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Anti-*Helicobacter pylori* activity of bioactive components isolated from *Hericium erinaceus*

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ABSTRACT

Ethnopharmacological relevance: The fungus *Hericium erinaceus* (*Bull.*) Pers is used in Chinese traditional medicine to treat symptoms related to gastric ulcers. Different extracts from the fungus were assessed for anti-*Helicobacter pylori* activity to investigate the antibacterial activity of the ethanol extracts from *H. erinaceus* and verify the traditional indication of use.

Materials and methods: The fruiting bodies of *H. erinaceus* were concentrated with ethanol by HPD-100 macroporous resin and the whole extract was partitioned by petroleum ether and chloroform to afford fractions with using a silica gel column. Several pure compounds of petroleum ether extracts were obtained and analyzed using nuclear magnetic resonance (NMR). The activity of the extracts and fractions towards *H. pylori* was assessed by the microdilution assay and by the disk diffusion assay *in vitro*. From the most active fraction, two pure compounds were isolated and identified as the main components with anti-*H. pylori* activity from the fungus *H. erinaceus*. The cytotoxicity of these two compounds against the human erythroleu-kemia cell line K562 was also evaluated.

Results: The crude ethanol extracts from the fungus *H. erinaceus* were inhibitory to *H. pylori*. The petroleum ether extracts (PE1s, PE2s) and the chloroform extracts (TEs) demonstrated strong inhibition to *H. pylori*. The inhibition of *H. pylori* was observed through an agar dilution test with minimal inhibition concentration (MIC) values from 400 μg/mL to 12.5 μg/mL. Two pure compounds, 1-(5-chloro-2-hydroxyphenyl)-3-methyl-1-butanone and 2,5-bis(methoxycarbonyl)terephthalic acid were isolated from the petroleum ether fractions and identified using ^{1}H NMR and ^{13}C NMR spectra analysis. The MIC value for 1-(5-chloro-2-hydroxyphenyl)-3-methyl-1-butanone was 12.5–50 μg/mL and the MIC value for 2,5-bis (methoxycarbonyl)terephthalic acid was 6.25–25 μg/mL. Both two compounds showed weak cytotoxicity against K562 with $IC_{50} < 200$ mM.

Conclusions: This study revealed that the extracts from petroleum ether contribute to the anti-*H. pylori* activity. The compounds obtained from petroleum ether extracts, 1-(5-chloro-2-hydroxyphenyl)-3-methyl-1-butanone and 2,5-bis(methoxycarbonyl)terephthalic acid, inhibit the growth of *H. pylori*.

1. Introduction

Hericium erinaceus (*Bull.*) Pers (Houtoujun in China, yamabushitake in Japan and the lion's mane mushroom in the US) belongs to the Basidiomyeota, Basidiomycetes, Agariomycetes, Russulales, and *Hericiaceae* families and is the traditional medicinal food for the treatment of gastricism in China and Japan (Zhu et al., 2014; Li et al., 2012). Traditionally, *H. erinaceus* is a luxurious food in China, and a number of processed products derived from *H. erinaceus* flood the market. It contains important pharmacological constituents such as polysaccharides, bioactive proteins, terpenoids and hericenone. Previous studies have demonstrated that extractions from the fruiting bodies exhibit antitumor activity, hypoglycemic activity, anti-bacterial activity and anti-inflammatory properties (Sheu et al., 2013; Han et al., 2013; Patel and Goya, 2012). Li et al. (2014) demonstrated that the extracts from *H. erinaceus* were active against gastric cancer NCI-87 cells *in vitro* and tumor xenografts bearing in SCID mice *in vivo*, and these extracts had the potential for development into anticancer agents for the treatment of gastrointestinal cancer used alone and/or in combination with clinical used chemotherapeutic drugs. These studies have identified the bioactive polysaccharide. Other low-molecular-weight bioactive compounds have also exhibited

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Table 1
Growth inhibition of *H. pylori* by *H. erinaceus* ethanol extracts.

Tested sample[a]	*H. pylori* ATCC 43504	*H. pylori* SS1	*H. pylori* DXF	*H. pylori* W_2504	*H. pylori* 9	*H. pylori* 64	*H. pylori* 78	*H. pylori* 83
CK	−	−	−	−	−	−	−	−
EEs	+	+	+	+	+	+	+	+
PE1s	++*	+	+	+	+	++*	+	+
PE2s	+	+	+	+	+	+	+	+
TEs	+	+	+	+	+	+	+	+
MTZ	+++	+++	+++	+++	+++	+++	+++	+++

(−) not effective; (+) slightly effective; (++) moderately effective; and (+++) highly effective.

* Statistically singnificant at $P < 0.05$. $n=7$ in each group. The inhibition zone values of the extracts were compared to determine variation in their efficacy against the isolates.

[a] 75% EtOH in volume (CK); ethanol extracts (EEs); first petroleum ether extracts (PE1s); second petroleum ether extracts (PE2s); chloroform extracts (TEs); MTZ, metronidazole.

bioactivity. Hericenone L, isolated from *H. erinaceus*, demonstrated anti-cytotoxic activity on the EC109 cell line (Ma et al., 2012a). Amycenone, in *H. erinaceus,* increased intracerebral NGF and reduced depression and anxiety (Inanaga, 2012). Ergosterol peroxide, isolated from *H. erinaceus*, inhibited the growth of *Staphylococcus atzreus, Bacillus megaterium, Bacillus thuringiensis, Bacillus subtilis* and *Escherichia coli* (Ma et al., 2012b).

Helicobacter pylori is an important factor in gastric disease. The identification of an anti-*H. pylori* substance could be used to treat gastric disease (Wu et al., 2014; Chang et al., 2012). There are many therapeutic strategies, including chemotherapy that can treat gastric disease. However, these treatments are systemically toxic and drug resistance has limited their success. New therapeutic strategies, with improved immunity potential less toxic side effects, are being developed to treat gastric disease. Wang et al. (2008) reported that the *H. pylori* exterminate rate with the Houtoujun tablet was significantly higher than that with sesapride (216 cases of clinical validation). A low dose of decolored polysaccharides significantly improved the repair of a gastric mucosal injury (Jiang et al., 2014). The flavonoids isolated from *Piper carpunya* contributed to the anti-*H. pylori* activity. The Bi^{3+} with lower content to HEP from *H. erinaceus* exhibited strong inhibition effects on *H. pylori* (Quílez et al., 2010; Zhu et al., 2014). The methyl antcinate B, antcins A and K, from the fruiting bodies of *A. camphorata*, demonstrated anti-*H. pylori* activity (Geethangili et al., 2010). Haiying Rong et al. (2012) reported that the *H. pylori* exterminate rate of *H. erinaceus* with antibiotics was superior to antibiotics alone in the treatment of patients with peptic ulcer, with reduced recurrence.

Previously, we examined the antimicrobial activity of the ethanol extracts of 14 species of mushrooms using *H. pylori*. The extract of *H. erinaceus* inhibited *H. pylori in vitro* as demonstrated by the minimal inhibition concentration test (MIC < 10 mg/mL) *in vitro* (Shang et al., 2013). This study evaluated the antibacterial effects of the compounds on anti-*H. pylori* and antineoplastic activity against human erythroleukemia cell lines K562.

2. Materials and methods

2.1. Materials and reagents

The fruiting bodies of *H. erinaceus* were obtained at Fengxian District, Shanghai, China, and authenticated by Dr. Shang Xiaodong at the Institute of Edible Fungi, Shanghai Academy of Agricultural Science, China. A voucher specimen (*H. erinaceus* 0605) was deposited at the Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences (Dr. Tan Qi, 2012, Shanghai, China). Analytically pure dry powders of MTZ (metronidazole) and TET (tetracycline) were purchased from Amresco (Solon, OH, USA). Chemical grade reagents were purchased from China National Pharmaceutical Group Corporation (Sinopharm).

2.2. Preparation of extracts

The entire fruiting body (200 g) was macerated with 2 L deionized water at room temperature. Chemical grade ethyl alcohol (95%) was used for extraction. Macerated material (100 mL) was mixed tenfold in HPD-100 macroporous resin (Cangzhou Bon Adsorber Technology Co., Ltd. Cangzhou, Hebei Province, China). The mixtures were absorbed and concentrated on a constant temperature shaker (Shanghai Sukun Industry & Commerce Co., Ltd. Shanghai, China) at 110 rpm and 25 °C for 24–48 h. They were eluted with three times the column bed volume (BV) of water. Ethanol (75%, 50 mL) was added to the concentrate and filtered at 110 rpm and 25 °C on the constant temperature shaker for 2 h. The ethanol was evaporated to obtain the crude extract. Bioassay-guided results indicated that the ethanol fraction was efficient at inhibiting *H. pylori* (Table 1).

The crude ethanol extract (100 mL) was at reflux (5 mL of 1 g mL^{-1} KOH and a small amount of zeolite at 80 °C) for 1 h using a condensation recycling installment to remove the saponification solvent. The slurry was cooled to room temperature (RT) and then successively extracted by petroleum ether to yield PE1s. The other solution was titrated to a pH between 2.0 and 3.0 using concentrated sulfuric acid. The liquid was extracted by petroleum ether to obtain PE2s and the extraction residue was concentrated with chloroform to yield the TEs. The anti-*H. pylori* activity of PE1s, PE2s, and TEs was determined.

2.3. Isolation of the active compounds

The active PE2s extraction (4 g) was analyzed on a CC (silica gel, 50 g; column, 100–200 mesh), then eluted with a petroleum ether–C_3H_6O gradient (50:1–0:1, v/v) using a thin-layer chromatography (TLC) control. It was then eluted with methyl alcohol. Ten fractions were obtained (II-1-6, 215.7 mg; II-7-9, 319.9 mg; II-10-13, 306.1 mg; II-14-18, 311.0 mg; II-19-30, 355.1 mg; II-31-45, 363.9 mg; II-46-53, 184.5 mg; II-54-58, 96 mg; II-59-63, 78.1 mg and II-64-78, 425.4 mg). The anti-bacterial activity of these components was determined (Table 2). The subfractions were evaporated by rotary evaporators and volatilized under fume cupboards at room temperature. Fractions II-10-13 and II-54-58 were recrystallized with acetone after Sephadex LH-20 to yield Compound **1** and Compound **2**.