

## *In vitro* Anti-*Helicobacter pylori* Activity of Capsaicin

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### Abstract

Worldwide, peptic ulcer and gastritis considered to be one of the biggest health challenge, *Helicobacter pylori* is responsible for more than eighty percent of chronic active gastritis where continual infection remains for decennary. However, the success of commercially available drugs for the management of *H. pylori* has overwhelmed by antibiotic-resistant strains, especially, metronidazole and clarithromycin, therefore, an urgent need arise to search for new options for treatment with enhanced anti- *H. pylori* activities, while being less toxic to human cells. Naturally occurring plant products, including spices, are one of these strategies that showed activity against *H. pylori*. Present study aim to test the antibacterial activity of capsaicin and other pure plant-derived compounds against a standard (NCTC 11916) *H. pylori* strain *In vitro* and to test for possible synergistic effect when combined with conventional therapy. Capsaicin shows good antibacterial activity on regular antimicrobial sensitivity testing methods (Anti-MSTM) and titration checkerboard assay MIC (0.0625 mg/ml), whereas piperine MIC was (0.125 mg/ml). While for curcumin no inhibition was found. The strain was found to be resistant to metronidazole with (MIC=250 µg/ml). When combining capsaicin with metronidazole, (FIC) Fractional inhibitory concentration values shown a synergistic effect, While the additive effect was found for capsaicin combination with piperine. Our obtained data indicate that capsaicin possesses promising anti *H. pylori* bioactivity and synergistic activity when combined with metronidazole but more work is necessary to examine the mechanisms by which these happened. Furthermore, it is necessary to ensure its activity against *H. pylori* *In vivo* and clinical settings.

**Keywords:** *H. pylori*, Capsaicin, Spices, Synergism

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## INTRODUCTION

*Helicobacter pylori* is a type of gram-negative bacteria. *H. pylori* Infection occurs in more than 50 percent of the world's population (Tanih *et al.*, 2008), which leads to develop chronic gastritis that could steps forward to be peptic ulcer disease, adenocarcinomas, (MALT) gastric mucosa-associated lymphoid tissue lymphoma, and other gastroduodenal diseases (Ernst and Gold, 2000). *H. pylori* was the first bacterium which classified as a class I carcinogen according to the WHO, (Peek and Blaser, 2003).

Various treating options designed for *H. pylori* infections; however, no single antibiotic can eradicate treatment so that, a combination of different antibiotics has been used. These antibiotics are often combined with Antisecretory agents such as proton pump inhibitors (PPIs) and/or histamine receptor (H2) antagonists (Gonzalez *et al.*, 2014; McColl, 2010; Kusters *et al.*, 2006; Nakayama and Graham, 2004).

However, the success of commercially available drugs for the management of *H. pylori*-associated gastric disorders has overwhelmed by the various adverse effects associated with these drugs, noncompliance with patients, economical problem due to high price of the antibiotic regimens, in addition to the increase in antibiotic-resistant strains (especially to metronidazole and clarithromycin) (Graham, 2004; De, Kundu, 2009).

This leads to an insistent require to search for novel complementary and alternative treatment regimens with enhanced activity against *H. pylori* while being less toxic to human cells. One of these strategies is to go back to nature.

Traditionally Arabic communities including Jordanians used a lot of herbal preparations to treat minor complaints mainly gastrointestinal and respiratory-related problems (Abu-Irmaileh, and Afifi, 2003; Humam *et al.*, 2020). Many of these herbs have a wide spectrum of antimicrobial and antifungal properties (Abu-Qatouseh *et al.*, 2013). Previous *in vitro* and *in vivo* studies have shown support that plant products, such as spices, are effective against *H. pylori* (Toyoda, *et al.*, 2015; O'Mahony *et al.*, 2005). So it is time to go toward the use of natural sources against this bacterial infection and to find a strategy to prevent it. To do this, three compounds derived from commonly used spices in Jordan as daily food ingredients

have been evaluated for their antimicrobial effectiveness against *H. pylori in vitro*, which are capsaicin, piperine, and curcumin.

Capsaicin, the chili peppers active ingredient, which represented more than 80% of extract, obtain from Capsicum plants (family Solanaceae) the most famous chili use worldwide (Sharma *et al.*, 2013; Ali *et al.*, 2019; Al-Samydai *et al.*, 2019).

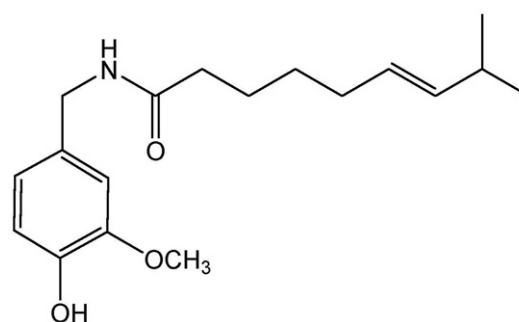


Fig. 1. The molecular structure of Capsaicin (Al-Samydai *et al.*, 2019)

On the other hand, alkaloid Piperine, found in Black pepper (*Piper nigrum*) which is known as the “king of spices” (Tharmalingam, 2014) from the *Piperaceae* family. Finally, Curcumin is the principal constituent of turmeric which extracted from *Curcuma longa* rhizomes (family; *Zingiberaceae*) commonly used in food as spices and coloring agent (Mario *et al.*, 2007; Mehmood *et al.*, 2011).

This study aim to study the antimicrobial activity of Capsaicin (*in vitro*) on *H. pylori* standard strain, test the antimicrobial effect of capsaicin in combination with other spices against *H. pylori* strains and to study the possible synergetic effect of such combinations with conventionally used antibiotics to maximize the therapeutic effect or to reduce the side effects of them.

## MATERIALS AND METHODS

All chemicals used in experiments were HPLC grade. Dimethyl sulfoxide (DMSO) (Xilong chemicals, China), Capsaicin (Fluka, Switzerland) purchased from Sigma-Aldrich Chemicals, Piperine (Merck, Germany) and Curcumin (Sigma, USA).

### Bacterial Strain and Growth Conditions

Columbia blood agar base (Oxoid, Hampshire, UK) was sterilized by autoclaving (121 °C for 15 min) then enriched with laked horse

blood in 7 % (Oxoid, Hampshire, UK) and supplied with *Helicobacter pylori* selective supplement (Dent) (Oxoid, Hampshire, UK) (Mehmood *et al.*, 2011). In this study, (NCTC 11916) standard strain of *H. pylori* was used and colonized to be used for testing.

Continuous subcultures of *H. pylori* were performed to increase the colony's yield and recovery. To achieve the needed microaerophilic conditions, plates were incubated in Candle jar 2.5L (Oxoid, Hampshire, UK) and 98% relative humidity at 37°C. The suitable environment was achieved by using CampyGen Gas Pak (Oxoid, Hampshire, UK) and incubated for 7 Days 21. Selected colonies were picked with a sterile loop and transferred to Muller Hinton Broth media supplemented with fetal bovine serum 10% and glycerol 20% and then stored at -80°C for subsequent tests and long term preservation (Abu-Qatouseh, 2016).

*H. pylori* growth, confirmed by characteristic of colony morphology, Gram staining, and conventional biochemical tests (positive for oxidase, catalase, and urease) (Abu-Sini, 2017).

#### Anti-MSTM and MIC Determination

Sterile blank (6 mm) discs (Oxoid, Hampshire, UK) were soaked by 25 µL of the tested compounds that which prepared in a concentration of 1 mg/ml dissolved in DMSO. Discs soaked with ciprofloxacin 1mg/ml dissolve in DMSO had been used as a positive control, whereas DMSO soaked discs alone used as a negative control.

The freshly prepared bacterial suspension was prepared in sterile Phosphate Buffered Saline (PBS) to attuned turbidity to 6x10<sup>8</sup> CFU/ml (2 McFarland) (Abu-Qatouseh, 2011) and consequently homogeneously spread on solid Columbia blood agar medium.

Discs placed on the surface of each agar plate and incubated for 5-7 days under suitable cultivation environment (Abu-Qatouseh, 2011). Experiments were performed in triplicates. MICs obtained by two-fold agar dilution method (Abu-Qatouseh, 2011). Accordingly, all compound was in sequence diluted with DMSO and incorporated to agar plates with laked horse blood 7% (v/v). On the surface of each plate, spots of *H. pylori* suspension (6x 10<sup>8</sup>CFU) were applied. After 7 days, visible colonies growth (37°C under microaerophilic conditions) was determined. The

MIC was triplicate measured.

#### In-vitro Interaction Determination

Interaction between capsaicin and metronidazole In vitro, and between capsaicin and piperine against a standard *H. pylori*, was obtained by Checkerboard test (Abu-Sini *et al.*, 2017; Odds, 2003). Tested compounds were dissolved in serially diluted with DMSO then homogenized mixed with molten Columbia blood agar media triplicately. FICs were calculated as follows:

$$FIC = \left( \frac{\text{MIC of drug A in combination}}{\text{MIC of drug A alone}} + \frac{\text{MIC of drug B in combination}}{\text{MIC of drug B alone}} \right)$$

The FIC index, interpreted as follows: ≤0.5, synergy; 0.5–1, additive; 1–4.0, indifference; >4.0, antagonism (Abu-Sini *et al.*, 2017; Odds, 2003).

#### Urease Inhibition Assay

Quantitative determination of urease inhibition was performed using the standard colorimetric method (Abu-Sini *et al.*, 2017). In brief, 10 µl of *H. pylori* suspension in PBS (adjusted to an absorbance of 1.5 at a wavelength of 600 nm) were mixed with an equivalent quantity of serially diluted solution of capsaicin in 96 well plates upto 30 minutes at 37°C. 200 µl of detecting reagent composed of 50mM PB (pH 6.8) of urea (500 mM) and phenol red (0.02%) (Oxoid,CM0053, UK) add to each well. The develop color was monitored at 555 nm measuring OD in a 5-minute interval for 2 hrs. bacteria with DMSO and bacteria with PBS as Negative controls. Other controls included capsaicin alone without bacteria. PBS and DMSO were used as blanks.

The percentage of inhibition was calculated by the equation; Percentage of Inhibition = [ (activity without capsaicin - activity with capsaicin) / activity without capsaicin ] × 100

Compartion was done with a urease inhibitor; 3mg/ml Acetohydroxamic acid (AHA) as reference [Sigma, USA].

## RESULTS AND DISCUSSION

### Antimicrobial Activity against Metronidazole-Resistant *H. pylori*

Our preliminary screening showed that capsaicin and piperine possess considerable antibacterial activities against *H. pylori* in

comparison to ciprofloxacin. Similar antibacterial activities of the above compounds were previously reported (Zeyrek and Oguz, 2005; Jones *et al.*, 1997; Lee *et al.*, 2007). On the other hand, curcumin did not show any effect against *H. pylori* as reported previously by other researchers (Sarkar and Mukhopadhyay, 2016). This controversy could be explained by different factors including variations in strains used and their susceptibility or resistance to antimicrobial agents, in addition to the methodology applied in this study.

Capsaicin and piperine showed strong inhibition effect against *H. pylori* with an average zone of inhibition of 15 mm and 12 mm, respectively. The tested *H. pylori* strain is considered sensitive for both capsaicin and piperine with zones of inhibition exceed 11 mm (Adeniyi, 2012) obtained results shown agreement with a study done by Zeyreket *et al.*, 2005, which concluded that capsaicin began to inhibit both metronidazole resistant and metronidazole sensitive *H. pylori* clinical isolates at the minimum concentration 25 µg/ml. The mechanism by which this is happening is still unknown. Thus, it might be recommended to take small amounts of hot pepper for patients with gastric or duodenal ulcers since heavy ingestion of high doses of the compound has been associated with necrosis, ulceration and even carcinogenesis (Surh and Lee, 1996; Rollyson, 2014). Toyoda *et al.*, 2015 demonstrated that capsaicin shown anti-inflammatory activity against *H. pylori*- by inhibition of inflammatory factors such as *Tnf-α*.

Taken together, these findings suggest that capsaicin or chili peppers hold a promise in the treatment and prevention of *H. pylori* linked diseases, which might be related to the inhibition of growth and colonization of *H. pylori* resistant strains of and for patients which preferred natural products rather than synthetic antibiotics. It was also demonstrated the piperine inhibition activity against *H. pylori* growth and found that it inhibits growth completely at a concentration of 0.125 mg/ml as shown in our MIC findings.

Tharmalingam *et al.* 2014 investigated the inhibitory action of piperine on *H. pylori* growth and adhesion. Their study showed that piperine has suppressed the level of *H. pylori* adhesion to gastric adenocarcinoma cells in a dose-dependent manner by suppressing the flagellar hook gene *flgE* and integral membrane component of the export

apparatus gene *flhA* expression. Suppression of both genes leads to less motility which results in less attacking of *H. pylori* to the gastric epithelial cells which might be the possible reason in the adhesion inhibition.

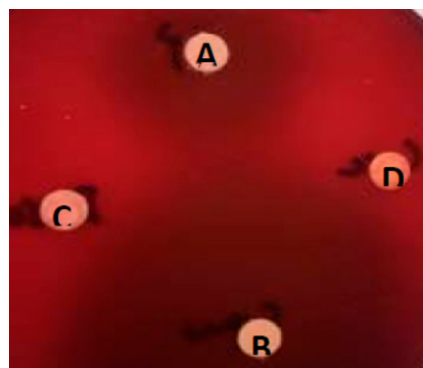
Besides, piperine suppressed expression of many inflammatory factors *Il-1β*, *Tnf-α* and *Il-6*, and the infiltration of neutrophils and mononuclear cells have been also suppressed both in the antrum and corpus of *H. pylori*-infected gerbils when treated with 100 ppm piperine Toyoda, 2015.

Our results propose that piperine could have potential as an alternative agent for the eradication of *H. pylori* infection and preventing chronic gastritis linked with it. Present findings recommend that combination of capsaicin and piperine could be used as part of the regimen for the management of *H. pylori*.

#### **In vitro interactions between capsaicin and piperine and between capsaicin and metronidazole**

It has been reported that the high rate of metronidazole resistant *H. pylori* is increasingly reported in Jordan due to the misuse of metronidazole (the frequently prescribed drugs by the general practitioners for the treatment of protozoal infections and gynecological problems) (Abu-Qatouseh *et al.*, 2016). Based on these findings it is worthy to study the synergistic effects between metronidazole and capsaicin.

To the best of our knowledge, there are no reports about the use of capsaicin and metronidazole for their synergistic effect against *H. pylori*.

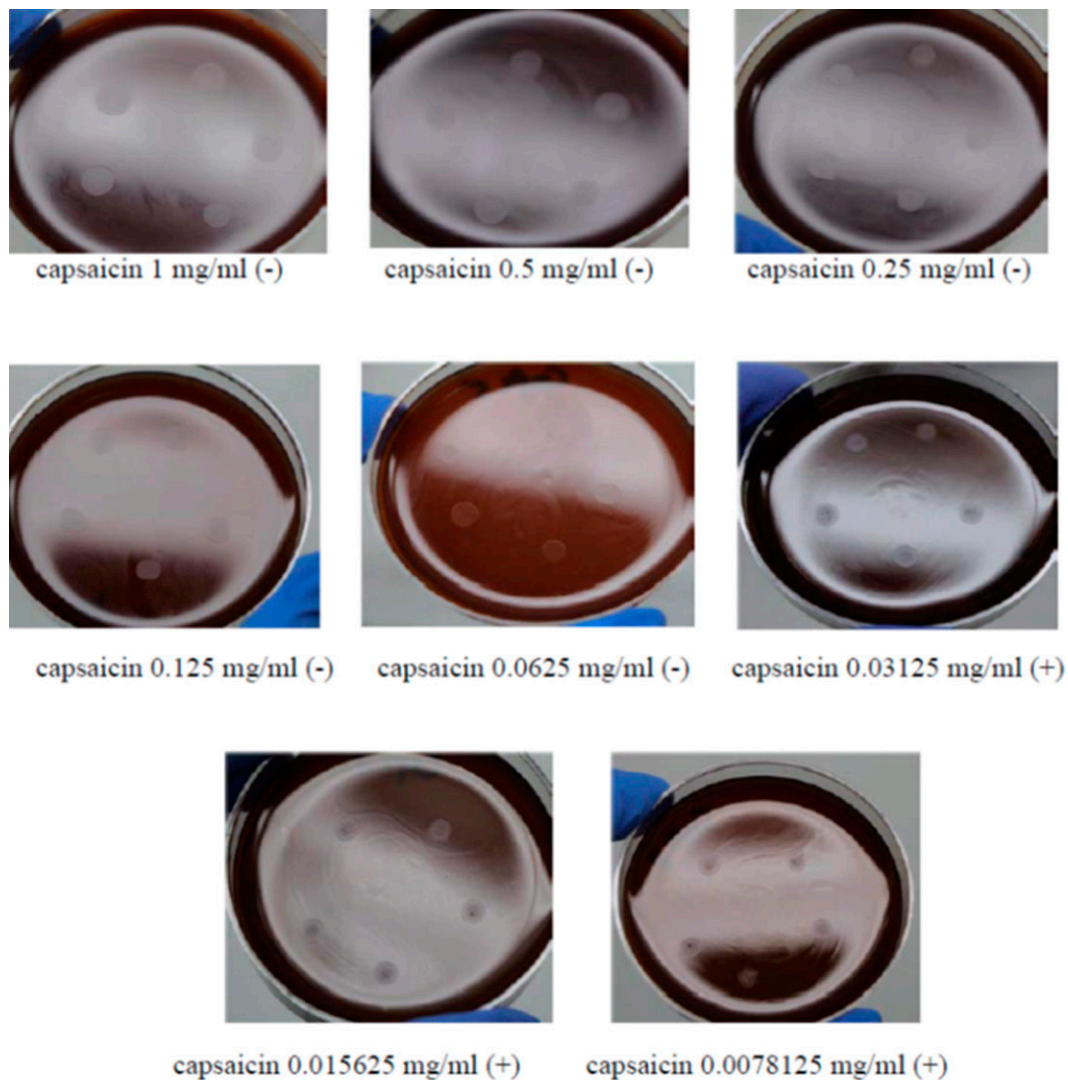


**Fig. 2.** Antimicrobial susceptibility tests (disc diffusion) of 1 mg/ml of A: capsaicin, B: ciprofloxacin, C: DMSO and D: curcumin against *H. pylori* strain.

The combination showed that MIC for metronidazole decreased by eight -folds when combined with capsaicin. The MIC for capsaicin was 62.5 µg/ml and the MIC for capsaicin in combination was 15.625 µg/ml. The MIC for metronidazole was 250 µg/ml and the MIC for metronidazole in combination was 31.25 µg/ml. Metronidazole and capsaicin FIC value means were 0.126 and 0.25 respectively, FIC index for their combination was 0.376 which indicated that this combination was synergic. Capsaicin has potential synergy with antibiotic metronidazole and thus suggested that higher eradication success rates with this antibacterial combination using lower

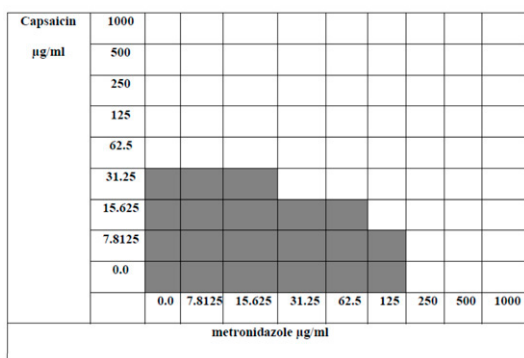
doses of metronidazole, thus minimizing toxicity and the chance for resistance issues and thus lowering the cost of treatment.

However, in our studies, the mechanism of this synergistic activity was not investigated. Although it might affect one of the metronidazole resistance mechanisms, that are caused by the failure of enzymatic reduction and prevented the production of antibacterial metabolites. This resistance mechanism has been elucidated with mutations of genes encoding certain electron transport proteins operative in the reduction process *rdxA* and *frxA*. Other mechanisms have been suggested as the cause of metronidazole



**Fig. 3.** Minimum inhibitory concentration (mg/ml) of capsaicin against reference strain

resistance in *H. pylori* such as involvement of efflux pump gene *hefA* 31 Since it was reported that capsaicin has reduced the MIC of ciprofloxacin by inhibiting the NorA efflux pump, thus reducing the intracellular invasion of *Staphylococcus aureus*. Whereas, capsaicin combination with piperine showed additive effect where MIC for capsaicin was 62.5µg/ml and the MIC for capsaicin combination with metronidazole was 15.625µg/ml as shown in figure 4.



**Fig. 4.** Checkerboard method showing the prepared diluted concentrations of capsaicin and metronidazole (bolded squares refers to a positive bacterial growth)

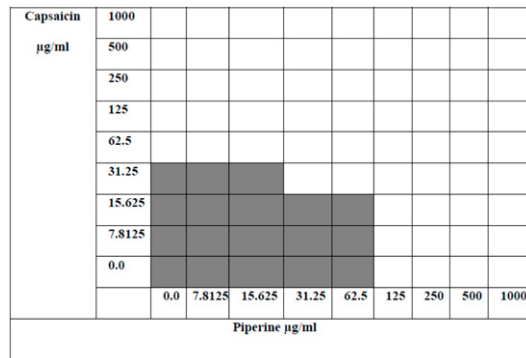
While for piperine MIC was 125µg/ml and the MIC for piperine in combination was 31.25 µg/ml as shown in figure 5. FIC value means of capsaicin and piperine were 0.5 and 0.25 respectively, and the FIC index for the combination was (0.75) which indicated that this combination has an additive effect.

Although the additive antibacterial effect was reported for such combinations, this combination may have a potential effect when it goes to *in vivo* interaction where piperine might improve the bioavailability of capsaicin and hence improve its activity and lead to the use of lower doses of capsaicin while maintaining its antibacterial activity.

**Inhibitory Effect Capsaicin against *H. pylori* Urease Enzyme**

Several studies have suggested a role for *H. pylori* urease in the survival and pathogenesis of the *H. pylori*. In this study, evaluation of the urease inhibitory potential of capsaicin was achieved by using the colorimetric method to

determine the percent inhibitory activity of capsaicin over a concentration range of 31.25-1000 µg/ml. Compounds achieving less than 60% inhibition are considered to have no significant effect Abu-Qatouseh *et al.*, 2013. Capsaicin showed insignificant inhibitory activity (< 15% inhibition) over the tested concentration range. The previous study by Modolo *et al.*, 2015 showed that inhibiting urease activity by capsaicin was achieved at concentrations reaching 10mg/ml.



**Fig. 5.** Checkerboard method showing the prepared diluted concentrations of capsaicin and piperine (bolded squares refers to a positive bacterial growth)

**CONCLUSIONS**

The antibacterial activity of capsaicin and piperine against *H. pylori* was confirmed and thus could be considered for added values of the treatment of *H. pylori* particularly if the *in vivo* studies proved this concept. Our obtained data indicate that they possess promising anti-*H. pylori* bioactivity but more work is necessary to examine the inhibitory mechanism of capsaicin and piperine against *H. pylori*. Furthermore, it is necessary to ensure their activity against *H. pylori* *in vivo* and on clinical settings taking into consideration the possibility of synergistic effect of capsaicin when combined with metronidazole, so that it might be used as dietary supplements to complement and expedite current treatment regimens although more future work is needed to determine the factors that could affect the efficacy of capsaicin including dosage forms, presence of delivery system, addition of anti-acids, etc. and the synergistic mechanism by which it improves the activity of metronidazole.

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None.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

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## DATA AVAILABILITY

All datasets analyzed in the study are included in the manuscript and presented as tables and figures.

## ETHICS STATEMENT

Not applicable.

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