WATER-SOLUBLE PALM FRUIT EXTRACT: POTENTIAL ANTI-DIABETIC MECHANISMS

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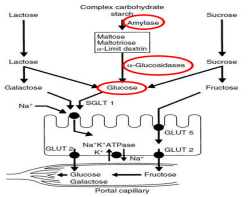
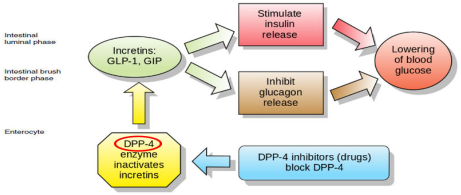
INTRODUCTION

- Type 2 diabetes mellitus (T2DM) was projected to increase to 642 million cases worldwide by 2040 (Chatterjee *et al.*, 2017)
- The water-soluble part of the oil palm fruit is rich in phenolic acids, which could be recovered from the aqueous vegetation liquor during the palm oil milling process (Sambanthamurthi *et al.*, 2011a)
- Water-Soluble Palm Fruit Extract (WSPFE) has potential anti-diabetic activities, as demonstrated in the Nile rat (*Arvicanthis niloticus*) (Sambanthamurthi *et al.*, 2011b; Bolsinger *et al.*, 2014)
- We sought to investigate the possible mechanisms by which WSPFE samples confer potential protection against T2DM



SGLT1 – Sodium/glucose cotransporter 1; GLUT2 – Glucose transporter 2; GLUT5 – Glucose transporter 5
http://www.surgicalcore.org/popup/92049



GLP-1 – Glucagon-like peptide 1; GIP – Gastric inhibitory polypeptide; DPP-4 – Dipeptidyl peptidase IV
http://en.wikipedia.org/wiki/Incretin

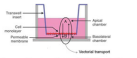
METHODOLOGY

Preparation of WSPFE Samples

WSPFE from Labu Pilot Plant

- Spray Drying to Obtain Spray Dried (SD) WSPFE
- Freeze Drying to Obtain Freeze Dried (FD) WSPFE
- Solvent Extraction to Obtain WSPFE Ethyl Acetate Fraction (EAF)
- Preparative HPLC to Obtain WSPFE Fractions

- Total Phenolic Content Analysis
- Filter Sterilisation



In Vitro Caco-2 Cell Monolayer Model

Positive Controls
- *GLUT2 Inhibitor: Phloretin*
- *SGLT1 Inhibitor: Phloridzin*

Preparation of Human Colonic Caco-2 Cells

- Complete Medium with Fetal Bovine Serum
- 5% Carbon Dioxide in Air at 37°C

Caco-2 Cell Viability Assays

- Promega CellTiter-Glo (CTG) Cell Viability Assay Kit ((Luminescence)

Preparation of Caco-2 Cell Monolayers

- Collagen Coated Corning Costar Filter Inserts

Glucose Uptake Assays

- Glucose Hexokinase Assay Kit

1) Sample
- *SD WSPFE*
- *FD WSPFE*
- *WSPFE EAE*
- *7 WSPFE Fractions*

2) Concentration (μg/mL)
- *0*
- *100*
- *200*
- *300*
- *400*
- *500*

3) Exposure Time
- *Acute (1 Hour)*
- *Chronic (20 Hours)*

4) Presence of Sodium
- *Present (Active and Facilitated Transport)*
- *Absent (Facilitated Transport Only)*

Complementary Experiments on Mouse Everted Intestinal Sacs

- ICR mice
- SD WSPFE
- Glucose Oxidase Assay Kit

http://www.scielo.br/img/revistas/bjps/v40n1/4298.gif
Ex Vivo Intestinal Segment Model

α-Glucosidase Assays

- Abcam Alpha-Glucosidase Activity Assay Kit (Colorimetric)
- Positive Control: Acarbose

α-Amylase Assays

- Abcam Alpha-Amylase Activity Assay Kit (Colorimetric)
- Positive Control: Acarbose

DPPIV Assays

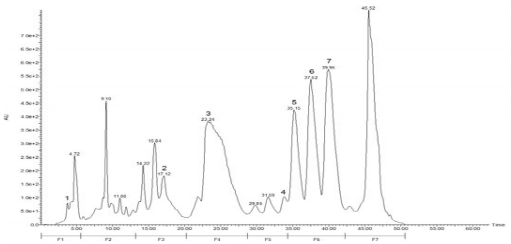
- Promega DPPIV-Glo Protease Assay Kit (Luminescence)
- Positive Control: Sitagliptin

Statistical Analyses

- SPSS Statistics software
- One-Way Analysis of Variance (ANOVA) with Tukey's Honestly Significant Difference (HSD) or Dunnett's Post-Hoc Test against Negative Control
- Differences with $p < 0.05$ Statistically Significant

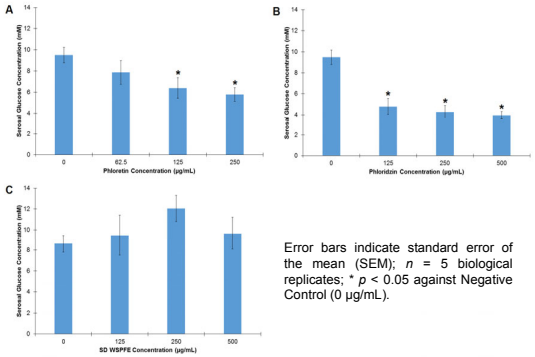
RESULTS & DISCUSSION

Figure 1: Preparative liquid chromatogram of WSPFE fractions viewed at 280 nm ultraviolet wavelength.



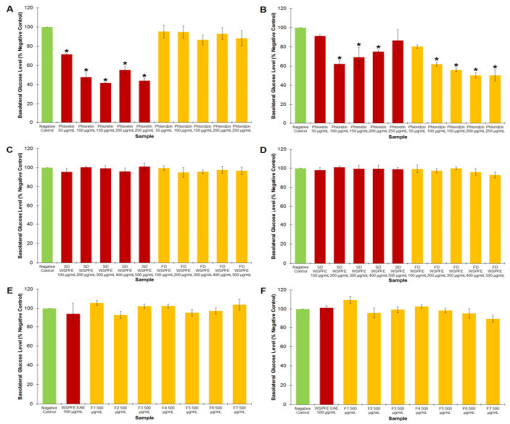
Peaks – 1: Shikimic acid; 2: Protocatechuic acid; 3: *p*-hydroxybenzoic acid; 4: Indoleacetic acid derivative; 5: 5-O-caffeoylshikimic acid; 6: 3-O-caffeoylshikimic acid; 7: 4-O-caffeoylshikimic acid.

Figure 2: Glucose uptake assay results on everted mouse intestinal sacs using positive controls and SD WSPFE of different doses. (A) Phloretin; (B) Phloridzin; (C) SD WSPFE.



Error bars indicate standard error of the mean (SEM); $n = 5$ biological replicates; * $p < 0.05$ against Negative Control (0 μg/mL).

Figure 3: Glucose uptake assay results on human colonic Caco-2 cell monolayers in sodium uptake buffer. (A) Positive controls in acute treatment; (B) Positive controls in chronic treatment; (C) SD WSPFE and FD WSPFE in acute treatment; (D) SD WSPFE and FD WSPFE in chronic treatment; (E) WSPFE EAF and F1 – F7 (500 μg/mL) in acute treatment and (F) WSPFE EAF and F1 – F7 (500 μg/mL) in chronic treatment. Results were similar for assays using sodium-free uptake buffer.



Error bars indicate SEM; $n = 3$ biological replicates; * $p < 0.05$ against Negative Control.

Table 1: IC_{50} values of WSPFE samples against Caco-2 cell viability.

Sample	IC_{50} (μg/mL)
SD WSPFE	*
FD WSPFE	*
WSPFE EAF	*
F1	*
F2	1265 ± 107 [a]
F3	544 ± 48 [bc]
F4	1007 ± 99 [ab]
F5	666 ± 107 [b]
F6	571 ± 178 [bc]
F7	737 ± 78 [b]
Phloretin	131 ± 31 [c]
Phloridzin	*

IC_{50} were expressed as means ± SEM from 3 biological replicates. Means in a column with different letters are significantly different ($p<0.05$). *indicates IC_{50} was not achieved at the highest concentration tested (2000 μg/mL).

Table 2: IC_{50} values of WSPFE samples against α-glucosidase, α-amylase and DPPIV.

Sample	IC_{50} (μg/mL)		
	α-glucosidase	α-amylase	DPPIV
SD WSPFE	*	158 ± 1 [b]	*
FD WSPFE	*	232 ± 13 [a]	*
WSPFE EAF	179 ± 11 [b]	108 ± 13 [b]	224 ± 6 [b]
F1	210 ± 10 [b]	*	*
F2	162 ± 23 [b]	*	162 ± 7 [c]
F3	213 ± 18 [b]	*	291 ± 16 [a]
F4	214 ± 18 [b]	*	196 ± 2 [bc]
F5	210 ± 13 [b]	239 ± 29 [a]	185 ± 4 [c]
F6	*	*	*
F7	*	160 ± 2 [b]	*
Positive Control	440 ± 64 [a]	0.324 ± 0.016 [c]	0.006333 ± 0.000384 [d]

IC_{50} were expressed as means ± SEM from 3 technical replicates. Means in a column with different letters are significantly different ($p < 0.05$). * indicates IC_{50} was not achieved at the highest concentration tested (500 μg/mL). Acarbose was used as the positive control for α-glucosidase and α-amylase assays. Sitagliptin was used as the positive control for DPPIV assays.

- WSPFE samples did not inhibit glucose uptake, as shown through *ex vivo* everted mouse intestinal sac and *in vitro* Caco-2 cell monolayer experiments
- The concentrations of WSPFE samples used in the glucose uptake experiments were only up to 500 μg/mL
- α-glucosidase, α-amylase and DPPIV experiments showed that WSPFE EAF which contained all seven individual WSPFE fractions, consistently had stronger inhibitory effects as compared to SD WSPFE and FD WSPFE
- SD WSPFE had better α-amylase inhibitory effects than FD WSPFE
- For individual fractions, F2 demonstrated the strongest inhibitory effects against α-glucosidase and DPPIV

CONCLUSIONS

- Although WSPFE samples did not inhibit glucose uptake, they showed inhibitory effects on the three enzymes tested, especially WSPFE EAF and F2
- Further studies to investigate their effects on carbohydrate digestion and postprandial hyperglycaemia are warranted

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