In vitro studies of antimicrobial activity of (curcuma longa L.) rhizomes against helicobacter pylori
Najah Ali

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, 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. 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. This reaction is characterized by a mucosal infiltration of inflammatory cells, especially neutrophils, which is mediated by enhanced expression of proinflammatory chemokines and cytokine. H. pylori infections can lead to peptic ulcers, and also lead to more serious complications such as internal bleeding, obstruction, perforation, and peritonitis. 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. 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. 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. 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. 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.

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. In view of the incomplete cure achieved with conventional therapy because of increasingly resistant strains, undesirable side effects, the cost of the antibiotic regimens, development of bacterial resistance to the available antibiotics has led researchers to investigate the antibacterial compounds from plants sources.

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. 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, antiviral activities, antioxidant property and antifungal activity of curcumin.

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

Material and Methods

Helicobacter Pylori Culture

Helicobacter. 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% O₂, 10% CO₂, 85% N₂) (double gas incubator; Heraeus, Langenselbold, Germany) for 3–6 days. Stock cultures were maintained at −70°C.

**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 with¹⁵ 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.**

<table>
<thead>
<tr>
<th>Agent or inhibitor</th>
<th>The mean of inhibition zone (mm ± SD) <em>H. pylori</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>7.7 ± 2.7*</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>8.3 ± 3.10</td>
</tr>
<tr>
<td>DMSO</td>
<td>0</td>
</tr>
</tbody>
</table>

*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. The antimicrobial activities of this plant have already been studied with different microorganisms.

**References**


**Conflict of Interest**

None.