Study of Antiglycation Activities of Young Shoot Leaves of Nine Edible Plants Used in Thai Cuisine as Health Promoter

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INTRODUCTION

Glycation is a natural process in which reducing sugars react with protein amino groups, forming advanced glycation end products (AGEs) linked to metabolic diseases. Plant-based antioxidants like phenolic compounds, flavonoids, and tannins have shown potential in inhibiting this process[1,2]. The 9 plants commonly used in Thai cuisine (Fig.1) were selected to evaluate their antiglycation activity using an in vitro BSA-glucose model and analyzed for phenolic, flavonoid, and tannin content. This study aimed to identify natural, plant-based inhibitors of glycation to help prevent AGE-related health problems.

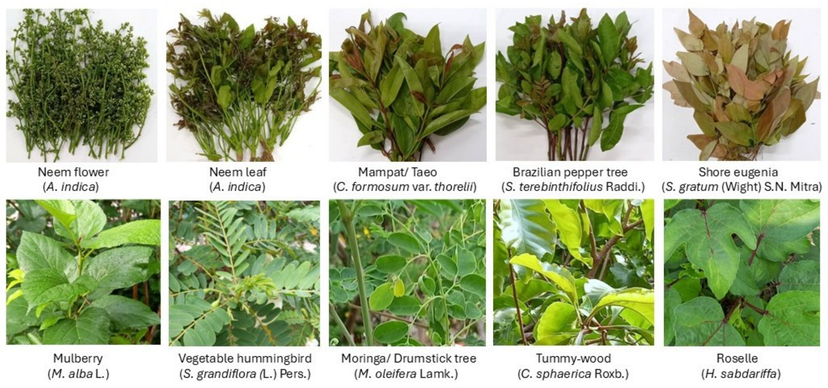

Fig.1 leaves of 9 edible plants used in Thai cuisine as vegetables

METHODS

Plant preparation and extraction: Fresh young shoot leaves of 9 plants were washed and dried at 60°C for 4 h in a hot air oven, then pulverized. 1 g of each plant was weighed and soaked with 10 mL of 75% EtOH at room temperature for 24 h. Then, the mixture was sonicated for 20 min. at 35-37ºC, and the supernatant was collected after centrifugation at 3500 rpm for 10 min. The residual was re-extracted four times with 5.0 mL of 75% EtOH. All supernatants were pooled and filtered using a syringe filter (0.45 µm) and transferred into a tight container and stored at 4-8°C until used. The resulting liquid extracts corresponded to 1 mg of dry powder per 30 µL. Each plant was extracted in triplicate.

Assay of phytochemical substances and antioxidant capacity: (Table 1)

Table 1: Standard methods to analysis of total phenolic, flavonoid, and tannin content and antioxidant capacity.

Substances	Methods	Standards
Total phenolic (TPC)	Folin-Ciocalteu method[3]	Gallic acid
Total flavonoid (TFC)	Aluminum chloride colorimetric method[4]	Quercetin
Total tannin (TT)	Methyl cellulose precipitable tannin method[5]	Aqueous (-)-epicatechin
DPPH scavenging activity	carried out according to the method described by Baliyan et al.[6]	Ascorbic acid

Antiglycation study: Stock solutions of BSA and glucose were prepared in phosphate-buffered saline (PBS), and each plant extract was incubated with these solutions in a microcentrifuge tube, using PBS to adjust the final volume. Positive controls included aminoguanidine HCl, quercetin, and gallic acid, and all samples were incubated at 40 °C for 7–14 days. Four glycoxidation-related parameters were measured using fluorescence at specific wavelengths to detect total AGEs, methylglyoxal-BSA (MGO-BSA), dityrosine, and pentosidine. Antiglycation activity was evaluated by the decrease in fluorescence intensity, and percentage of AGEs inhibition was calculated using a standard formula.

Statistic analysis: Data were expressed as mean ± S.D. Calibration curves were calculated using linear regression. Statistical significance was determined using ANOVA with Bonferroni correction and Pearson correlation in STATA 18.5 BE.

RESULTS AND DISCUSSION

The results of compounding analysis of total phenolic, flavonoid, and tannin content and antioxidant capacity demonstrated on Table 2

Table 2: Total phenolic, flavonoid, and tannin contents, and DPPH scavenging activity of leaf extract

Plants	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	TT (mg EE/g DW)	DPPH scavenging activity (mg AAE/g DW)
A. indica (flower)	31.37 ± 0.90	5.47 ± 0.31	31.61 ± 2.13	30.70 ± 0.369
A. indica (leaf)	50.27 ± 2.64	20.13 ± 0.51	60.75 ± 3.57	53.52 ± 0.39
C. sphaerica	**124.80 ± 2.61**	**7.97 ± 0.59**	**305.07 ± 22.31**	**203.86 ± 21.64**
C. formosum	**167.80 ± 1.61**	**14.03 ± 1.22**	**210.73 ± 7.94**	**124.48 ± 15.57**
H. sabdariffa	26.33 ± 1.00	8.10 ± 0.35	25.97 ± 3.59	18.10 ± 0.77
M. oleifera	35.47 ± 1.78	10.83 ± 0.50	37.88 ± 2.15	21.78 ± 1.25
Morus alba	18.63 ± 0.72	7.57 ± 2.06	25.75 ± 1.10	16.09 ± 1.08
S. terebinthifolius	**149.03 ± 13.92**	**15.70 ± 0.30**	**307.80 ± 14.82**	**264.31 ± 25.45**
S. grandiflora	19.03 ± 1.01	4.07 ± 0.31	31.49 ± 2.36	3.18 ± 0.43
S. gratum	**97.20 ± 2.95**	**11.87 ± 0.51**	**174.47 ± 12.40**	**180.66 ± 1.11**

Antiglycation activity of plant extracts at 1 mg/mL was evaluated using the BSA-glucose model, with aminoguanidine HCl, quercetin, and gallic acid as positive controls. Results showed that while native BSA maintained stable fluorescence, glycated BSA exhibited a 2- to 4-folds increase in AGEs fluorescence over 7 - 14 days of incubation. (Fig.2)

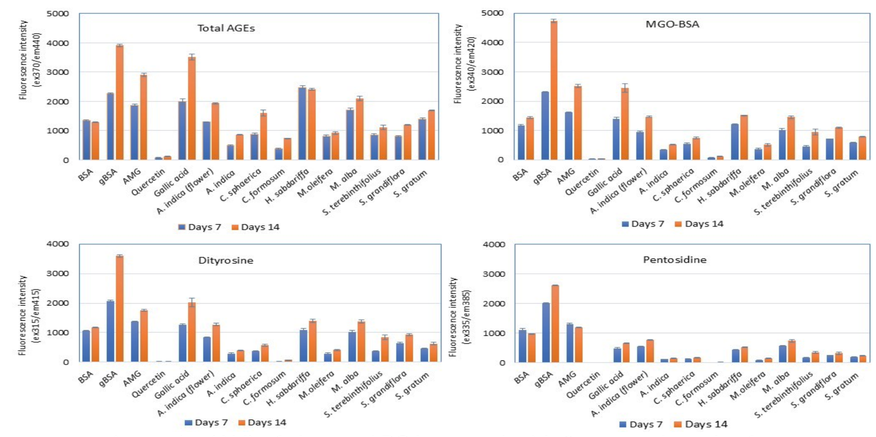

Fig.2 Fluorescence intensity (AU) of glycated BSA treated with 9 young shoot leaf extracts, and controls

Antiglycation activity of 9 leaf extracts, 1 flower extract, and some natural substances on the total AGEs, MGO-BSA, dityrosine, and pentosidine formation was determined by BSA–glucose assay after incubating at 40ºC for 7 days. Each value (n = 3) represents means ± SD. (Table 3)

Table 3: Inhibiting percentages on the several AGEs formation

Plants or controls	Total AGEs (ex370/em440)	MGO-BSA (ex340/em420)	Dityrosine (ex330/em415)	Pentosidine (ex335/em385)
Glycate BSA	0.00	0.00	0.00	0.00
AMG	17.47 ± 1.41	30.02 ± 0.94	33.30 ± 0.76	35.42 ± 1.58
Quercetin	96.84 ± 0.46	98.69 ± 0.09	99.21 ± 0.33	99.74 ± 0.19
Gallic acid	12.16 ± 3.84	39.71 ± 2.76	38.80 ± 1.37	75.93 ± 1.11
A. indica (flower)	42.82 ± 0.69	59.05 ± 1.10	59.39 ± 0.96	72.44 ± 0.05
A. indica (leaf)	78.07 ± 0.72	85.40 ± 0.47	85.79 ± 0.72	94.29 ± 0.32
C. sphaerica	61.42 ± 1.77	76.65 ± 1.10	81.36 ± 0.12	93.86 ± 0.92
C. formosum	**82.84 ± 0.93**	**97.21 ± 0.29**	**98.59 ± 0.26**	**99.50 ± 0.14**
H. sabdariffa	-9.25 ± 2.40	47.52 ± 0.97	47.68 ± 2.52	78.46 ± 0.73
M. oleifera	63.94 ± 1.19	84.27 ± 1.07	85.95 ± 0.95	95.45 ± 0.40
M. alba	24.75 ± 2.70	56.05 ± 2.49	50.55 ± 2.19	71.50 ± 0.71
S. terebinthifolius	62.18 ± 1.37	80.40 ± 1.02	81.78 ± 0.88	91.57 ± 0.64
S. grandiflora	64.73 ± 1.10	69.15 ± 0.21	68.71 ± 1.24	87.64 ± 0.37
S. gratum	38.16 ± 1.71	74.29 ± 0.63	78.00 ± 0.82	90.06 ± 0.23

Inhibition of MGO-BSA, dityrosine, and pentosidine showed strong correlation with phenolic and flavonoid content, while tannin had a moderate effect, suggesting these phytochemicals play key roles in antiglycation. Tannin-rich leaves like *C. sphaerica*, *C. formosum*, *S. terebinthifolius*, and *S. gratum* demonstrated high antiglycation activity and potential for use in nutraceuticals, functional foods, or cosmetics. (Table 4)

Table 4: Pearson correlation of phytochemical substances and antioxidant capacity and antiglycation activities

	Correlation (r)				
substances	antioxidant	Total AGEs	MGO-BSA	Dityrosine	Pentosidine
TPC	0.8600** (p = 0.0001)	0.4758* (p = 0.0079)	0.6659** (p = 0.0001)	0.7017** (p = 0.0001)	0.6405** (p = 0.0001)
TFC	0.4015* (p = 0.0279)	0.4568* (p = 0.0112)	0.6526** (p = 0.0001)	0.6290** (p = 0.0002)	0.5907** (p = 0.0006)
TT	0.9582** (p = 0.0001)	0.3796* (p = 0.0385)	0.5091* (p = 0.0041)	0.5659* (p = 0.0011)	0.5091* (p = 0.0041)

* Correlation is statistically significant at $p<0.05$, and ** at $p<0.001$

CONCLUSIONS

The ethanolic extracts of 9 edible plants showed promising in vitro antiglycation activity, likely through interactions between phytochemicals and BSA that reduce glucose binding. Among them, *C. formosum* leaf extract was the most effective, highlighting the potential of these commonly consumed Thai plants as natural agents for preventing glycation-related health issues.

ACKNOWLEDGEMENT

The authors are thankful to the Department of Pharmacognosy and Pharmaceutical Chemistry, the Department of Pharmaceutical Technology, and the Cosmetic and Herbal Innovation Center (CHIC) for providing the facilities to complete this research successfully.

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