Anti-*Helicobacter pylori* Activity of Herbal Medicines

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The extracts of *Coptidis japonica* (rhizoma), *Eugenia caryophyllata* (flower), *Rheum palmatum* (rhizoma), *Magnolia officinalis* (cortex) and *Rhus javanica* (galla rhois) potently inhibited the growth of *Helicobacter pylori* (HP). However, these herbal extracts showed no inhibitory effect on HP urease except *Galla rhois*. Among the components separated from active herbal extracts by silica gel column chromatography, the inhibitory effects of decursinol angelate and decursin on the growth of HP were the most potent, followed by magnolol, berberine, cinnamic acid, decursinol and gallic acid. Minimum inhibitory concentrations (MICs) of decursin and decursinol angelate were 6 —20 μg/ml

Key words *Helicobacter pylori*; *Angelica gigas*; *Coptis japonica*; berberine; magnolol; decurcin

Helicobacter pylori (HP) was isolated from the gastric antrum of chronic gastritis patients by Warren and Marshall in 1983.[1] HP also produces a vacuolating toxin and its toxicity may be potentiated by urease-mediated ammonia production.[2] HP urease is considered to play critical roles in the pathogenesis of gastritis and peptic ulcer. Therefore, eradication of this bacteria and inhibition of the HP urease are important for the treatment of patients with gastroduodenal diseases.[3]

Several trials in U.S.A. and western Europe have shown that HP could be eradicated by mixed therapeutic agents such as antibiotics, bismuth subsalicylate, proton pump inhibitors and H_2-blockers.[4] All these drugs were administered for eradication over a long period and adverse side-effects often occurred in patients. However, anti-HP activity of traditional medicines has not been studied except the reports: Imamura *et al.*[5] that ethanol extract of some traditional herbs inhibited the growth; Jones *et al.*[6] that capsaicin inhibited HP growth; and Kim *et al.*[7] that water extract of some traditional herbs inhibited the growth and the urease of HP. Therefore, it is valuable to screen what kinds of herbs can inhibit the growth of HP. In the present study, water extracts of more than fifty herbal medicines were investigated to determine their inhibitory effects against urease activity and growth of HP *in vitro*.

MATERIALS AND METHODS

Materials Brucella agar and brucella broth were purchased from Difco Co. (U.S.A.). Horse serum, gallic acid and cinnamic acid were from Sigma Chem. Co. (U.S.A.). AnaeroPak Campylo was from Mitsubishi Gas Chemical Co. Inc. (Japan). HP ATCC43504, NCTC11637 and NCTC11638 were purchased from ATCC and NCTC, respectively. The other HPs (HP82516, 82548, 4), clinical isolates selected from Korean gastroscopic samples, were kindly donated by Dr. J.-P. Park, Korean Reseach Institute of Chemical Technology. Herbal medicines used here are listed in Table 1, were obtained from a drug store (Heungin Yakup Co., Seoul) in Korea and identified taxonomically. These materials were extracted with boiling water for 6 h and the extracts were then evaporated to concentrate them. These extracts were sequentially fractionated with ether, ethylacetate and butanol.

Isolation of HP Growth-Inhibitory Components from Herbal Medicines To isolate the active component from the ether extract of rhizoma of *Angelica gigas*, this ether extract (50 g) was applied to silica gel column chromatography (5×80 cm) and eluted with 5.5 l of hexane–ethylacetate (9 : 1 to 2 : 1). The active fraction (10 g) was applied to silica gel column chromatography (2.5×27 cm) and eluted with 2.3 l of CH_2Cl_2–ethylacetate (9 : 1). Two compounds were isolated and recrystallized with EtOH: decursin (**1**, 230 mg) and decursinol angelate (**2**, 145 mg), compared with authentic compound (Chart 1).[8] After the hydrolysis of **2** (100 mg) with 5% KOH, the hydrolysates were adjusted to pH 3 with 10% H_2SO_4, extracted with ethylacetate and concentrated. The resulting powder (40 mg), decursinol (**3**) was recrystallized with MeOH. To isolate the active component from the ethylacetate extract of rhizoma of *Coptidis japonica*, this (2 g) was applied to silica gel column chromatography (2.5×30 cm) and eluted with 2 ls of $CHCl_3$–MeOH (4 : 1). Four compounds were isolated and recrystallized with MeOH. Then the anti-*Helicobacter pylori* activities were assayed for these compounds. Among them, the most active compound (0.18 g) was berberine, compared with authentic compound.[9] To isolate the active component from the ether extract of cortex of *Magnoliae officinalis*, this fraction (20 g) was applied to silica gel column chromatography (4×60 cm) and eluted with 3.2 l of benzene–ethylacetate (9 : 1). The active compound (1.5 g) was isolated and recrystallized with benzene, and was identified magnolol (**4**), compared with authentic compound.[10]

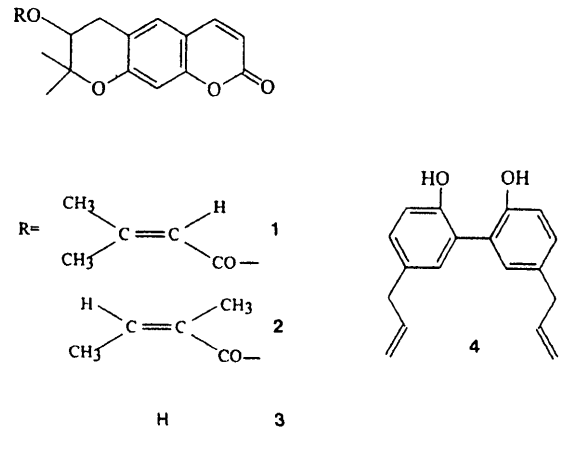

Chart 1

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Preparation of Urease from HP and Its Assay HP was inoculated from an agar plate into 30 ml of brucella broth supplemented with 10% fetal bovine serum in a 100 ml flask, which was placed in an anaerobic jar with AnaeroPak Campylo. The harvested cells were washed with 10 ml of 20 mM phosphate buffer, pH 7.0, sonicated and centrifuged at 5000×***g*** for 30 min. The resulting supernatant was used as the crude enzyme. Urease activity was determined according to the method of Gutmann and Bergmeyer.[11]

Assay of HP Growth-Inhibitory Activity One ml of each extract or isolated compound was placed in a petri dish containing 7 ml of unsolidified brucella agar supplemented with 7% horse serum. Final concentration of each extract was 1 mg/ml, and final concentration of each isolated compound was 200, 100, 80, 60, 40, 25, 20, 10, 8, 4, 2, 1, 0.5, 0.2 or 0.1 μg/ml. Approximately 5×10^5 colony forming unit (CFU) of HP was inoculated into the agar plates and cultured microaerobically for 3 d at 37 °C in an anaerobic jar (85% N_2, 10% CO_2, 5% O_2). The mininum inhibitory concentration (MIC) range was determined after an incubation period of 72 h. Ampicillin was used as a positive control. All experiments were conducted in triplicate.

RESULTS AND DISCUSSION

The inhibitory effect of the water extract of each herbal medicine on urease activity and growth of HP is shown in Table 1. Although the extracts of *Cimicifuga heracleifolia*, *Rhus javanica* and *Mentha arvensis* var. *piperascins* weakly inhibited the urease activity at 0.3 mg/ml, most of the herbal medicines showed no inhibitory effect. However, the extracts of *Coptidis japonica*, *Eugenia caryophyllata*, *Rheum palmatum*, *Magnolia officinalis* and *Rhus javanica* very strongly inhibited the growth of HP at 1 mg/ml. *Angelicae gigas*, *Cinnamomum cassia*, *Angelica dahurica*, *Scutellaria baicalensis*, *Perilla sikokiana*, *Vitex rotundifolia* and *Phenodendri Cortex* also strongly inhibited growth. These extracts were sequentially fractionated with ether, ethylacetate and butanol and the inhibitory effects of the fractions on HP urease were tested. Polar solvent-fractionated extract had only weak inhibitory activity on HP urease, but non-polar solvent-fractionated extract strongly inhibited the HP growth. These results suggested that the growth of HP is hard to control using the inhibitory action of these herbal extracts on its urease. Therefore, we separated the active components from these non-polar solvent-fractionated extracts and measured their inhibitory potency (Table 2). Among the isolated compounds, decursin (**1**) and decursinol angelate (**2**) were the most potent: MIC was 6—20 μg/ml. MICs of magnolol (**4**), gallic acid, berberine and cinnamic acid were 10—40, >200, 8—200 and 80—200 μg/ml, respectively. Compared to thiosulfinate and (+)-protolichesterinic acid, previously isolated

Table 1. Inhibitory Effects of Herbal Medicines on the Urease and Growth of *Helicobacter pylori*

Herbal medicine[a]	Family name	Inhibition		Herbal medicine	Family name	Inhibition	
		Urease (%)	Growth[b]			Urease (%)	Growth
Achyranthes japonica (radix)	Amaranthaceae	0	−	*Glycyrrhiza uralensis* (radix)	Leguminosae	0	−
Amomum xanthioides (seed)	Zingiberaceae	0	−	*Hordeum vulgare* (seed)	Gramineae	0	−
Amomum xanthioides (dried semem)	Zingiberaceae	0	−	*Liriope platyphylla* (tuber)	Liliaceae	0	−
Angelica dahurica (radix)	Umbelliferae	0	++	*Lithospermum erythrorhizon* (radix)	Boraginaceae	0	−
Angelica gigas (radix)	Umbelliferae	0	++	*Lonicera japonica* (flower)	Caprifoliaceae	0	−
Angelica koreana (radix)	Umbelliferae	0	−	*Lycium chinense* (fructus)	Solanaceae	0	−
Angelica tenuissima (radix)	Umbelliferae	0	−	*Magnolia officinalis* (cortex)	Magnoliaceae	0	+++
Anthriscus sylvestris (radix)	Umbelliferae	0	−	*Massa medicata* fermentata		0	−
Aralia cordata (radix)	Araliaceae	0	−	*Mentha arvensis* var. *piperascins* (herba)	Labiatae	29	−
Artemisia capillaris (herba)	Compositae	0	−	*Paeonia albiflora pallas* var. *trichocarpa* (radix)	Ranunculaceae	0	−
Astragalus membranaceus (radix)	Leguminosae	0	−	*Paeonia moutan* (cortex radicis)	Paeoniaceae	0	−
Atractylodes japonica (rhizoma)	Compositae	0	−	*Panax ginseng* (radix alba)	Araliaceae	0	−
Atractylodes japonica (rhizoma alba)	Compositae	0	−	*Perilla sikokiana* (herba)	Labiatae	0	++
Bupleurum falcatum (radix)	Umbelliferae	2	−	*Phellodendron amurense* (cortex)	Rutaceae	10	++
Cassia obtusifolia (seed)	Leguminosae	0	−	*Pinellia ternata* (tuber)	Araceae	0	−
Chrysanthemum indicum (flower)	Compositae	0	−	*Platycodon grandiflorum* (radix)	Campanulaceae	0	−
Cimicifuga heracleifolia (rhizoma)	Ranunculaceae	42	−	*Poncirus trifoliata* (fruit)	Rutaceae	0	−
Cinnamomum cassia (ramulus)	Lauraceae	0	++	*Poria cocos*	Polyporaceae	0	−
Citrus aurantium (fruit)	Rutaceae	0	−	*Prunus armeniaca* var. *ansu* (seed)	Rosaceae	0	−
Citrus aurantium subsup. *nobilis* (pericarpium)	Rutaceae	0	−	*Pueraria thunbergiana* (radix)	Leguminosae	0	−
Cnidium officinale (rhizoma)	Umbelliferae	0	−	*Rheum palmatum* (rhizoma)	Polygonaceae	0	+++
Coptis japonica (rhizoma)	Ranunculaceae	16	+++	*Rhus javanica* (galla rhois)	Anacardiaceae	37	+++
Cyperus rotundus (rhizoma)	Cyperaceae	0	+	*Saussurea lappa* (radix)	Compositae	0	−
Eugenia caryophyllata (flower)	Myrtaceae	0	+++	*Schizandra chinensis* (fruit)	Schizandraceae	0	−
Forsythia viridissima (fruit)	Oleaceae	0	−	*Scutellaria baicalensis* (radix)	Labiatae	0	++
Fritillaria thunbergii (bulbus)	Lililaceae	0	−	*Vitex rotundifolia* (fruit)	Verbenaceae	0	++
Gardenia jasminoides (fruit)	Rubiaceae	0	−	*Zingiber officinale* (rhizoma)	Zingiberaceae	0	−

a) Final concentration of each herbal extract for the inhibitory activity of HP urease was 0.3 mg/ml. *b*) Inhibitory potency of each herbal extract on HP growth was preliminarily estimated as follows: very strongly inhibited (+++; MIC, <1 mg), strongly inhibited (++; MIC, 1—2 mg), weakly inhibited (+; MIC, 2—5 mg) and not inhibited (−; MIC, >5 mg). *H. pylori* ACTC 43504 was used here.

Table 2. MIC of Compounds Separated from Some Herbs on the Growth of *Helicobacter pylori*

Compound	MIC (μg/ml)					
	HP ATCC43504	HP NCTC11637	HP NCTC11638	HP 82516	HP 82548	HP 4
Cinnamic acid	100	80	80	80	80	200
Decursin	20	10	10	10	6	10
Decursinol angelate	20	10	10	10	6	10
Decursinol	200	200	60	60	40	100
Berberine	40	20	8	40	20	200
Magnolol	20	10	10	20	10	40
Gallic acid	>200	>200	>200	>200	>200	>200
Ampicillin	1	1	0.5	1	2	2

from natural plants,[12,13] decursin and decrusinol angelate strongly inhibited the growth of HP. However, these compounds did not inhibit HP urease except gallic acid. Thus, the HP growth inhibitory modes of these compounds were different from that of the urease inhibitors, *p*-hydroxyhippurohydroxamic acid, omeprazole and rabeprazole.[14—16]

Compared to these antibiotics which have been used in clinics, the isolated compounds showed inhibitory effects on growth of HP at one-order higher concentration. However, the resistant pathogens for these antibiotics and their side effects have appeared.[17,18] Therefore, herbal extracts and the isolated compounds are believed to contribute to the prevention of the gastritis to some degree, even if they are not potent growth inhibitor.

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