Fig. 1. A procedure for the isolation of phenolic compounds from *Morus alba* cv. *Kuksang*.

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Isolation and Identification of Inhibitory Compounds from *Morus alba* cv. *Kuksang* on α-amylase and α-glucosidase

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Abstract

▪ *Morus alba* cv. *Kuksang* has been used to treat diabetes, stroke, and beriberi disease, so diabetes is divided into three types those dependant on insulin, independent on insulin and of those desiring insulin types, a further 91% are independing on insulin type which mainly occurs after 40 years because of low insulin activating or producing small amounts of insulin. In this study, we tried to obtain basic diabetes data to determine and develop functional food materials which have inhibitory effects on α-amylase and α-glucosidase of isolated phenolic compounds from *Morus alba* cv. *Kuksang* extracts.

Introduction

▪ Many phenolic compounds in plant are good sourece on biological activity. The objective of this study were to dete rmine biological activities of *Morus alba* cv. *Kuksang*.

Materials & Methods

- Total phenolic assay by Folin-Denis' method.
- Measurement of Xanthine Oxidase inhibitory activity by Stirp and Corte method.
- Measurement of Angiotensin converting enzyme(ACE) inhibitory activity by Cushman and Ondetti method.

Result

Table 1. Inhibitory activity of water and ethanol extracts from various mulberry leaves (*Morus alba* L.) against α-amylase and α-glucosidase (1)

Scientific names	Inhibitory activity (%)			
	α-Amylase		α-Glucosidase	
	Water extracts	60% Ethanol extracts	Water extracts	60% Ethanol extracts
Control	0	0	0	0
Morus alba cv. *Cheongilpong*	0.00	75.00	8.09	12.69
Morus alba cv. *Cheongolpong*	36.62	33.49	57.0	34.2
Morus alba cv. *Gosu* 9	42.65	30.29	67.7	56.6
Morus alba cv. *Geumsang*	53.81	39.67	59.9	54.4
Morus alba cv. *Sawonpong*	39.67	13.13	26.4	53.6
Morus alba cv. *Waryong*	28.65	21.94	32.8	54.0
Morus alba cv. *Subongpong*	41.17	13.13	38.5	58.2
Morus alba cv. *Geomseolpong*	11.31	0.00	37.1	56.4
Morus alba cv. *Cheongwoonpong*	60.18	9.47	59.3	54.2
Morus alba cv. *Daeryukpong*	30.29	67.19	58.0	53.1
Morus alba cv. *Hasusang*	60.18	10.58	57.8	57.7
Morus alba cv. *Yongcheonpong*	13.81	10.17	52.5	48.4
Morus alba cv. *Sinilpong*	21.58	34.19	61.7	46.2
Morus alba cv. *Yangmyeonsang*	0.00	9.84	60.1	46.5
Morus alba cv. *Singwanpong*	13.81	15.48	65.8	48.0
Morus alba cv. *Suwonpong*	11.14	11.94	59.5	49.5
Morus alba cv. *Donae*	26.56	9.51	58.7	46.3
Morus alba cv. *Dangsang*	0.00	11.22	53.2	57.1
Morus alba cv. *Chukmu*	16.44	37.37	52.7	55.3
Morus alba cv. *Hongolpong*	31.37	11.22	59.5	47.0
Morus alba cv. *Suseongpong*	0.00	8.42	52.3	46.8
Morus alba cv. *Kuksang* 27	13.81	13.81	64.9	47.9
Morus alba cv. *Sugyeopong*	11.14	21.58	63.4	47.1
Morus alba cv. *Sangilpong*	0.00	21.58	63.8	50.6
Morus alba cv. *Dahojosaeng*	5.67	0.00	59.9	49.6
Morus alba cv. *Gamraksang*	26.56	40.51	62.3	54.7
Morus alba cv. *Wonjugojo*	91.14	24.49	24.9	34.6
Morus alba cv. *Jeokmok*	9.31	45.53	27.2	39.9
Morus alba cv. *Nopal*	81.64	18.16	48.0	29.8
Morus alba cv. *Geumsang*	100.00	53.96	42.9	31.0
Morus alba cv. *Mayudiemoreji*	93.14	70.02	46.6	36.2
Morus alba cv. *Sanghansibmunja*	80.60	0.02	32.4	25.5
Morus alba cv. *Gakjayongsan*	100.00	68.70	33.4	22.5
Morus alba cv. *Daejeongseon*	100.00	68.70	34.6	36.0
Morus alba cv. *Kuksang* 70	87.25	65.98	37.1	40.1
Morus alba cv. *Gabseon*	75.00	30.57	45.5	27.2
Morus alba cv. *Gaeryangseonmunja*	88.08	22.41	37.1	32.4

Continued. (2)

Scientific names	Inhibitory activity (%)			
	α-Amylase		α-Glucosidase	
	Water extracts	60% Ethanol extracts	Water extracts	60% Ethanol extracts
Control	0	0	0	0
Morus alba cv. *Kuksang* 10	92.50	60.20	38.0	28.0
Morus alba cv. *Kuksang*	93.75	28.57	48.7	29.1
Morus alba cv. *Suwonsang* 2	88.08	0.00	42.7	16.2
Morus alba cv. *Dangsang* 1	85.49	2.39	31.7	20.0
Morus alba cv. *Baekgwang* 6	60.20	55.56	38.3	13.2
Morus alba cv. *Sacheongeum*	81.64	15.99	45.6	27.6
Morus alba cv. *Suwonsang* 1	73.80	13.79	47.8	31.5
Morus alba cv. *Gimpasyo*	72.57	0.00	43.3	32.9
Morus alba cv. *Baekasibmunja*	72.57	2.39	37.8	27.6
Morus alba cv. *Buljeon* 2	63.14	0.00	10.3	0
Morus alba cv. *Baekwoon* 32	68.70	9.31	7.7	0
Morus alba cv. *Onodamaguwa*	75.00	24.49	11.7	0
Morus alba cv. *Yamanakaske*	70.02	4.72	0.7	0
Morus alba cv. *Gukbu*	63.14	68.70	0	0
Morus alba cv. *Somok*	58.68	48.99	17.1	5.2
Morus alba cv. *Baekchunil*	63.14	68.70	23.4	0
Morus alba cv. *Lisang*	0.00	50.68	11.7	0
Morus alba cv. *Daedangsang*	2.39	43.76	0	2.5
Morus alba cv. *Suhoik* 20	18.16	9.31	15.67	0.00
Morus alba cv. *Chuncheon* 1	18.16	18.16	17.72	7.85
Morus alba cv. *Palbang*	4.72	11.57	28.83	13.61
Morus alba cv. *Guan*	4.72	0.00	31.71	13.61
Morus alba cv. *Yageunnosang*	34.48	7.03	25.54	0.00
Morus alba cv. *Cheongsibjosaeng*	9.31	0.00	3.74	0.00
Morus alba cv. *Gukhwa*	2.39	4.72	11.14	16.90
Morus alba cv. *Baenggal*	2.39	0.00	0.00	18.55
Morus alba cv. *Yulmok*	4.72	0.02	0.00	7.85
Morus alba cv. *Sugwang*	4.72	11.57	18.55	0.00
Morus alba cv. *Gwasu*	9.31	9.31	4.56	10.73
Morus alba cv. *Byeongmusang*	4.72	11.57	7.44	2.09
Morus alba cv. *Suwonsang* 3	13.79	2.39	15.67	19.78
Morus alba cv. *Nogukyasang*	0.02	4.72	23.89	10.32
Morus alba cv. *Simbaek*	22.41	18.16	9.49	5.79
Morus alba cv. *Busa*	0.02	0.02	21.01	5.79
Morus alba cv. *Gerusaresteri*	4.72	7.03	0.00	4.15
Morus alba cv. *Sabangso*	0.02	4.72	0.00	0.00
Morus alba cv. *Mijiro*	40.83	19.46	39.22	0.00

Continued. (3)

Table 2. Content of total phenolics in water and 60% ethanol extracts from *Morus alba* cv. *Kuksang*

Sample	Contents of phenolics (mg/g)	
	Water extracts	40% Ethanol extracts
Morus alba cv. *Kuksang*	9.7±0.2	14.3±0.2

Each value represents the mean±SD (n=6)

Table 3. Inhibition of extracts from *Morus alba* cv. *Kuksang* on α-amylase activity

Sample	Water extracts		60% Ethanol extracts		Positive control (Acarbose)
	clear zone (cm²)	Inhibition (%)	clear zone (cm²)	Inhibition (%)	Inhibition (%)
Control	13.9±0.6	–	13.9±0.6	–	–
Morus alba cv. *Kuksang*	0.9±0.2	93.8±1.1	9.9±0.1	28.6±0.8	40.0±2.3

Each value represents the mean±SD (n=6), concentration of sample was 200 μg/mL phenolics

Table 4. Inhibition of extracts from *Morus alba* cv. *Kuksang* on α-glucosidase activity

Sample	Water extracts		60% Ethanol extracts		Positive control (Acarbose)
	PNP (μg/mL)	Inhibition (%)	PNP (μg/mL)	Inhibition (%)	Inhibition (%)
Control	3.5±0.2	–	3.5±0.2	–	–
Morus alba cv. *Kuksang*	1.8±0.1	48.7±2.9	2.5±0.1	29.1±3.6	67.9±2.1

Each value represents the mean±SD (n=6), concentration of sample was 200 μg/mL phenolics

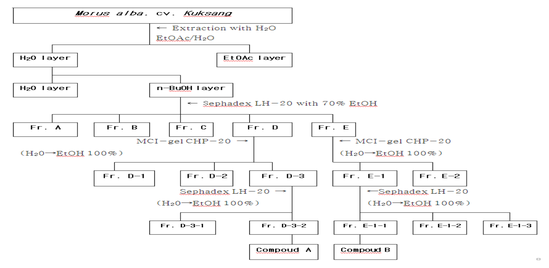

Fig 1. A procedure for the isolation of phenolic compounds from *Morus alba cv. Kuksang.*

Table 5. The contents of phenolic compounds and inhibition effect of solvent fraction from *Morus alba* cv. *Kuksang* extracts on α-amylase and α-glucosidase activity

Solvent	Phenolics content (mg/20 g)	Inhibition activity (%)	
		α-Amylase	α-Glucosidase
Ethyl acetate layer	154.4±0.3	100	9.6±0.1
Butanol layer	105.3±1.6	100	27.6±0.1
H_2O layer	216.2±1.7	54.2±0.9	12.5±0.2

Phenolics content in extracts were 200 μg/mL for inhibitory activity on α-amylase and α-glucosidase activity

Table 6. Content of phenolic compounds and inhibition activities of α-amylase and α-glucosidase from phenolics fractions by Sephadex LH-20 column chromatography

Fraction	Phenolics content (μg/mL)	Inhibition activity (%)	
		α-Amylase	α-Glucosidase
Control	–	–	–
A	0.0±0.1	–	–
B	1.4±0.1	1.6±0.5	–
C	12.6±0.5	76.4±5.6	3.9±0.2
D	32.2±1.2	100.0±3.7	12.6±1.4
E	29.4±1.1	100.0±6.8	19.4±1.1

Phenolics content in extracts were 200 μg/mL for inhibitory activity on α-amylase and α-glucosidase activity

Table 7. Inhibitory activities of phenolics fraction from Sephadex LH-20 on α-amylase and α-glucosidase

Fraction		α-Amylase		α-Glucosidase	
		Glucose (μg/mL)	Inhibition (%)	PNP (μg/mL)	Inhibition (%)
Control		320.8±5.6	–	3.66±0.21	–
D	D-1	323.2±4.7	–	3.64±0.60	–
	D-2	321.5±1.6	–	3.65±0.42	–
	D-3	151.9±6.2	52.7±6.3	2.75±0.23	24.9±1.7
E	E-1	215.1±3.8	32.9±3.2	2.37±0.33	35.3±1.4
	E-2	325.5±3.0	–	3.66±0.38	–

Each value represents the mean±SD (n=6), phenolics content in extracts were 200 μg/mL for inhibitory activity on α-amylase and α-glucosidase activity

Table 8. Inhibitory activities of phenolics fractions by MCI-gel CHP-20 on α-amylase and α-glucosidase

Fraction		α-Amylase		α-Glucosidase	
		Glucose (μg/mL)	Inhibition (%)	PNP (μg/mL)	Inhibition (%)
Control		325.6±3.9	–	3.56±0.18	–
D-3	D-3-1	323.9±6.7	–	3.56±0.53	–
	D-3-2	101.8±8.3	68.7±2.2	2.51±0.61	29.5±0.7
E-1	E-1-1	167.9±5.2	48.4±2.3	2.15±0.12	39.6±1.2
	E-1-2	326.6±2.7	–	3.57±0.14	–
	E-1-3	325.2±8.8	–	3.55±0.69	–

Each value represents the mean±SD (n=6), phenolics content in extracts were 200 μg/mL for inhibitory activity on α-amylase and α-glucosidase activity

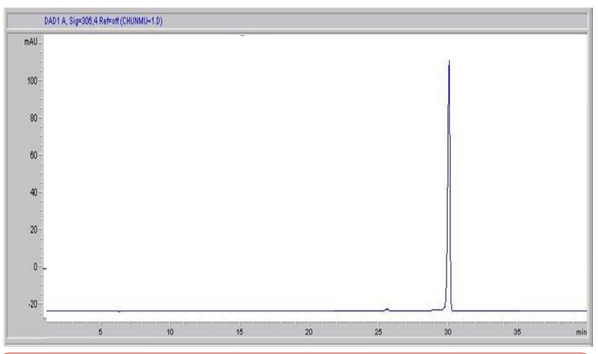

Fig 2. Chromatography of purified D-1-3 and F-1-1 compounds from *Morus alba* cv. *Kuksang* extracts.

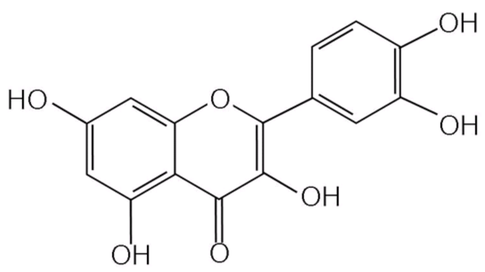

Fig 3. The structure of the purified compound.

Table 10. Spectroscopic data of purified compound with inhibitory activity on α-amylase and α-glucosidase from *Morus alba* cv. *Kuksang*.

	Type	Yellow crystal
	FAB-MS (m/z)	[338]
	Melting point	313-314℃
Purified compound	IR (cm^{-1})	3382 (OH) 1667 (unsaturated ketone) 1615 and 1509 (aromatic C=C)
	^{1}H-NMR	6.16 ppm(1H, d, J=2 Hz, 6-H) 6.39 ppm(1H, d, J=2 Hz, 8-H) 6.82 ppm(1H, d, J=16 Hz, 3'-H) 7.56 ppm(1H, dd, J=2, 8 Hz, 6'-H) 7.71 ppm(1H, d, J=8 Hz, 2'-H) 12.71 ppm(1H, brs, aromatic-H)
	^{13}C-NMR	178 (C-4), 164 (C-7), 160 (C-5) 156 (C-9), 148 (C-2), 136 (C-3) 103 (C-10), 98 (C-6), 94 (C-8) 146 (C-4'), 145 (C-3'), 122 (C-1') 116 (C-5'), 115 (C-2')

Conclusion

▪ The inhibitory activity of the water extracts (200 μg/mL phenolics) against α-amylase and α-glucosidase was determined as 93.8% and 48.7% respectively. The purification of inhibitory compounds was carried out by Sephadex LH-20 and MCI-gel CHP-20 column chromatography using a gradient elution procedure of increasing ethanol in H_2O. The quercetin was confirmed to be the chemical structure of the inhibitory compound against α-amylase and α-glucosidase by spectroscopic analysis of FAB-MS, NMR and IR spectrum.

Reference

▪ AOAC (1989) Official method of analysis. 14th ed. Association of official analytical chemists, Washington DC **43**, 1-3.
▪ Baik SC, Kim JB, Cho MJ, Kim YC, Park CK, Ryou HH, Choi HJ, and Rhee KH (1990) Prevalence of *Helicobacter pylori* infection among normal Korean adults(in Korean). *J Korea Soc Mic* **25**, 455-462.