorbance was taken at 520 nm using a spectrophotometer.
  - DPPH scavenging (%) = \([(A_0 - A_t)/A_0]\) \times 100

- Ao – absorbance of control, At – absorbance of sample

- BHT and ascorbic acid were used as a reference standard.
2. Reducing Power Activity

(Iron (III) to iron (II) reduction)

The concentration of Fe$^{2+}$ is monitored by measuring the formation of Perl’s Prussian blue.
3. Phosphomolybdenum assay

- Based on the reduction of Mo (VI) to Mo (V) formation of green Mo (V) complexes in acidic medium.
- Plant extract was mixed with the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate).
- The samples were incubated at 95 °C for 90 min, cooled to room temperature and absorbance was measured at 695 nm and methanol was used as blank.
Methodology...

• The antioxidant activity was expressed as milligram butylated hydroxytoluene equivalent/gram of dried extract (mg BHTE/g) based on the calibration curve.

\[ y = 0.43x + 0.08 \]
\[ R^2 = 0.99 \text{ (p < 0.01)} \]

Colorless \rightarrow \text{Green}

Fig 5 BHT calibration curve
Methodology...

Alpha-amylase inhibition activity

• 1 mL of α-amylase (from *Aspergillus oryzae*) was added to test samples 1 mL (1 mg/mL) in phosphate buffer solution.

• 1 mL of 1% boiled potato starch solution in phosphate buffer solution was added.

• After incubation, the reaction was stopped by adding 1 mL of DNSA color reagent (1.0 g of 3, 5- dinitrosalicylic acid, 20 mL of 2 M NaOH and 30 g of sodium potassium tartarate in 100 mL distilled water).

• The sample test tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature.

• Absorbance was read at 540 nm
Methodology...

- **Alpha-amylase inhibition activity**

\[ \% \text{ inhibition} = \left( \frac{A_{\text{control}} - (A_{\text{sample}} - A_{\text{blank}})}{A_{\text{control}}} \right) \times 100 \]

Acarbose was used as reference.
Methodology...

**Fig 6** Reaction of DNSA with reducing sugar

- Reducing sugar reduces DNSA to 3-amino-5-nitrosalicylic acid (red–brown color).
- The magnitude of the absorbance (red–brown) is directly proportional to the amount of reducing sugar produced as a result of hydrolysis of starch.
Methodology...

Statistical analysis

• A triplicate data were analyzed by one way analysis of variance (ANOVA) using SPSS 20.0 statistical software.
• Mean separation was conducted using Duncan’s multiple range tests at $p < 0.05$.
• The (IC$_{50}$) was calculated from the dose–response curves (Origin 8 software).
Result and Discussion

TPC/TFC

Table 1 TPC (mg GAE/g DE) and TFC (mg QE/g DE) of various extracts of red data paste.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolic (mg GAE/g)</th>
<th>Total flavonoid (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>$9.69 \pm 0.87^b$</td>
<td>$8.82 \pm 0.58^d$</td>
</tr>
<tr>
<td>Water</td>
<td>$7.22 \pm 1.21^a$</td>
<td>-</td>
</tr>
<tr>
<td>Acetone</td>
<td>$14.98 \pm 0.76^d$</td>
<td>$22.05 \pm 0.87^c$</td>
</tr>
<tr>
<td>Methanol</td>
<td>$8.55 \pm 0.73^{ab}$</td>
<td>$5.17 \pm 0.08^a$</td>
</tr>
<tr>
<td>Aqueous: methanol (20:80, v/v)</td>
<td>$11.64 \pm 0.43^c$</td>
<td>$16.19 \pm 0.14^b$</td>
</tr>
</tbody>
</table>
1. DPPH scavenging

Result ...

Plant extract

\[ \text{N} - \text{N(C}_6\text{H}_5\text{)}_2 \]

\[ \text{H} + \text{AH} \]

\[ \text{N} - \text{N(C}_6\text{H}_5\text{)}_2 \]

DPPH

antioxidant

DPPH-H

\[ \text{NO}_2 \]

\[ \text{NO}_2 \]

\[ \text{NO}_2 \]

\[ \text{NO}_2 \]
Result...

- **DPPH scavenging activity**

![Graph showing DPPH radical scavenging activity (%)](image)

**Figure 7** DPPH radical scavenging activity (%) of various solvent extracts from dried Datta paste and controls (L-ascorbic acid and BHT). Values are average of triplicate measurements (mean ± SD).
Result…

- **DPPH scavenging IC50 (µg/mL)**

![Bar chart showing DPPH scavenging IC50 values for various solvents and controls. The values are as follows:
- Petroleum ether: 94.22 µg/mL
- Water: 795.96 µg/mL
- Acetone: 392.13 µg/mL
- Methanol: 99.44 µg/mL
- Aqueous: methanol (20:80, v/v): 36.56 µg/mL
- BHT: 23.38 µg/mL
- Ascorbic acid: 87.84 µg/mL

The graph indicates that water has the highest IC50 value, followed by methanol, acetone, and petroleum ether. Ascorbic acid and BHT show lower IC50 values, with ascorbic acid being the lowest.](image-url)
2. Ferric reducing activity

Acetone (11.06) > pet. ether (3.10) > methanol 80% (2.10) > methanol (0.90) > water (0.40) mg AAE/g DE
3. Phosphomolybdenum assay  

Acetone (0.62) > pet. ether (0.43) > methanol  
80% (0.32) > methanol (0.19) > water (0.18)  
mg BHTE/g DE
Result...

Alpha-amylase inhibition activity

C- Control, 1- Water extract (red), 3- Methanol extract (red), 5- Methanol 80% extract (red), 7- Pet. ether extract (red), 9- Acetone extract (red),
Result…

Alpha-amylase inhibition activity

Acarbose (74.50%) > Acetone (56.02%) > methanol (45.60%) > pet. ether (44.95%) > methanol 80% (44.80%) > catechin (34.51%) > water (25.41%)
Result...

Correlation analysis

Figure 8 Correlations between TPC: DPPH scavenging (%) (A) and ferric reducing power (B)
Result...

**Figure 9** Correlations between TPC: TA (C) and alpha-amylase inhibition activity (D)
Figure 10 Correlations between TFC: DPPH scavenging (%) (A) and ferric reducing power (B)
Result...

**Figure 11** Correlations between TFC: TA (C) and alpha-amylase inhibition activity (D)
Conclusion

• TPC and TFC of Datta paste were highest in the acetone extract.
• This extract showed highest value of antioxidant activity and α-amylase inhibition activity.
• This suggests that the antioxidant and α-amylase inhibition activities of the tested extracts were closely associated with their phenolic constituents.
• Datta paste has significant antioxidant and α-amylase inhibition activities, which can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications.
Recommendation

- Evaluation of antioxidant activity of each raw material to see the existence of synergism.
- Isolation of individual compounds to elucidate their different antioxidant mechanisms.
- Effect of the extracts on protein digestibility and sensory quality of food products in the view of the phenolic content and protein precipitation capacity of the extracts.
- Further study on toxicity
- Effect of storage on antioxidant activity and the stability of bioactive compounds on export Ethiopian spices and herbs (ginger, korarima, coriander etc).
Recommendation...

- Effect of thermal treatment (cooking temp, and time)
- further studies on the bioavailability and *in-vivo* antioxidant of various solvent extracts and individual compounds in various animal models.
- Conducting *in vivo* and clinical investigation on isolated bioactive molecules for confirming the activity of antidiabetic agents.
Acknowledgement

- Hawassa College of Education.
- Department of Environmental Sciences, Dalhousie Agricultural College, Canada.
Thank you