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# Anti-*Helicobacter pylori* Diarylheptanoid Identified in the Rhizome of *Alpinia officinarum*

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**The antibacterial activity of materials derived from the rhizome of *Alpinia officinarum* (Zingiberaceae) against *Helicobacter pylori* ATCC 43504, ATCC 700392, and ATCC 700824 was examined using paper-disc diffusion and agar dilution methods. Results were compared with those following treatment with currently used antibiotics: amoxicillin, metronidazole, and tetracycline. The bioactive principle was characterized as the diarylheptanoid 5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-3-heptanone by spectroscopic analysis. This compound was isolated from *A. officinarum* leaves as a new anti-*H. pylori* principle. Against *H. pylori* ATCC 43504, ATCC 700392, and ATCC 700824, the antibacterial activity of the diarylheptanoid (48, 24, and 24 μg/mL) was comparable to that of metronidazole (32, 16, and 16 μg/mL) but less effective than that of either amoxicillin (0.06, 0.06, and 0.03 μg/mL) or tetracycline (0.5, 1, and 0.5 μg/mL), based on minimum inhibitory concentrations. *A. officinarum* rhizome-derived materials, particularly the diarylheptanoid isolated, merit further study as potential antibacterial functional food products or therapeutic products for prevention or eradication from humans from diseases caused by *H. pylori*.**

**Key words:** Alpinia officinarum, *diarylheptanoid, 5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-3-heptanone,* Helicobacter pylori, *natural antibacterial agent*

In humans, *Helicobacter pylori* is a microaerophilic Gram-negative bacterium that colonizes the stomachs of approximately half of the world's populations [Dunn *et al*., 1997]. This organism is found to be highly associated with a number of the most important diseases of the upper gastrointestinal tract including gastric inflammation, chronic superficial gastritis, duodenal and gastric ulcers, gastric adenocarcinoma, and non-Hodgkin's lymphomas of the stomach [Blaser, 1992; Taylor and Parsonnet, 1995; Dunn *et al*., 1997]. In developing countries, 70-90% of population carries *H. pylori* [Taylor and Parsonnet, 1995; Frenck and Clemens, 2003], whereas the prevalence of infection in developed countries is lower, ranging from 25 to 50% [Taylor and Parsonnet, 1995]. Most infections by *H. pylori* are acquired in childhood and persist lifelong if not eradicated properly.

*H. pylori* eradication has principally been done with the use of antibacterial drugs, including potent combination therapies consisting of two or three antibiotics such as amoxicillin, clarithromycin, and/or metronidazole with bismuth or a proton pump inhibitor, which are still the most effective. They have often serious side effects such as taste disturbances, nausea, diarrhea, dyspepsia, headache, and angioedema [Goddard and Longan, 1996; Dunn *et al*., 1997; Ohsaki *et al*., 1999] as well as disturbing human gastrointestinal microflora [Ahn *et al*., 2000; Zoppi *et al*., 2001]. The cost of combination therapy is also significant. Additionally, widespread use of antibiotics in combination therapies has often resulted in the development of resistance [Goddard and Longan, 1996; Graham *et al*., 1996; Dunn *et al*., 1997]. These

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**Abbreviations:** ATCC, American Type Culture Collection; FT-IR, Fourier transform infrared spectroscopy; HPLC, high-performance liquid chromatograph; MIC, minimum inhibitory concentration; MS, mass spectrometry; NMR, nuclear magnetic resonance spectrometry; TLC, thin-layer chromatography; TMS, tetramethylsilane; UV, ultraviolet spectroscopy

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problems highlight the need for the development of new treatment strategies for selective *H. pylori* eradication.

Plant preparations, in part, of their often multiple targets, have less adverse effects and lower potential to cause resistance, and have been suggested as a source of anti-*H. pylori* products [Raskin *et al*., 2002]. These potential new antibacterial products can be applied to humans in the same manner as the anti-*H. pylori* drugs currently used. Additionally, some plant preparations are highly effective against antibiotic resistant strains of *H. pylori* [Fahey *et al*., 2002; Fukai *et al*., 2002]. Therefore, much effort has been focused on plant preparations and their constituents as potential sources of commercial anti-*H. pylori* products [Mitsher *et al*., 1972; Fabry *et al*., 1996; Cowan, 1999; Yesilada *et al*., 1999]. In this paper, we assess the antibacterial activity of the constituents that comprise the rhizome of lesser galangal, *Alpinia officinarum* Hance (Zingiberaceae), against *H. pylori*. The antibacterial activity of the plant rhizome constituent was then compared with three commonly used antibiotics, amoxicillin, metronidazole, and tetracycline, against *H. pylori* strains ATCC 43504, ATCC 700392, and ATCC 700824.

Mature whole *A. officinarum* Hance was collected from Hainan Island, P. R. China, in September 2003 and identified by Dr. Sang-Cheol Shin, Department of Forest Environment, Korea Forest Research Institute (Seoul, Korea). A voucher specimen (PRC-1) was deposited in the Research Institute for Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University.

Three bacterial strains were incubated on Brucella agar supplemented with 5% bovine calf serum at 37°C for 3 days in anaerobic jars (Hirayama, Tokyo, Japan), then colonies were suspended in 10 mL of Brucella broth. The inoculum (0.1 mL) was prepared to contain $1\times10^{7\text{-}8}$ CFU/mL by adjusting the turbidity of the suspension. Three quantities (0.01, 0.1 and 1 mg) of *A. officinarum* rhizome-derived material in 0.1 mL of methanol solution were applied by a micropippet to paper discs (Advantec, 8-mm diameter and 1-mm thickness, Toyo Roshi, Tokyo, Japan). After drying in a fume hood, the paper discs were separately placed on the Brucella agar surface, each inoculated with a *H. pylori* strain. All plates were incubated at 37°C for 3 days in a condition of 5% $O_2$, 15% $CO_2$ and 80% $N_2$ in an anaerobic jar. Diameters of inhibition zones were recorded.

Air-dried rhizomes (1.5 kg) of *A. officinarum* were pulverized and extracted twice with 5 liters of methanol at room temperature for 2 days and filtered. The combined filtrate was concentrated under vacuum at 40°C to yield approximately 9% as a dark brownish tar (based on the weight of the dried rhizome). The extract (40 g) was sequentially partitioned into hexane (6.4 g), chloroform (20.2 g), ethyl acetate (9.0 g), butanol (2.6 g), and water soluble (1.8 g) portions for subsequent bioassay. Significant differences in the antibacterial activity toward *H. pylori* ATCC 43504 were observed across the various fractions of the extract (Table 1), and were used to identify peak activity fractions for the next step in purification. At a quantity of 0.1 mg/disc, the chloroform soluble fraction exhibited the most pronounced antibacterial activity and was used for further fractionation. The strong and moderate antibacterial activity was obtained from the hexane and ethyl acetate soluble fractions, respectively. At 1.0 mg/disc, the butanol and water soluble fractions exhibited no antibacterial activity.

**Table 1. Antibacterial activity of the solvent fraction from methanolic extracts of *Alpinia officinarum* rhizome against *Helicobacter pylori* ATCC 43504 using paper-disc diffusion bioassay**

| Material | Inhibition zone (mm) | | |
|---|---|---|---|
| | Dose (mg/disc) | | |
| | 1 | 0.1 | 0.01 |
| Methanol | 23 | 16 | 8 |
| Hexane | 35 | 20 | 8 |
| Chloroform | 35 | 25 | 10 |
| Ethyl acetate | 23 | 16 | 8 |
| Butanol | 8 | 8 | 8 |
| Water | 8 | 8 | 8 |

Tests were conducted in three replicates.

The most active chloroform soluble fraction (10 g) was chromatographed on a 70×5.5 cm silica gel column (600 g) and eluted with a gradient of chloroform and methanol (90:10, 70:30, 50:50, and 0:100, v/v). Column fractions were monitored by TLC on silica gel plates with hexane and ethyl acetate (55:45, v/v). Fractions with similar $R_f$ values on the TLC plates were pooled. Of three fractions, the bioactive C2 fraction (6.4 g) was chromatographed on the preparative TLC plates under hexane and ethyl acetate (55:45, v/v). HPLC was used to further separate the constituents from the active fraction (0.62 g). The column was a 7.8×300 mm μPorasil (Milford, CA) using a mobile phase of hexane and ethyl acetate (1:1, v/v) at a flow rate of 2 mL/min. Chromatographic separations were monitored using a UV detector at 284 nm. Finally, a potent active compound **1** (0.23 g) was isolated at a retention time of 8.97 min. The structure of the isolate was identified the diarylheptanoid 5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-3-heptanone (Fig. 1) by spectroscopic analysis, including MS and NMR. The interpretations of proton signals of this compound were

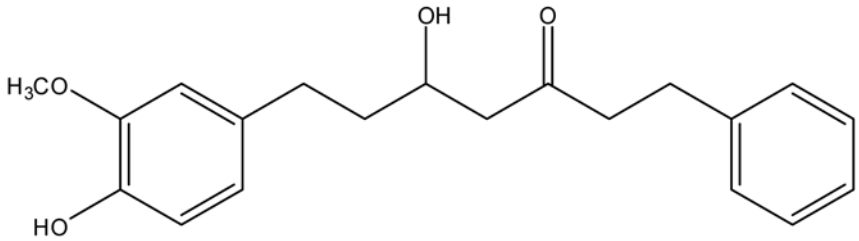


**Fig. 1. Structure of 5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-3-heptanone.**

largely consistent with those of Inoue *et al*. [1978] and Liu *et al*. [2005]. This is the first report of the antibacterial activity of a diarylheptanoid isolated from the *A. officinarum* rhizome against *H. pylori*. It was identified on the basis of the following evidence: colorless powder; $[\alpha]_D^{25}$ +1.2° (EtOH); UV $\lambda_{max}$ (MeOH) nm (ε): 246 (5.4); FT-IR $cm^{-1}$: 3400, 1700, 1600, 1500; EI-MS *m/z*: 328 ($M^+$), 310, 205, 177, 137, 105, 91, 77; $^1H$ NMR ($CDCl_3$, 400 MHz) and $^{13}C$ NMR ($CDCl_3$, 100 MHz): given in Table 2.

The MICs of the test compounds were determined according to the method of Malekzadeh *et al*. [2001]. Briefly, the MICs were determined by adding various quantities (0.01-128 μg/mL) of the isolate and the antibiotics amoxicillin, metronidazole, and tetracycline to Brucella agar media containing 5% bovine calf serum before inoculation with *H. pylori* suspension. The MIC was defined as the concentration leaving no survivors after 3 days of incubation at the conditions stated above. The anti-*H. pylori* activity of the isolated diarylheptanoid along with amoxicillin, metronidazole, and tetracycline toward the test strains was evaluated by comparing the MIC values estimated from the agar dilution bioassay (Table 3). As judged by the MIC values, the antibacterial activity of the isolated diarylheptanoid (48 μg/mL) was comparable to that of metronidazole (32 μg/mL) but less effective than that of either amoxicillin (0.06 μg/mL) or tetracycline (0.5 μg/mL) against *H. pylori* ATCC 43504. Against *H. pylori* ATCC 700392, MIC values of the diarylheptanoid isolate, amoxicillin, metronidazole, and tetracycline were 24, 0.06, 16 and 1 μg/mL, respectively. Similar differences in the *H. pylori* ATCC 700824 response against the test compounds were observed.

The antibacterial activity of the diarylheptanoid isolate towards the three *H. pylori* strains was comparable to that of metronidazole but less effective than either amoxicillin or tetracycline. However, antibiotics such as tetracycline had potent antibacterial activity toward various lactic acid-producing bacteria such as bifidobacteria and lactobacilli at low concentrations [Chae *et al*., 1999; Ahn *et al*., 2000]. Based on our results, *A. officinarum* rhizome-derived diarylheptanoid may hold promise as a novel and effective anti-*H. pylori* product, although the exact antibacterial mode of action of the compound remains to be determined. Additionally, the diarylheptanoid

**Table 2. $^{13}C$ (100 MHz) and $^1H$ NMR (400 MHz) data for compound 1 ($CDCl_3$, TMS)**

| Position | $\delta_C$ (ppm) | $\delta_H$ (ppm)[a] |
|---|---|---|
| 1 | 30.5 | 2.81 m |
| 2 | 45.9 | 2.78 m |
| 3 | 211.5 | |
| 4 | 51.2 | 2.54 m |
| 5 | 68.2 | 4.01 m |
| 6 | 40.4 | 1.66 m |
| 7 | 32.3 | 2.63 m |
| $CH_3O$ | 56.3 | 3.80 s |
| 1' | 142.9 | |
| 2' | 129.3 | 7.14 m |
| 3' | 129.4 | 7.23 d (*J*=6.0) |
| 4' | 127.0 | 7.14 m |
| 5' | 129.4 | 7.21 d (*J*=5.2) |
| 6' | 129.3 | 7.14 m |
| 1" | 134.9 | |
| 2" | 113.2 | 6.74 d (*J*=2.0) |
| 3" | 148.5 | |
| 4" | 145.5 | |
| 5" | 116.1 | 6.69 d (*J*=8.0) |
| 6" | 121.8 | 6.60 dd (*J*=8.0, 2.0) |

[a]Coupling constants (Hz) are in parentheses.

**Table 3. MICs of 5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenyl-3-heptanone (isolate) and three antibiotics against *Helicobacter pylori* strains using agar dilution bioassay**

| Compound | MIC (μg/mL) | | |
|---|---|---|---|
| | *H. pyroli* ATCC 43504 | *H. pyroli* ATCC 700392 | *H. pyroli* ATCC 700824 |
| Isolate | 48 | 24 | 24 |
| Amoxicillin | 0.06 | 0.06 | 0.03 |
| Metronidazole | 32 | 16 | 16 |
| Tetracycline | 0.5 | 1 | 0.5 |

isolate possesses inhibitory and bacterial activity against enteropathogenic *Escherichia coli* (EPEC) clinical isolates and efficiently suppress EPEC lipopolysaccharide-induced inflammation in human peripheral blood mononuclear cells [Liu *et al*., 2005].

In conclusion, *A. officinarum* rhizome-derived materials, particularly the diarylheptanoid isolate, could be useful as sources for therapeutic products for the prevention or eradication of *H. pylori* induced diseases in human. The antibacterial action of the diarylheptanoid may be an indication of at least one of the pharmacological actions of *A. officinarum* rhizome. For the practical use of *A. officinarum* rhizome-derived materials as novel anti-*H. pylori* products, further research is needed to establish whether this activity could be exerted in vivo after consumption of the product by humans. Additionally, their antibacterial modes of action need to be established and formulations for improving antibacterial potency and stability need to be developed.

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