

Screening test for anti-*Helicobacter pylori* activity of traditional Chinese herbal medicines

Feng Ma, Ye Chen, Jing Li, He-Ping Qing, Ji-De Wang, Ya-Li Zhang, Bei-Guo Long, Yang Bai

Feng Ma, Ye Chen, Jing Li, He-Ping Qing, Ji-De Wang, Ya-Li Zhang, Yang Bai, Department of Gastroenterology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, China

Bei-Guo Long, Department of Microbiology, School of Basic Medical Sciences, Southern Medical University, Guangzhou 510515, Guangdong Province, China

Author contributions: Ma F and Chen Y performed the majority of experiments, wrote and edited the manuscript; Li J and Qing HP had supportive contributions; Long BG and Wang JD provided the vital reagents and analytical tools; Bai Y and Zhang YL provided the financial support for this work; Bai Y designed the study and edited the manuscript.

Supported by The Cooperation Project in Industry, Education and Research of Guangdong Province and Ministry of Education of China, No. 2009B090300280

Correspondence to: Dr. Yang Bai, Department of Gastroenterology, Nanfang Hospital, Southern Medical University, Tonghe Road 1838, Guangzhou 510515, Guangdong Province, China. baiyang1030@hotmail.com

Telephone: +86-20-61641535 Fax: +86-20-87280770

Received: June 1, 2010 Revised: August 3, 2010

Accepted: August 10, 2010

Published online: November 28, 2010

suggesting that traditional Chinese herbal medicines have anti-inflammatory and antibacterial effects and can thus be used in treatment of *H. pylori* infection.

CONCLUSION: Rhizoma Coptidis, Radix Scutellariae and Radix isatidis are the potential sources for the synthesis of new drugs against *H. pylori*.

© 2010 Baishideng. All rights reserved.

Key words: Chinese herbal medicines; *Helicobacter pylori*; Minimum inhibitory concentration; Gastric; Oral

Peer reviewer: Seng-Kee Chuah, MD, Division of Hepatogastroenterology, Kaohsiung Chang Gung Memorial Hospital, 123, Ta-Pei Road, Niasung Hsiang, Kaohsiung 833, Taiwan, China

Ma F, Chen Y, Li J, Qing HP, Wang JD, Zhang YL, Long BG, Bai Y. Screening test for anti-*Helicobacter pylori* activity of traditional Chinese herbal medicines. *World J Gastroenterol* 2010; 16(44): 5629-5634 Available from: URL: <http://www.wjg-net.com/1007-9327/full/v16/i44/5629.htm> DOI: <http://dx.doi.org/10.3748/wjg.v16.i44.5629>

Abstract

AIM: To evaluate the anti-*Helicobacter pylori* (*H. pylori*) activity of 50 traditional Chinese herbal medicines in order to provide the primary evidence for their use in clinical practice.

METHODS: A susceptibility test of water extract from 50 selected traditional Chinese herbal medicines for *in vitro* *H. pylori* Sydney strain 1 was performed with broth dilution method. Anti-*H. pylori* activity of the selected Chinese herbal medicines was evaluated according to their minimum inhibitory concentration (MIC).

RESULTS: The water extract from Rhizoma Coptidis, Radix Scutellariae and Radix isatidis could significantly inhibit the *H. pylori* activity with their MIC less than 7.8 mg/mL,

INTRODUCTION

Helicobacter pylori (*H. pylori*), a microaerophilic, Gram-negative spiral bacterium which was first detected in 1984 by Marshall *et al*^[1], is one of the most common chronic bacterial pathogens in humans. Approximately 50% of people in the world are infected with it, and its prevalence is significantly higher in developing countries than in developed countries^[2]. *H. pylori* infection is an important etiologic impetus usually leading to chronic gastritis, gastroduodenal ulcer and low grade gastric mucosa-associated lymphoid tissue lymphoma. Epidemiological data show that a high *H. pylori* infection rate is related to the high incidence of gastric cancer and gastric adenocarcinoma^[3]. World Health Organization has categorized *H. pylori* as

a class 1 carcinogen^[4]. Fortunately, its eradication with antibiotics can result in ulcer healing, prevent peptic ulcer recurrence and reduce the prevalence of gastric cancer in high-risk populations^[5]. However, it is not always successful because of its resistance to one or more antibiotics and other factors such as poor patient compliance, undesirable side effects of the drugs and significant cost of combination therapy. Worrel *et al*^[6] reported that over 15% of the patients undergoing antibiotics therapy would experience therapeutic failure. In developing countries, since the application of antibiotics is still under a poor management as a whole, there is a growing need for finding new anti-*H. pylori* agents that can hopefully eradicate the invasion and presence of survived *H. pylori* strains to avoid relapse of gastric ulcer. Hence, a considerable variety of studies involving tests for medicinal plants showing antimicrobial activity and discrepant susceptibility test results are available due to variations in the methods and conditions used for its susceptibility testing. It was reported that Garlic extracts exhibit a weak or modest anti-*H. pylori* activity^[7,8]. *Pteleopsis suberosa*^[9], Cinnamon^[10], Cranberry juice^[11], *Aristolochia paucinervis* Pomel^[12], black Myrobalan^[13], *etc.*, have also been found to have anti-*H. pylori* activities. Ndip *et al*^[14] reported that *Ageratum conyzoides*, *Scleria striatinux* and *Lycopodium cernua* show a very potent antibacterial activity. Fifty-four herbal medicines from Korea have been screened for their anti-*H. pylori* activity, of which, *Rheum palmatum*, *Rhus javanica*, *Coptis japonica* and *Eugenia caryophyllata* have a strong anti-*H. pylori* activity^[15]. Extracts and fractions from 7 Turkish plants also demonstrate anti-*HP* activities^[16]. Traditional medicinal plants from Pakistan and *Psoralea corylifolia* L. demonstrate a strong anti-*H. pylori* activity^[17]. Some compounds even have been isolated and their anti-*H. pylori* activity has also been testified, for example, Myroxylon Peruferum from the Brazilian medicinal plants^[18]. In addition, some flavonoids and isoflavonoids isolated from licorice, such as licochalcone A and licoisoflavone B, have been reported to exhibit inhibitory activities against *H. pylori*^[19].

In China, traditional Chinese medicine and pharmacology play an indispensable role in the health care system, especially in prevention and management of chronic diseases. Studies^[20,21] revealed that some traditional herbal medicines are efficient against gastrointestinal diseases, including chronic gastritis and peptic ulcer disease, a major outcome of *H. pylori* infection, indicating that the medicinal plants may contain constituents, which have antibacterial and anti-inflammatory activities. The present study was to evaluate the anti-*H. pylori* activity of some selected medicinal plants to identify the potential sources for synthesis of new drugs against *H. pylori*. In this study, 50 traditional Chinese medicinal herbs (Table 1) were examined and screened for their anti-*HP* activity according to their minimum inhibitory concentration (MIC).

MATERIALS AND METHODS

Extract of medicinal plants

A total of 50 traditional Chinese medicinal herbs, pur-

chased from Tongren Drugstore, were identified by Professor Chang-Qing Wang, Beijing Academy of Agriculture and Forestry Sciences. Medicinal herbs were selected according to their traditional use in Chinese pharmacology as anti-inflammatory or antibacterial drugs. Radix Scutellariae Baicalensis (20 g) was soaked for 2 h in about 1000 mL distilled water at room temperature, decocted for 1 h (first quickly, then slowly), and filtered through filter paper. Their residue was decocted for a second time and filtered. The filtrate was collected and centrifuged at 3500 r/min for 15 min. Finally, the liquid was concentrated to 10 mL (2 g/mL) in a rotary vacuum evaporator, then capped and autoclaved for 15 min at 121°C. Under the sterile environment, 12 sterile 7.5 cm × 1.3 cm capped tubes were arranged in a row in the rack, marked with No. 1, No. 2, ..., No. 12 tubes, then 1 mL sterile distilled water was transferred to each tube with a fresh transferpettor and decocted. A Radix Scutellariae Baicalensis stock solution (2 g/mL) was added into No. 1 tube and mixed thoroughly. Then 1 mL solution was transferred from No. 1 tube to No. 2 tube, diluted twice until no extract dilution solution was added into the No. 10, No. 11 and No. 12 tubes as controls. After that, 1 mL two-fold concentration of modified brucella broth (control of diarrheal diseases in China Research, Shanghai Regent Supply) supplemented with 10% sterile fetal calf serum (Guangzhou Ruite Ltd., China) was added into each of the tubes. The final concentration of medicinal plants reached 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 2.0 and 1.0 mg/mL in No. 1-10 tubes, respectively. Extraction was performed in duplicate from other medicinal plants and their extracts were stocked at 4°C prior to use.

Inocula

H. pylori Sydney strain 1 (SS1), obtained from Laboratory of Medical Microbiology, Southern Medical University, was stored in brain-heart infusion broth supplemented with 20% (v/v) glycerol and 1% yeast extract at -70°C. After freeze thawing under a superclean bench (Shanghai Anting Scientific Instrument Ltd., China), a drop of *H. pylori* was inoculated into a single-fold concentration of modified sterile brucella broth (as above), cultured at 37°C in a shaking incubator containing 5% O₂, 10% CO₂, and 85% N₂ at 150 r/min for 3 d. *H. pylori* SS1 was identified with routine diagnostic procedures, according to their colony morphology, Gram-staining, test of oxidase, catalase and urease reaction, as well as molecular identification based on the amplified species-specific sequences of 16s rRNA by polymerase chain reaction (PCR). Bacterial suspension was transferred quickly into 0.9% sterile physiological saline and diluted to the McFarland standard 0.5 (1 × 10⁸ cfu/mL) as the standardized bacterial suspension. The turbidity was verified by spectrophotometrically measuring the absorbance of the suspension ($A_{625\text{ nm}}$).

Susceptibility testing

Ten microliters of standardized bacterial suspension (1 × 10⁸ cfu/mL) was inoculated into No. 1-11 tubes within

Table 1 Fifty anti-*Helicobacter pylori* traditional Chinese herbal medicines

Chinese name	English name	Pharmaceutical name	Botanical name	MIC (mg/mL)
Huanglian	Coptis Rhizome	Rhizoma Coptidis	Coptis chinensis Franch.	3.9 < MIC ≤ 7.8
Huangqin	Baikang skullcap Root	Radix Scutellariae	Scutellaria Baicalensis Georgi.	3.9 < MIC ≤ 7.8
Banlangen	Indigowoad Root	Radix isatidis	Isatis tinctoria L.	3.9 < MIC ≤ 7.8
Jinyinhua	Honeysuckle Flower	Flosloniceriae Japonicae	L. Similis Hemsl.	7.8 < MIC ≤ 15.6
Qinpi	Largeleaf Chinese Ash Bark	Cortex Fraxini	F. Bungeana DC.	7.8 < MIC ≤ 15.6
Zihuadiding	Tokyo Violet Herb	Herba Violae cum Radice	Viola yedoensis Mak.	15.6 < MIC ≤ 31.2
Huangbai	Chinese Corktree Bark	Cortex Phellodendri	Chinese Schneid	15.6 < MIC ≤ 31.2
Daqingye	Indigowoad Leaf	Folium isatidis	Isatis	15.6 < MIC ≤ 31.2
Pugongying	Dandelion	Herba Taraxaci Mongolici cum Radice	Taraxacum mongolicum	15.6 < MIC ≤ 31.2
Dahuang	Rhubarb	Rhizoma Rhei	R. Officinale bail	31.2 < MIC ≤ 62.5
Shandougen	Tonkin sophora Root	Radix Sophorae Tonkinensis	Sophora Subprostrata Chun et T. Chen	31.2 < MIC ≤ 62.5
Longdancao	Chinese Gentian Root	Radix Gentianae Scabrae	Gentiana Scabra Bge. In	31.2 < MIC ≤ 62.5
Hezi	Medicine Terminalia Fruit	Fructus Terminaliae Chebulae	Terminalia chebula Retz.	31.2 < MIC ≤ 62.5
Machixian	Purslane Herb	Radix Sophorae Subprostratae	Sophora Subprostrata Chun et T. Chen	62.5 < MIC ≤ 125
Banzhilian	Barbed Skullcap Herb	Herba Scutellariae Barbatae	Scutellaria Barbata D. Don	62.5 < MIC ≤ 125
Yuxingcao	Heartleaf Houttuynia Herb	Herba Houttuyniae Cordatae	Houttuynia cordata Thunb.	62.5 < MIC ≤ 125
Tufuling	Glabrous Greenbrier Rhizome	Rhizoma Smilacis Glabrae	Simlax glabra Roxb.	62.5 < MIC ≤ 125
Niubangzi	Great Burdock Achene	Fructus Arctii Lappae	Fructus Arctii	125 < MIC ≤ 250
Juhua	Chrysanthemum Flower	Flos Chrysanthemi Morifolii	Chrysanthemum morifolium Ramat.	125 < MIC ≤ 250
Baijiangcao	Whiteflower Patrinia Herb	Herba Whiteflower Patrinia Herb	Patrinia Scabiosaefolia Fisch.	125 < MIC ≤ 250
Tianhuafen	Snakegourd Root	Radix Trichosanthis	Thichosanthes kirilowii Maxim.	125 < MIC ≤ 250
Yadanzhi	Java Brucea Fruit	Fructus Bruceae Javanicae	Brucea Javanica Merr.	125 < MIC ≤ 250
Niu Huang	Cow-bezoar	Calculus Bovis	Bos taurus domesticus Gmelin	250 < MIC ≤ 500
Mabo	Puff-ball	Fructus lasiosphaerae	lasiosphara fenslli Reich.	250 < MIC ≤ 500
Zisu	Perilla Leaf	Folium Perillae Frutescentis	Perilla	250 < MIC ≤ 500
Chaihu	Chinese Thorowax Root	Radix Bupleuri	Bupleurum scorzoneraefolium	250 < MIC ≤ 500
Rendongteng	Honeysuckle Stem	Caulis Lonicerae	Lonicera Japonica Thunb.	250 < MIC ≤ 500
Kushen	Lightyellow Sophora Root	Radix Sophorae Flavescentis	Sophora Flavescens Ait	250 < MIC ≤ 500
Rougui	Cassia Bark	Cortex Cinamomi Cassiae	Cinnamomum cassia Presl.	> 500
Congbai	Fistular Onion Stalk	Herba Alii Fistulosi	Allium fistulosum L.	> 500
Xiangru	Haichow Elsholtzia Herb	Herba Elsholtziae Splendentis	E. Haichowensis Sun.	> 500
Bohe	Wild Mint	Herba Menthae	Mentha haplocalyx Briq.	> 500
Qinghao	Sweet Wormwood	Herba Artemisiae Apiaceae	Artemisia apiacea Hance	> 500
Wuzhuyu	Medicinal Evodia Fruit	Fructus Evodiae Rutaecarpae	Evodia Rutaecarpa Benth.	> 500
Chishao	Red Peony Root	Radix Paeoniae Rubra	Paeonia lactiflora Pall.	> 500
Wumei	Smoked Plum	Fructus Pruni Mume	Prunus mume Sieb. et Zucc	> 500
Mudanpi	Tree Peony Bark	Cortex Moutan Radicis	Paeonia suffruticosa Andr	> 500
Xuanshen	Figwort Root	Radix Scrophulariae Ningpoensis	Scrophularia ningpoensis Hemsl	> 500
Ganjiang	Dried Ginger	Rhizoma Zingiberis Officinalis	Zingiber officinale Rose	> 500
Fuzi	Root of Common Monkshood	Radix Aconiti Carmichaeli Praeparata	Aconite carmichaeli Debx.	> 500
Huajiao	Bunge pricklyash	Fructus Zanthoxyli Bungeani	Zanthoxylum bungeanum Maxim.	> 500
Gaoliangjiang	Lesser Galangal Rhizome	Rhizoma Alpiniae Officinarum	Alpinia officinarum Hance.	> 500
Dingxiang	Clove Flower-bud	Flos Caryophylli	Eugenia caryophyllata Thunb.	> 500
Shiliupi	Rind Peel	Pericarpium Punicae Granati	Punica granatum L.	> 500
Xixin	Manchurian wildginger Herb	Herba Asari cum Radice	Asarum sieboldii Miq.	> 500
Cangzhu	Swordlike Atractylodes Rhizome	Rhizoma Atractylodis	Atractylodes lancea Thumb.	> 500
Lugen	Reed Rhizome	Rhizome Phragmitis Communis	Phragmites communis	> 500
Baitouweng	Chinese pulsatilla Root	Radix pulsatillae Chinensis	Pulsatilla Chinensis Reg.	> 500
Xiaohuixiang	Fennel Fruit	Fructus Foeniculi vulgaris	Foeniculum vulgare Mill.	> 500
Zhizi	Cape Jasmine Fruit	Fructus Gardeniae Jasminoidis	Gadernia Jasminoides Ellis	> 500

MIC: Minimum inhibitory concentration.

15 min. No. 11 tube was used as a growth control (broth with bacterial inoculum, no extract) and No. 12 tube was used as a sterility control (broth only). All tubes were cultured in a shaking incubator containing 5% O₂, 10% CO₂, and 85% N₂. At the same time, 10 µL bacterial suspension from No. 11 tube was diluted quickly with 10 mL 0.9% sterile physiological saline at 1:1000, then 100 µL 0.9% sterile physiological saline was transferred onto the surface of three Campylobacter plates [control of diarrheal diseases in China Research, Shanghai Regent Supply;

each liter containing bio-polyone (10 g), bio-lysate (10 g), bio-myotone (3 g), corn starch (1 g), sodium chloride (5 g), agar (13.5 g), pH 7.3, autoclaved at 121°C for 15 min] containing 5% (v/v) of sterile defibrinated sheep blood (Guangzhou Ruite Ltd., China), cultured at 37°C in a jar system (Refrigerating Machine Factory, Yiwu City, Zhejiang Province, China) containing 5% O₂, 10% CO₂, and 85% N₂ to verify the absence of contamination and calculate the colonies. Susceptibility test for other medicinal plants was performed in duplicate.

RESULTS

After incubation, the tubes were visually examined to determine whether the *H. pylori* strains grew. *H. pylori* strains in No. 11 tube grew well and no bacterial growth was observed in No. 12 tube. The colonies in 3 agar plates grew well with an average number of about 50. The lowest concentration (highest dilution) of extract that inhibited the visible growth of *H. pylori* strains (no turbidity) was defined as MIC. For further confirmation, 10 μ L of bacterial suspension from the clearly visible tubes was diluted quickly with 10 mL 0.9% sterile physiological saline at 1:1000, then 100 μ L 0.9% sterile physiological saline was transferred onto the surface of three Campylobacter plates and cultured for 3 d. When the average number of colonies in the 3 agar plates was less than 5 or no colony was found, the MIC was considered less than or equal to the concentration. When the growth of *H. pylori* strains occurred in all dilutions containing the extract, the MIC was considered greater than the highest concentration. When no growth of *H. pylori* strains occurred in any concentration tested, the MIC was considered less than the lowest concentration. When a tube with visible growth of *H. pylori* strains, e.g. growth at 500, 250 and 62.5 mg/mL, but not at 125 mg/mL, was called a skipped tube and ignored. Growth of *H. pylori* strains in isolated tubes indicated contamination, the test should be repeated. The results are listed in Table 1. The MIC of Rhizoma Coptidis, Radix Scutellariae and Radix Isatidis was less than 7.8 mg/mL. The MIC of Flosloniceræ Japonicæ and Cortex Fraxini was less than 15.6 mg/mL. The MIC of Herba Violæ cum Radce, Cortex Phellodendri and Folium Isatidis, Herba Taraxaci Mongolici cum Radice was less than 31.2 mg/mL. The MIC of Rhizoma Rhei, Radix Sophoræ Tonkinensis, Radix Gentianæ Scabrae, Fructus Terminaliæ Chebulæ was less than 62.5 mg/mL. The MIC of Radix Sophoræ Subprostratæ, Herba Scutellariæ Barbatae, Herba Houltuyniæ Cordatæ and Rhizoma Smilacis Glabrae was less than 125 mg/mL. The MIC of Fructus Arctii Lappæ, Flos Chrysanthemum Morifolii, Herba Whiteflower Patrinia Herb, Radix Thichosanthis and Fructus Bruceæ Javanicæ was less than 250 mg/mL. The MIC of Calculus Bovis, Fructus Lasiosphaeræ, Folium Perilac Frutescentis, Radix Bupleuri, Caulis Loniceræ, Radix Sophoræ Flavescentis was less than 500 mg/mL, and the MIC of other medicinal herbs was greater than 500 mg/mL. Authority books, such as Pharmacopoeia of People's Republic of China (Committee of National Pharmacopoeia, 2005 edition), Chinese Herbal Medicine (Gong-Wang Liu, Li-Ya Gao, 2000, Hua Xia Publishing House), Modern Clinical Chinese Herbal Medicines (Dong Kun-Shan, Wang Xiu-Qin, Dong Yi-Fan, 2001, Chinese Traditional Medicine Press), acclaimed that most selected medicinal plants have an activity against microscopic organisms, including various Gram-negative or -positive bacteria, fungi, viruses or parasites. In fact, many of them are the constituents of Chinese patent medicines used in treatment of stomach discomfort-related diseases. More importantly, most of them demonstrate a significant anti-*H. pylori* activity.

DISCUSSION

In this study, the MIC in 50 traditional Chinese herbal medicines was detected. Although a considerable variety of plants showing an antimicrobial activity have also been reported in other studies^[7-19], variation of MIC still exists due to the bioassay methods employed in different studies, the sources and age of the plants, the solvent used for extraction, and *H. pylori* strains. The susceptibility of *H. pylori* SS1 to water extracts was examined and screened in this study with broth dilution diffusion, a quantitative assay method, which is less time-consuming and less labor-intensive than agar dilution method, and cheaper than E test. All selected herbal medicines are the commonly used traditional Chinese herbal medicines prescribed by physicians of traditional Chinese medicine. Some plants are even recommended as a dietetic therapy for health preserving, such as Radix Isatidis, Herba Houltuyniæ Cordatæ. More importantly, all the selected herbal medicines have a same standard from the TonRen Corporation. Although the susceptibility of only an isolated *H. pylori* strain to such medicines was tested, the susceptibility of other clinical strains to these medicines should also be tested.

In this study, the water extract from Rhizoma Coptidis, Radix Scutellariae and Radix Isatidis had a stronger anti-*H. pylori* activity than that from other plants, indicating that the three plants can be used as useful sources for the synthesis of novel drugs against *H. pylori*. Traditional medical practitioners and biomedical specialists play an important role in pharmacodynamics and pharmacokinetics research. In order to find scientific evidence and rationalize the utility and efficacy of traditional Chinese medicines, they have tried to extract and analyze the active compounds of medicinal plants with various biomedical analytical techniques and assay methods, and evaluated their antibacterial and anti-inflammatory mechanism in animal experiments.

Rhizoma Coptidis contains berberine. Several protoberberine alkaloids of berberine, palmatine, coptisine and aporphinoid alkaloid of magnoflorine have been confirmed to be the major pharmacologically active constituents, and these alkaloids demonstrate a significant antimicrobial activity against a variety of organisms including bacteria, viruses, fungi, protozoans, helminthes, and Chlamydia^[22,23]. The pharmacological antibacterial activity of the 3 berberine alkaloids is berberine > coptisine > palmatine^[24]. An animal experiment suggested that the total alkaloid is a potent protective agent against *H. pylori* LPS which induces gastric mucosal inflammation^[25].

Radix Scutellariae contains over 30 kinds of flavonoid, such as baicalin, baicalein, wogonin, wogonin-7-glucuronide, oroxylin A, and oroxylin A 7-O-glucuronide^[26]. Active flavonoids, including baicalin, baicalein, wogonin, and wogonoside, have a variety of pharmacological activities, such as anti-inflammation, free radical scavenging and anti-oxidation^[27], and antibacterial action^[28]. All active flavonoids exert their anti-inflammatory effect mainly by inhibiting the inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) gene expression^[29]. It was

reported that Radix Scutellariae inhibits LPS-induced production of proinflammatory mediators, including NO, IL-3, IL-6, IL-10, IL-12p40, IL-17, IP-10, KC, and VEGF in mouse macrophages^[30].

Radix Isatidis is also officially documented. Organic acids, including syringic acid, 2-amino-benzoic acid, salicylic acid and benzoic acid among the main chemical active components, have been segregated and purified as a crystal^[31]. Some authors have even tested the potency sequence of the 4 organic acids (syringic acid > 2-amino-benzoic acid > salicylic acid > benzoic acid)^[32]. Furthermore, the 4 organic acids share a basic molecular structure, and the number, position and type of their functional groups on phenyl ring have great impacts on antibacterial activities. Extracts from Radix Isatidis can decrease the production of inflammatory mediators, such as nitric oxide, prostaglandin E2, and pro-inflammatory cytokines^[33].

Traditional Chinese medicines have a long history, due to their effectiveness and relatively low toxicity, and herbal medicines have drawn more and more attention during the past decades. Chemical compositions of Rhizoma Coptidis, Radix Scutellariae and Radix Isatidis have been extensively studied, some of which can act on *H. pylori* LPS and inhibit the production of proinflammatory mediators. In our study, water extracts from the medicinal plants demonstrated a strong anti-*H. pylori* activity, and a wide range of phytochemistry materials from medicinal plants could reduce the inflammatory response, indicating that the 3 herbal drugs can be used as anti-inflammatory or antibacterial agents. However, the strong *in vitro* anti-*H. pylori* activity of these water extracts does not necessarily imply that they have a strong *in vivo* anti-*H. pylori* activity. On the other hand, some of these plants may be more potent *in vivo* due to metabolic transformation of their components into highly active intermediates. However, further study is needed to confirm the effect of Rhizoma Coptidis, Radix Scutellariae, Radix Isatidis and other traditional Chinese medicines on alimentary tract diseases due to *H. pylori* infection.

It is well known that human beings are the main reservoir of *H. pylori*. World Health Organization pointed out that most subjects infected with *H. pylori* have no clinical symptoms, peptic ulceration and superficial chronic gastritis, but peptic ulcer, ulcer complications, and progression to gastric cancer will occur in approximately 17%, 4.25% and 1% of *H. pylori*-infected subjects, respectively^[34]. Besides, extragastric diseases involving the cardiovascular, hepatobiliary, dermatological, immunological, hematological systems^[35] are also related with *H. pylori* infection. Moreover, since *H. pylori* was isolated from human dental plaque^[36], *H. pylori* has been detected in oral cavity, suggesting that oral cavity diseases such as halitosis, glossitis, burning mouth syndrome, recurrent aphthous stomatitis, dental caries, are related with oral *H. pylori* infection. Anand *et al*^[37] reported that the prevalence of *H. pylori* is higher in dental plaque of patients with gastric *H. pylori* infection than in that of patients without gastric *H. pylori*. It has been shown that patients with poor oral hygiene have the most frequent recurrence of gastric *H. pylori* infection^[38]. Oral cavity is a potential reservoir of *H. pylori* and

oral *H. pylori* may influence the relapse of gastric *H. pylori* infection. It was reported that *H. pylori* in dental plaque is hardly eradicated by triple therapy^[39,40], suggesting that oral antibiotics have almost no effect on *H. pylori* in oral cavity.

With the better recognition of *H. pylori*, more diseases have been found to be related to *H. pylori*. Since oral cavity, as a residence of *H. pylori*, is as important as stomach, prevention and treatment of oral *H. pylori* infection should be put on the agenda. Antibiotics have been the main drugs against *H. pylori* since the bacterium was discovered. Further study is needed to solve the problems such as drug resistance, poor patient compliance, undesirable side effects and the significant cost of combination therapy. Traditional Chinese medicines have shown their advantages over Western drugs, including a lower price, a low toxicity and less adverse reactions.

It is exciting that Rhizoma Coptidis, Radix Scutellariae, Radix isatidis and other herbs with a strong anti-*H. pylori* activity may provide the potential sources of new drugs, thus reducing the morbidity of oral cavity diseases and improving the eradication rate and relapse of gastric *H. pylori* infection.

ACKNOWLEDGMENTS

The authors thank all members of the team for their assistance and Beijing Academy of Agriculture and Forestry Sciences for providing laboratory and experiment equipments, as well as Professor Chang-Qing Wang for providing Chinese herbal medicines.

COMMENTS

Background

Many diseases are related to *Helicobacter pylori* (*H. pylori*) infection. Although antibiotics can eradicate gastric *H. pylori*, antibiotics treatment can lead the problems, such as drug resistance, poor patient compliance and undesirable side effects. It has been reported that some herbal medicines have an anti-*H. pylori* activity. The herbal medicine resources are rich in China with a long history of practicing traditional Chinese medicine. However, few studies are available on the anti-*H. pylori* activity of herbal medicines. Herbal medicines may be potential sources of new drugs.

Research frontiers

Human beings are the main reservoir of *H. pylori*. World Health Organization estimates indicate that *H. pylori* infection is closely related with gastric and extra-gastric diseases involving the cardiovascular, hepatobiliary, dermatological, immunological, and hematological systems. *H. pylori* has been detected in oral cavity. Oral cavity diseases such as halitosis, glossitis, burning mouth syndrome, recurrent aphthous stomatitis, dental caries, may be related with *H. pylori* infection.

Innovations and breakthroughs

In vitro susceptibility test was performed for water extract from 50 selected traditional Chinese herbal medicines and their anti-*H. pylori* activity was evaluated according their MIC values. The active compounds of Rhizoma Coptidis, Radix Scutellariae and Radix Isatidis, were detected and their anti-*H. pylori* activity was analyzed.

Applications

Since Rhizoma Coptidis, Radix Scutellariae and Radix Isatidis have a strong anti-*H. pylori* activity, with a low toxicity, a low price and less adverse reactions, they can be used in preventing and treating gastric and/or oral *H. pylori* infection.

Peer review

This study described the strong anti-*H. pylori* activity of Rhizoma Coptidis, Radix Scutellariae and Radix Isatidis, thus adding some novel herbal medicines for preventing and treating gastric and/or oral *H. pylori* infection.

REFERENCES

- 1 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1**: 1311-1315
- 2 Goodwin CS, Mendall MM, Northfield TC. Helicobacter pylori infection. *Lancet* 1997; **349**: 265-269
- 3 NIH Consensus Conference. Helicobacter pylori in peptic ulcer disease. NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease. *JAMA* 1994; **272**: 65-69
- 4 Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 1-241
- 5 Sepulveda AR, Coelho LG. Helicobacter pylori and gastric malignancies. *Helicobacter* 2002; **7** Suppl 1: 37-42
- 6 Worrel JA, Stoner SC. Eradication of Helicobacter pylori. *Med Update Psychiatr* 1998; **3**: 99-104
- 7 Cellini L, Di Campli E, Masulli M, Di Bartolomeo S, Allocati N. Inhibition of Helicobacter pylori by garlic extract (Allium sativum). *FEMS Immunol Med Microbiol* 1996; **13**: 273-277
- 8 O'Gara EA, Hill DJ, Maslin DJ. Activities of garlic oil, garlic powder, and their diallyl constituents against Helicobacter pylori. *Appl Environ Microbiol* 2000; **66**: 2269-2273
- 9 Germanò MP, Sanogo R, Guglielmo M, De Pasquale R, Crisafi G, Bisignano G. Effects of Pteleopsis suberosa extracts on experimental gastric ulcers and Helicobacter pylori growth. *J Ethnopharmacol* 1998; **59**: 167-172
- 10 Tabak M, Armon R, Neeman I. Cinnamon extracts' inhibitory effect on Helicobacter pylori. *J Ethnopharmacol* 1999; **67**: 269-277
- 11 Bae EA, Han MJ, Kim NJ, Kim DH. Anti-Helicobacter pylori activity of herbal medicines. *Biol Pharm Bull* 1998; **21**: 990-992
- 12 Gadhi CA, Benharref A, Jana M, Lozniewski A. Anti-Helicobacter pylori activity of Aristolochia paucineris Pomel extracts. *J Ethnopharmacol* 2001; **75**: 203-205
- 13 Malekzadeh F, Ehsanifar H, Shahamat M, Levin M, Colwell RR. Antibacterial activity of black myrobalan (Terminalia chebula Retz) against Helicobacter pylori. *Int J Antimicrob Agents* 2001; **18**: 85-88
- 14 Ndip RN, Malange Tarkang AE, Mbullah SM, Luma HN, Malongue A, Ndip LM, Nyongbela K, Wirmum C, Efange SM. In vitro anti-Helicobacter pylori activity of extracts of selected medicinal plants from North West Cameroon. *J Ethnopharmacol* 2007; **114**: 452-457
- 15 Burger O, Ofek I, Tabak M, Weiss EI, Sharon N, Neeman I. A high molecular mass constituent of cranberry juice inhibits helicobacter pylori adhesion to human gastric mucus. *FEMS Immunol Med Microbiol* 2000; **29**: 295-301
- 16 Yeşilada E, Gürbüz I, Shibata H. Screening of Turkish anti-ulcerogenic folk remedies for anti-Helicobacter pylori activity. *J Ethnopharmacol* 1999; **66**: 289-293
- 17 Zaidi SF, Yamada K, Kadowaki M, Usmanghani K, Sugiyama T. Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against Helicobacter pylori. *J Ethnopharmacol* 2009; **121**: 286-291
- 18 Ohsaki A, Takashima J, Chiba N, Kawamura M. Microanalysis of a selective potent anti-Helicobacter pylori compound in a Brazilian medicinal plant, Myroxylon peruiferum and the activity of analogues. *Bioorg Med Chem Lett* 1999; **9**: 1109-1112
- 19 Fukai T, Marumo A, Kaitou K, Kanda T, Terada S, Nomura T. Anti-Helicobacter pylori flavonoids from licorice extract. *Life Sci* 2002; **71**: 1449-1463
- 20 Lu B, Chen MT, Fan YH, Liu Y, Meng LN. Effects of Helicobacter pylori eradication on atrophic gastritis and intestinal metaplasia: a 3-year follow-up study. *World J Gastroenterol* 2005; **11**: 6518-6520
- 21 Wang Y. Clinical observation on the method of supplementing qi, clearing away heat and promoting blood circulation for treating 53 cases of gastritis related to pyrolic Helicobacterium. *J Tradit Chin Med* 2003; **23**: 83-86
- 22 Chuang WC, Young DS, Liu LK, Sheu SJ. Liquid chromatographic-electrospray mass spectrometric analysis of Coptidis Rhizoma. *J Chromatogr A* 1996; **755**: 19-26
- 23 Ren L, Xue X, Zhang F, Xu Q, Liang X. High performance liquid chromatography-mass spectrometry analysis of protoberberine alkaloids in medicine herbs. *J Sep Sci* 2007; **30**: 833-842
- 24 Chen J, Wang F, Liu J, Lee FS, Wang X, Yang H. Analysis of alkaloids in Coptis chinensis Franch by accelerated solvent extraction combined with ultra performance liquid chromatographic analysis with photodiode array and tandem mass spectrometry detections. *Anal Chim Acta* 2008; **613**: 184-195
- 25 Lu JS, Liu YQ, Li M, Li BS, Xu Y. [Protective effects and its mechanisms of total alkaloids from rhizoma Coptis chinensis on Helicobacter pylori LPS induced gastric lesion in rats] *Zhongguo Zhongyao Zazhi* 2007; **32**: 1333-1336
- 26 Li HB, Jiang Y, Chen F. Separation methods used for Scutellaria baicalensis active components. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004; **812**: 277-290
- 27 Gao Z, Huang K, Yang X, Xu H. Free radical scavenging and antioxidant activities of flavonoids extracted from the radix of Scutellaria baicalensis Georgi. *Biochim Biophys Acta* 1999; **1472**: 643-650
- 28 Liu IX, Durham DG, Richards RM. Baicalin synergy with beta-lactam antibiotics against methicillin-resistant Staphylococcus aureus and other beta-lactam-resistant strains of S. aureus. *J Pharm Pharmacol* 2000; **52**: 361-366
- 29 Li C, Zhou L, Lin G, Zuo Z. Contents of major bioactive flavones in proprietary traditional Chinese medicine products and reference herb of radix Scutellariae. *J Pharm Biomed Anal* 2009; **50**: 298-306
- 30 Yoon SB, Lee YJ, Park SK, Kim HC, Bae H, Kim HM, Ko SG, Choi HY, Oh MS, Park W. Anti-inflammatory effects of Scutellaria baicalensis water extract on LPS-activated RAW 264.7 macrophages. *J Ethnopharmacol* 2009; **125**: 286-290
- 31 Fang JG, Wang SB, Xu H, Liu YH, Liu YW. Chemical constituents from radix isatidis. *Zhongcaoyao* 2004; **35**: 845-846
- 32 Kong W, Zhao Y, Shan L, Xiao X, Guo W. Thermochemical studies on the quantity-antibacterial effect relationship of four organic acids from Radix Isatidis on Escherichia coli growth. *Biol Pharm Bull* 2008; **31**: 1301-1305
- 33 Shin EK, Kim DH, Lim H, Shin HK, Kim JK. The anti-inflammatory effects of a methanolic extract from Radix Isatidis in murine macrophages and mice. *Inflammation* 2010; **33**: 110-118
- 34 Malaty HM. Epidemiology of Helicobacter pylori infection. *Best Pract Res Clin Gastroenterol* 2007; **21**: 205-214
- 35 Gasbarrini A, Franceschi F, Armuzzi A, Ojetti V, Candelli M, Torre ES, De Lorenzo A, Anti M, Pretolani S, Gasbarrini G. Extradigestive manifestations of Helicobacter pylori gastric infection. *Gut* 1999; **45** Suppl 1: I9-I12
- 36 Krajden S, Fuksa M, Anderson J, Kempston J, Boccia A, Petrea C, Babida C, Karmali M, Penner JL. Examination of human stomach biopsies, saliva, and dental plaque for Campylobacter pylori. *J Clin Microbiol* 1989; **27**: 1397-1398
- 37 Anand PS, Nandakumar K, Shenoy KT. Are dental plaque, poor oral hygiene, and periodontal disease associated with Helicobacter pylori infection? *J Periodontol* 2006; **77**: 692-698
- 38 Avcu N, Avcu F, Beyan C, Ural AU, Kaptan K, Ozyurt M, Nevruz O, Yalçın A. The relationship between gastric-oral Helicobacter pylori and oral hygiene in patients with vitamin B12-deficiency anemia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; **92**: 166-169
- 39 Czesnikiewicz-Guzik M, Loster B, Bielanski W, Guzik TJ, Konturek PC, Zapala J, Konturek SJ. Implications of oral Helicobacter pylori for the outcome of its gastric eradication therapy. *J Clin Gastroenterol* 2007; **41**: 145-151
- 40 Loster BW, Majewski SW, Czesnikiewicz-Guzik M, Bielanski W, Pierzchalski P, Konturek SJ. The relationship between the presence of Helicobacter pylori in the oral cavity and gastric in the stomach. *J Physiol Pharmacol* 2006; **57** Suppl 3: 91-100

S- Editor Cheng JX L- Editor Wang XL E- Editor Zheng XM