Antioxidant and *in vitro* antidiabetic activities of honey produced by different stingless bee species in Malaysia

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Introduction

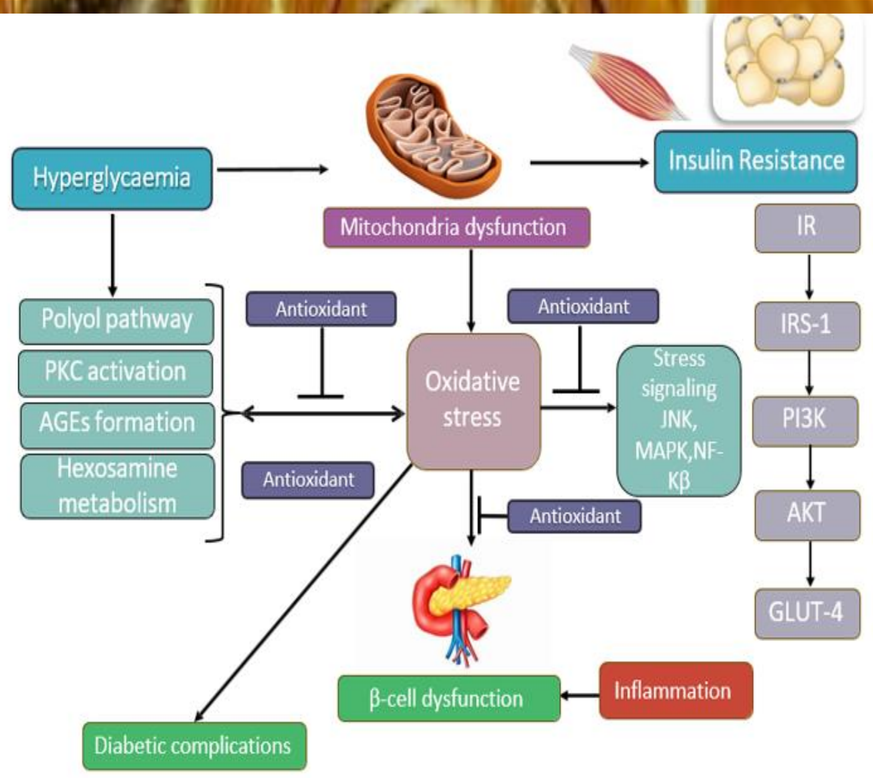
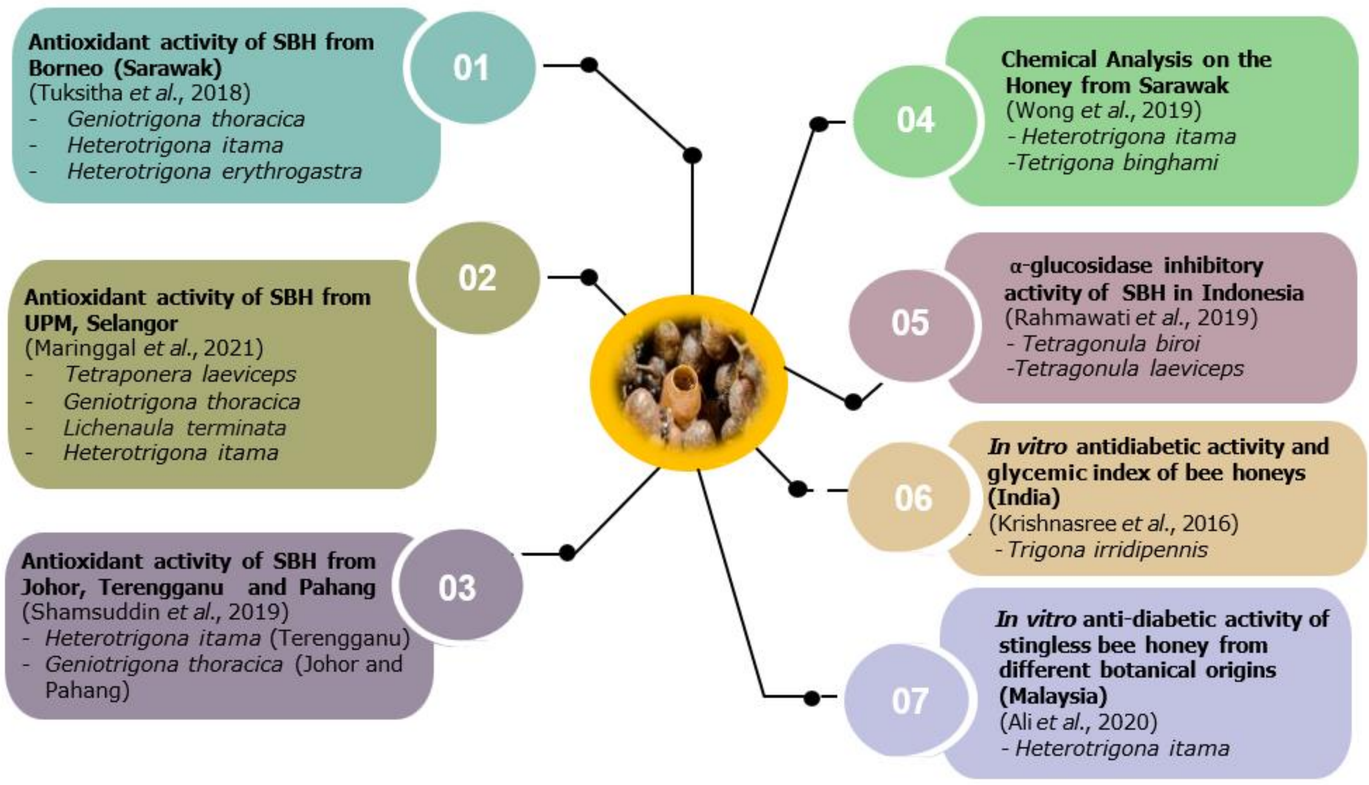


Figure 1: Pathogenesis of oxidative stress in diabetes and the role of antioxidants. (Source: Xu *et al.*, 2021)

Literature Review



Objective

General objective

To study the **antioxidant** and ***in vitro* antidiabetic** activities of honey from 3 different Malaysian stingless bee species.

Specific objective

1. To determine and compare **total phenolic, total flavonoid contents**, **antioxidant** (DPPH, ABTS, FRAP and phosphomolybdenum assays) and ***in vitro* antidiabetic** (α-amylase) activities of 3 different types of stingless bee honey.
2. To determine the correlation between **total phenolic, total flavonoid contents**, **antioxidant** and **α-amylase inhibitory activities**.

Methodology

Total phenolic content assay
(Biluca *et al.*, 2020)

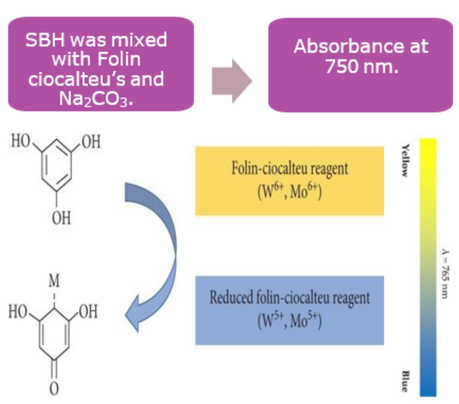


Figure 2: Principle of total phenolic content. (Ford *et al.*, 2019)

Total flavonoid content assay
(Dibacto *et al.*, 2021)

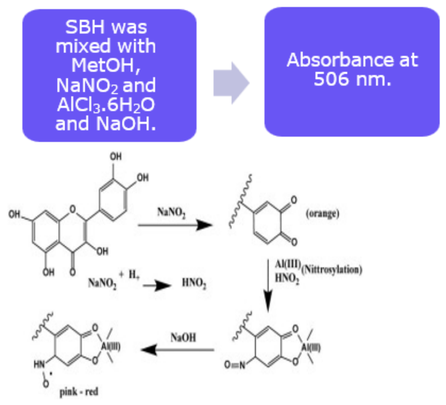


Figure 3: Principle of total flavonoid content. (Makuasa *et al.*, 2019)

Antioxidant activities of SBH

DPPH assay (Alsaleem *et al.*, 2020)

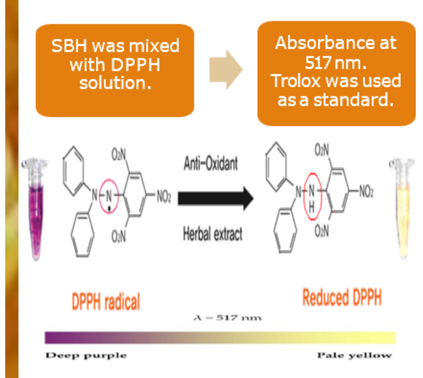


Figure 4: 2,2-diphenylhydrazyl (DPPH) reaction mechanism (Sadeer *et al.*, 2020)

ABTS assay (Echegaray *et al.*, 2021)

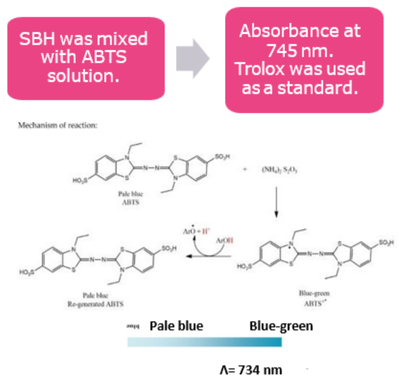


Figure 5: 2,2′-azino-bis (3-ethylbenzothiazoline- 6-sulfonic acid) (ABTS) reaction mechanism (Sadeer *et al.*, 2020)

FRAP assay
(Hsieh & Rajashekaraiah, 2021)

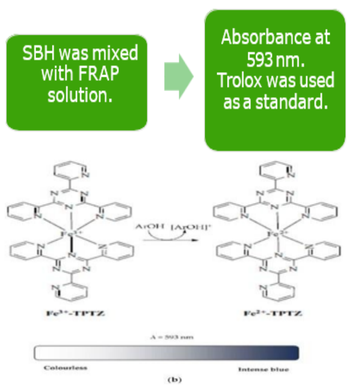


Figure 8: Ferric reducing antioxidant power (FRAP) reaction mechanism. (Sadeer *et al.*, 2020)

Phosphomolybdenum assay
(Batool *et al.*, 2019)

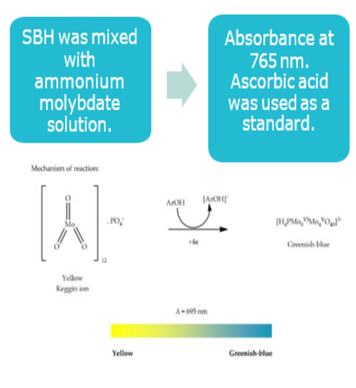


Figure 9: Phosphomolybdenum reaction mechanism. (Sadeer *et al.*, 2020)

***In vitro* antidiabetic activity**

α-amylase inhibitory assay
(Oyedemi *et al.*, 2017)

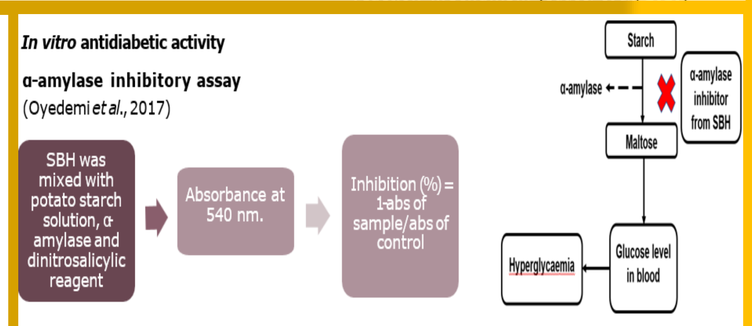


Figure 10: The role of α-amylase inhibitor.

Statistical analysis

- Statistical Package Science Social (SPSS) software version 26.
- One-way ANOVA with post-hoc Tukey HSD & Pearson correlation tests.
- $p<0.05$ (significant difference).

Results

Table 1: TPC, TFC, DPPH (IC_{50}), ABTS, FRAP, phosphomolybdenum and α-amylase (IC_{50}) of different types of SBH.

Type of SBH	TPC (mg GAE/100g SBH)	TFC (mg QE/100g SBH)	DPPH, IC_{50} (mg/ml)	ABTS (mg TEAC/100g SBH)	FRAP (mg $FeSO_4$ equivalent/100g SBH)	Phosphomolybdenum (mg AAE/100g SBH)	α-amylase, IC_{50} (mg/ml)
Heterotrigona itama	46.020±2.558[a]	44.484±0.932[a]	7.429±0.078[a]	28.801±2.526[a]	153.973±18.388[a]	242.203±4.095[a]	13.773±3.163[a]
Geniotrigona thoracica	21.499±2.002[b]	6.640±1.078[b]	88.730±8.353[b]	88.730±8.353[b]	45.625±6.123[b]	151.538±23.499[b]	26.442±3.388[b]
Lophotrigona canifrons	19.765±0.858[b]	9.620±1.061[c]	53.637±3.243[c]	53.637±3.243[b]	46.780±0.745[b]	169.924±25.889[b]	24.265±1.984[b]

Correlation

Table 2: Correlation matrix between TPC, TFC, DPPH (IC_{50}), ABTS, FRAP, phosphomolybdenum and α-amylase (IC_{50}).

Variables	TPC	TFC	DPPH (IC_{50})	ABTS	FRAP	Phosphomolybdenum	α-amylase (IC_{50})
TPC	1	0.995**	-0.894**	0.979**	0.980**	0.881**	-0.970**
TFC		1	-0.928**	0.972**	0.983**	0.865**	-0.961**
DPPH			1	-0.879**	-0.892**	-741*	-0.848**
ABTS				1	0.976**	0.819**	-0.916**
FRAP					1	0.869**	-0.914**
Phosphomolybdenum						1	-0.867**
α-amylase (IC_{50})							1

*Correlation significant at $p < 0.05$ level (2-tailed)
**Correlation is significant at $p<0.01$ level (2-tailed)

Discussion

- Research done by Shamsuddin *et al.*, 2021 also revealed that the antioxidant activity, phenolic and flavonoid contents in *H. itama* honey were significantly higher than *Apis* honey. High concentration of phenolic (ferulic acid, p-coumaric, gallic acid, and salicylic acid) and flavonoid compounds (taxifolin, myricetin and quercetin) in *H. itama* honey.
- The complex composition of honey, the interactions between various antioxidant compounds and potential synergistic relationships between them can also play an important role in antioxidant activities (Tuksitha *et al.*, 2018).
- Different value of phenolic and flavonoid compounds in honey were reported from different studies might be attributed to the different botanical origins, bee species, and geographical locations of honey (Maringgal *et al.*, 2021 Wong *et al.*, 2019).
- From this study, the strong positive correlation between TPC, TFC with antioxidant activities indicated phenolic and flavonoids compounds in SBH has great contribution to the antioxidant activities exhibited by DPPH, ABTS, FRAP and phosphomolybdenum assays.
- A strong negative correlation is seen between TPC and TFC and IC_{50} of α–amylase enzyme inhibition suggested that the greater TPC and TFC, greater will be the ability of SBH to inhibit α–amylase activity.

Conclusion

The honey produced by different stingless bee species (*H. itama*, *G. thoracica* and *L. canifrons*) in Perak displayed variation in phenolic and flavonoid contents. This study exhibited honey *H. itama* exhibited significantly high in TPC, TFC and antioxidant activities. This useful amount of phenolic and flavonoid compounds in the SBH that are able to act as natural antioxidants and also have significant anti-diabetic activity. This study revealed that bee species is important in determination of honey for therapeutic use.

Reference

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