# *In vitro* studies of antimicrobial activity of (*curcuma longa* l.) rhizomes against *helicobacter pylori*

Najah Ali[a]

[a]

Correspondence to Najah Ali (email: najahalim57@yahoo.com)

(*Submitted: 16 December 2016 – Revised version received: 27 January 2017 – Accepted: 13 February 2017 – Published online: 26 March 2017*)

**Objective** Studies on curcumin (*Curcuma longa*) powder have shown several biological actions such as antibacterial activity. Some microorganisms have an effect on human health by causing diseases, and one of these microorganisms is *Helicobacter pylori*. *H. pylori* has attracted great attention as a major cause of gastritis and peptic ulcer diseases. It is the first bacterium to be classified as a group I carcinogen by the International Agency for Research on Cancer. Because of increasing bacterial resistant strains, undesirable side effects, the cost of the antibiotic regimens, and other factors contributing to ineffectiveness, there is an urgent need to develop new treatment strategies for *H. pylori* infection. This project considered as an explorer study for the inhibitory effect of turmeric (*Curcuma longa*) powder against *H. pylori*.
**Methods** Curcumin extracted with dimethylsulfoxide (DMSO). The antibacterial activity of curcumin was determined by well diffusion method using Brain Heart Infusion agar (BHI) previously spread with 24 h old culture of the *H. pylori* organisms.
**Result** Curcumin exhibited good antibacterial activity against *H. pylori* (zone of inhibition in mm). The extract was effective in inhibiting the bacteria with zone of inhibition, 7.7 mm compared with the inhibition zone of Amoxicillin 8.5 mm.
**Conclusion** The results suggest that the curcumin exhibited good activity against *H. pylori* suggesting its potential as an alternative therapy, and further research may be needed to understand the in-depth mechanisms of an effective antibacterial material for eradication of this bacteria.
**Keywords** Curcuma longa L., *H. pylori*, antibacterial activity

## Introduction

*H. pylori* are spiral-shaped bacteria that grow in the digestive tract have a tendency to attack the stomach lining.[1] They have aptitude to induce diseases in some people, including peptic ulcers, and an inflammatory condition inside the stomach known as gastritis. The shape of *H. pylori* allows them to penetrate the stomach lining, where they are protected by mucus and the body immune cells and the acid are not able to reach them.[2] This reaction is characterized by a mucosal infiltration of inflammatory cells, especially neutrophils, which is mediated by enhanced expression of proinflammatory chemokines and cytokine.[3] *H. pylori* infections can lead to peptic ulcers, and also lead to more serious complications such as internal bleeding, obstruction, perforation, and peritonitis.[4] As virulence markers of *H. pylori* are not always associated with diseases, eradication of *H. pylori* from infected individuals remains the best choice for an effective treatment of *H. pylori* diseases.[5] Several triple therapies, consisting of the combined usage of two antibiotics and a proton pump inhibitor, gives a high eradication rate, producing a significant improvement in the status of the disease.[6] Curcumin 1, (7-bis 4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione; Diferuloylmethane, a yellow bioactive pigment, is the major component of turmeric, and a rich source of beneficial phenolic compounds.[7] It has been shown that curcumin has a wide spectrum of biological actions such as anti-inflammatory, and have a wide range of pharmacological uses.[7,8] It also has a long history of therapeutic use. Turmeric has been used for various purposes and through different routes of administration. It has been used topically on the skin for wounds, pemphigus and herpes zoster, for parasitic skin infections, and for acne. It has been used via oral administration for the common cold, liver diseases, urinary tract diseases, and as a liver purifier. For chronic rhinitis and coryza, it has been used via inhalation.[9,10]

Many studies have indicated that eradication by triple therapy is not always successful, and the acquisition by *H. pylori* of resistance to antibiotics, including metronidazole and clarithromycin, could represent a real problem that may reduce treatment efficacy.[11] In view of the incomplete cure achieved with conventional therapy because of increasingly resistant strains, undesirable side effects,[12] the cost of the antibiotic regimens,[13] development of bacterial resistance to the available antibiotics has led researchers to investigate the antibacterial compounds from plants sources.[14,15]

Herbs and spices have been found to reduce inflammation, protect against infection, help to detoxify the liver and cleanse the lungs and other organs and also protect from cell damage that can lead to rheumatoid arthritis, osteoporosis, heart disease and other degenerative diseases.[16] Evaluation of new analogs or new compounds of curcumin for their antibacterial effect is interesting for researchers. It has been shown that curcumin has a wide spectrum of biological actions such as anti-inflammatory, antidiabetic, anticarcinogenic,[17] antiviral activities,[18] antioxidant property[19] antifungal activity of curcumin.[15,20]

There are reports on synthesis of mono-carbonyl analogues of curcumin or preparation of bioactive conjugates of curcumin to increase antimicrobial and anticancer activity.[21,22]

## Material and Methods

### *Helecobacter Pylori Culture*

*Helecobacter. pylori* was isolated from 79 antral mucosal biopsy specimens of patients with chronic gastritis or

duodenal ulcers. In the Central Health Laboratories, the strains were identified on the basis of colony appearance, Gram staining, and positive reactions in biochemical tests (catalase, urease, and oxidase). *H. pylori* strains were cultured on brain heart infusion (BHI) agar (Difco Laboratories, Detroit, MI) supplemented with trimethoprim (5 g/ml), vancomycin (8 g/ml), and polymyxin B (10 g/ml). The plates were incubated at 37°C in a microaerophilic atmosphere (5% $O_2$, 10% $CO_2$, 85% $N_2$) (double gas incubator; Heraeus, Langenselbold, Germany) for 3–6 days. Stock cultures were maintained until use at (−70).

### Determination of Minimum Inhibitory Concentrations (MICs)

Frozen stock cultures were streaked on BHI agar and incubated for 3 days under microaerophilic conditions as mentioned earlier. Isolates were restreaked on fresh BHI agar and incubated for 24 h. Exponentially growing *H. pylori* was suspended in sterile phosphate-buffered saline (PBS) and adjusted to an optical density of 0.1 at 600 nm.

### Extraction Procedure

The dried rhizomes of curcumin were crushed with pestle and mortar. The powder was weighed and extracted with dimethylsulfoxide (DMSO). It was soaked in respective solvents for 3 days and then filtered with Whatman filter paper (pore size 0.2 µm). The residue left was re-extracted by soaking in the same solvent for three times. The combined filtrate was concentrated and weighed. The respective solvent was added to make final concentration of the extracts as 100 mg/ml. Same amount of extract was used for further studies such as antibacterial activity determining the minimum inhibitory concentration (MIC).[23]

### Preparation of Test and Standard Solutions

The test solutions of the curcumin were prepared in distilled DMSO at a concentration of 1, 5 and 20 mg/ml. Amoxicillin was used as standard and was dissolved in distilled DMSO to get a final concentration of 30 µg/ml. DMSO (0.1 ml) was used as solvent control.

### Antibacterial Activity Assay

Antibacterial activity was determined by well diffusion method using BHI agar plates previously spread with 24 h old culture of the *H. pylori* organism. Control plates were prepared by adding the respective solvents, and all the plates were incubated at 37°C for 24–48 h. The zone of inhibition was measured and the average diameter of zone of inhibition was recorded.[24]

### Statistical Analysis

The results were calculated as mean diameter of inhibition zone in mm ± standard deviation (mean ± SD). By ANOVA analysis.

## Results

Among 79 *Helicobacter pylori* strains tested against curcumin, 52 strains were isolated from patients with antral gastritis, whereas 21 and 6 cases were isolated from patients with duodenal ulcer and nonulcer dyspepsia, respectively. Curcumin, the main yellow bioactive component of turmeric powder, has been shown to have several biological effects such as antimicrobial activity.

The effects of plant extracts on bacteria have been researched in different parts of the world. It has been suggested that aqueous and ethanolic extracts from plants are a potential source of antiviral, anticancer and antimicrobial agents. In the present work, we studied the antibacterial activity of curcumin against *H. pylori*.

Curcumin exhibited very good activity against *H. pylori*, antibacterial activities (zone of inhibition in mm) of DMSO extract of Curcumin was studied. As shown in Table 1, curcumin was effective against the *H. pylori*.

The extract was effective in inhibiting the bacteria with zone of inhibition, 7.7 mm while the zone of inhibition of moxicillin was 8.5 mm (Fig. 1).

## Discussion

In last few decades research on spices has been directed to investigate their medicinal, antimicrobial and anticarcinogenic activities. Thus, spices like turmeric can protect the human body against bacterial infections and other metabolism related disorders. Herbal plants have a source of medicinal compounds since times immemorial. Plant extracts are used in different systems of medicine for the treatment of various human diseases, and for the treatment of viral and fungal infections.[25,26]

It is interesting to note that G+ve bacterial isolates were sensitive to curcumin extract. The present study is in agreement with[15] who reported that curcumin extract produce antibacterial activity against a broad range of microbes and especially G-ve strains and multiple antibiotic resistant bacteria. This result also correlates with the previous research[27]

Table 1. **Inhibition zone (mm) of extract DMSO of Curcumin against *H. pylori* compared with Amoxicillin.**

| Agent or inhibitor | The mean of inhibition zone (mm ± SD) *H. pylori* |
|---|---|
| Curcumin | 7.7 ± 2.7* |
| Amoxicillin | 8.3 ± 3.10 |
| DMSO | 0 |

*Significant at ≤ 0.05.

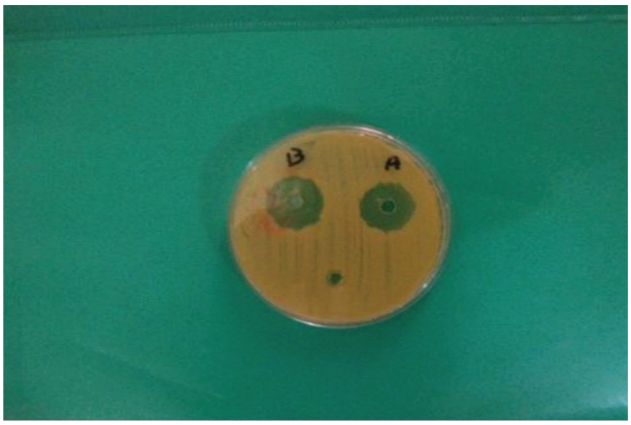


Fig 1. **Inhibition zone (mm) of curcumin extract against *H. pylori* compared with Amoxicillin . A = curcumin. B = Amoxicillin. Negative control (DMSO).**

who reported that curcumin had antibacterial effects against *H. pylori*, colonization in mice.

During extraction process, solvents diffuse into the solid plant material and soluble compounds of similar polarity. The polarity of solvent affects quantity and composition of secondary metabolite of an extract. Traditional healers primarily use water for extract preparation from plant extracts but organic solvents have been found to give more consistent antimicrobial activity compared to water extracts.[28] The antimicrobial activities of this plant have already been studied with different microorganisms.

## Conclusion

The results suggest that the curcumin exhibited good activity against *H. pylori* suggesting its potential as an alternative therapy, and further research may be needed to understand the in-depth mechanisms of an effective antibacterial material for eradication of this bacteria.

## Conflict of Interest

None.

## References

1. Lee A, O'Rourke J, De Ungria MC, Robertson B, Daskalopoulos G, Dixon MF. A standardized mouse model of Helicobacter pylori infection: introducing the Sydney strain. Gastroenterology. 1997;112:1386–1397.
2. Kundu P, de R, Pal I, Mukhopadhyay AK, Saha DR, Swarnakar S. Curcumin alleviates matrix metalloproteinase-3 and -9 activities during eradication of *Helicobacter pylori* infection in cultured cells and mice. PLoS One. 6,2011.
3. Backert S, Naumann M. What a disorder. Proinflammatory signaling pathways induced by *Helicobacter pylori. Trends. Microbiol*. 2010;18:479–486.
4. Koosirirat C, Linpisarn S, Changsom D, Chawansuntati K, Wipasa J. Investigation of the anti-inflammatory effect of Curcuma longa in Helicobacter pylori-infected patients. Int Immunopharmacol. 2010;10:815–818.
5. Toracchio S, Cellini L, Di Campli E, Cappello G, Malatesta MG, Ferri A, et al. Role of antimicrobial susceptibility testing on efficacy of triple therapy in Helicobacter pylori eradication. Aliment Pharmacol Ther. 2000;14:1639–1643.
6. Mohammadi K, Thompson KH, Patrick BO, Storr T, Martins C, Polishchuk E, et al. Synthesis and characterization of dual function vanadyl, gallium and indium curcumin complexes for medicinal applications. J Inorg Biochem. 2005;99:2217–2225.
7. Kawamori T, Lubet R, Steele VE, Kelloff GJ, Kaskey RB, Rao CV, et al. Chemopreventive Effect of Curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. Cancer Res. 2009;59:597–601.
8. Eigner D, Scholz D. Ferula asa-foetida and Curcuma longa in traditional medical treatment and diet in Nepal. J Ethnopharmacol. 1999;67:1–6.
9. Cordell GA. Biodiversity and drug discovery a symbiotic relationship. Phytochemistry. 2009;55:463–480.
10. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and curcumin: Biological actions and medicinal applications. Curr Sci. 2004;87:44–53.
11. Graham DY. Antibiotic resistance in Helicobacter pylori: implications for therapy. Gastroenterology. 1998;115:1272–1277.
12. Myllyluoma E, Veijola L, Ahlroos T, Tynkkynen S, Kankuri E, Vapaatalo H, Rautelin H, Korpela R. Probiotic supplementationimproves tolerance to *Helicobacter pylori* eradication therapy—a placebocontrolled, double-blind randomized pilot study. Aliment Pharmacol Ther. 2005;21:63–72.
13. Wong WM, Gu Q, Lam SK, Fung FM, Lai KC, Hu WH, et al. Randomized controlled study of rabeprazole, levofloxacin and rifabutin triple therapy vs. quadruple therapy as second-line treatment for Helicobacter pylori infection. Aliment Pharmacol Ther. 2003;17:553–560.
14. Anonymous *Curcuma* Linn. (Zingiberaceae) in The Wealth of India-Raw Material, Publication and Information Directorate, Council of Science and Industrial Research, New Delhi. 1950;2:401–406.
15. Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. Turmeric and curcumin: Biological actions and medicinal applications. Curr Sci. 2004;87:44–53.
16. Surh YJ. Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: a short review. Food Chem Toxicol. 2002;40:1097–1100.
17. Siddiqui AM, Cui X, Wu R, Dong W, Zhou M, Hu M, et al. The anti-inflammatory effect of curcumin in an experimental model of sepsis is mediated by up-regulation of peroxisome proliferator-activated receptor-gamma. Crit Care Med. 2006;34:1874–1882.
18. Si X, Wang Y, Wang J, Zhang J, Mc Manus BM, Luo H. Dysregulation of the ubiquitin-proteasome system by curcumin suppresses coxsackievirus B3 replication. J Virol. 2007;81:3142–3150.
19. Kewitz S, Volkmer I, Staege MS. Curcuma Contra Cancer? Curcumin and Hodgkin's Lymphoma. Cancer Growth Metastasis. 2013;6:35–52.
20. Aggarwal BB, Sundaram C, Malani N, Ichikawa H. Curcumin: the Indian solid gold. Adv Exp Med Biol. 2007;595:1–75.
21. Rai D, Singh JK, Roy N, Panda D. Curcumin inhibits FtsZ assembly: an attractive mechanism for its antibacterial activity. Biochem J. 2008;410:147–155.
22. Liang G, Yang S, Jiang L, Zhao Y, Shao L, Xiao J, et al. Synthesis and anti-bacterial properties of mono-carbonyl analogues of curcumin. Chem Pharm Bull. 2008;56:162–167.
23. Talaro KP, Talaro A. Drugs, microbes, host-The elements of chemotherapy. In: Foundations in Microbiology. 4th ed. McGraw-Hill, New York, pp. 2002;348–379.
24. Forbes BA, Sahm DF, Weissfeld AS. Laboratory methods and strategies for antimicrobial susceptibility testing. In: Bailey & Scott's Diagnostic Microbiology. 12th ed. Mosby, St. Louis, pp. 2007;187–214.
25. Shahi SK, Shukla AC, Bajaj AK, Banerjee U, Rimek D, Midgely G, et al. Broad spectrum herbal therapy against superficial fungal infections. Skin Pharmacol Appl Skin Physiol. 2000;13:60–64.
26. Dilis V, Trichopoulon A. Antioxidant intakes and food sources in greekadults. J Nutr. 2010;140:1247–9.
27. De R, Kundu P, Swarnakar S, Ramamurthy T, Chowdhury A, Nair GB, et al. Antimicrobial Activity of Curcumin against *Helicobacter pylori* Isolates from India and during Infections in Mice. Am Soc Microbiol. 2009;53:1592–1597.
28. Qa'dan F, Thewaini A, Ali D, Afifi R, Elkhawad A, Matalka KZ. The antimicrobial activities of extracts *Curcuma Longa* L. to acne-developing organisms. Am J Chinese Med. 2005;33:197–204.
29. Qadan F, Thewaini AJ, Ali DA, Afifi R, Elkhawad A, Matalka KZ. The antimicrobial activities of Psidium guajava and Juglans regia leaf extracts to acne-developing organisms. Am J Chin Med. 2005;33:197–204.