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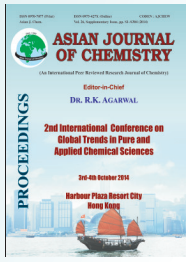

Antimicrobial Activity of Garlic Against *Helicobacter pylori*

GULCIHAN GUZELDAG[1,*], HATICE AYSUN MERCIMEK[1] and FATIH MATYAR[2]

[1]Faculty of Art and Science, Department of Molecular Biology and Genetics, Kilis 7 Aralik University, 79000 Kilis, Turkey
[2]Faculty of Education, Department of Science and Technology Education, Cukurova University, 01330 Saricam, Adana, Turkey

*Corresponding author: Fax: +90 348 8222351; Tel: +90 348 82223 50-1451; E-mail: gguzeldag@gmail.com, gguzeldag@kilis.edu.tr

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This study shows that garlic extracts produce levels of inhibition to *Helicobacter pylori* similar to those of the commercial materials. The research described here represents an important starting point in the fight against Helicobacter diseases. The extracted material can be used by direct application and involves a simple and economical extraction procedure that avoids isolation or purification techniques.

Keywords: Antimicrobial activity, Garlic, *Helicobacter pylori*.

INTRODUCTION

In recent years, the studies regarding the anti-*Helicobacter pylori* activity of medicinal plants have increased considerably. Attention has been given to the screening of medicinal plants all over the world as a means to identify low-cost sources of new drugs against *H. pylori*, a human gastric pathogen with high morbidity rate. The search for new chemical compounds with bactericidal or bacteriostatic effects against *H. pylori* is a challenge for the world's medical and scientific communities[1].

Garlic (*Allium sativum*) has been known one of the most used plants in traditional medicine and the wider cited in the literature for its medicinal properties[2] and proven to have antimicrobial effects[3].

This study shows that garlic extracts produce levels of inhibition to *Helicobacter pylori* similar to those of the commercial materials. Garlic has a wide spectrum of actions, not only is it antibacterial, but it also has beneficial effects on the cardiovascular and immune systems. The research described here represents an important starting point in the fight against and/or prevention of peptic ulcers, as well as other pathologies associated with helicobacter diseases such as gastric cancer. The extracted material can be used by direct application and involves a simple and economical extraction procedure that avoids isolation or purification techniques.

EXPERIMENTAL

Plant extraction: Garlic (*Allium sativum*) used for this investigation is free of any pre-harvest chemical treatment (organic products). Samples freshly harvested were sorted for uniformity and absence of defects and stored at 4 °C for prior analyses.

Aqueous extractions were carried out according to Freedman *et al.*[5] using distilled water as a homogenizer. For the preparation, dried garlic bulbs were peeled, weighed (20 g) and surface sterilized using 95 % ethanol. The ethanol was allowed to evaporate in a sterile laminar flow chamber and the garlic was macerated separately in 200 mL of solvent in glass bottles and put in an orbital shaker for 24 h at room temperature. The solvent were then removed under reduced pressure in a rotary evaporator at 55 °C approximately 60 min and then extract was first filtered with Whatman No. 1 and evaporated to dryness at room temperature.

Oil extractions were prepared with Soxhlet extraction with *n*-hexane, acetone and methanol for 6 h. The oils were obtained after the solvents wererecycled and suspended under reduced temperature and pressure and refluxing at 70 °C so as to remove any excess solvent used for the oil extracted. Extracts were then collected, dried under reduced pressure, weighed and stored at -20 °C before use[6].

Culture of *H. pylori*: *H. pylori* was supplied by the microbiology culture collection of the department. Bacteria inocula were prepared by using *H. pylori* agar plate and incubated at 37 ºC for 3 days under microaerophilic conditions within GasPak anaerobic system (Oxoid). Authenticity of the culture was confirmed by gram negative staining and testing for urease activity.

Antimicrobial activity: *Helicobacter pylori* was adjusted to the turbidity of the 0.5 McFarland standard in 3 mL of saline. The diluted inocula were swabbed on the surface of

TABLE-2
MIC VALUES OF EXTRACTS AGAINST *H. pylori*

	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096
DW	++	++	++	++	++	++	++	+	+	-	-
Acetone	++	++	++	++	++	++	++	++	++	+	-
Hexane	++	++	++	++	++	++	++	++	++	-	-
Methanol	++	++	++	++	++	++	++	++	++	++	+

Mueller Hinton agar plate using a sterile cotton swab. Agar wells were prepared with the help of sterilized cork borer with radius 6 mm. Using a micropipette, 100 µL of extracts were added to the each well of the plate. DMSO (10 %) was used as a negative control and 0.05 µg/mL clarithromycin was added as positive control included in all experiments, respectively. The plates were incubated in an upright position at 37 ºC for 72 h. The diameter of inhibition zones measured in mm and the results were recorded.

MIC determination: Determination of minimum inhibitory concentration (MIC) of garlic was evaluated by serial dilutions to various concentrations in reference to the cited literatures[7]. In the same way that the dilution method in Brucella broth medium, the oil is first diluted in ethanol and Tween 80 to obtain a homogeneous mixture. It is incorporated into the agar medium during cooling to obtain dilutions of 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024, 1/2048, 1/4096, respectively. Inoculum solution at 4 µL was added to every well. Being incubated for 72 h at 37 °C under microaerophilic conditions, the plates were monitored for turbidity growth and non-turbidity as no growth. After the incubation time the MIC was read as the lowest garlic extract concentration showing no visible bacterial growth on the culture plates. This was determined graphically, by plotting zone diameter (mm) A single colony or barely visible haze was considered negative when the agar dilution end point read. Solvent blanks and positive controls were also included. All the tests were tested in duplicate.

RESULTS AND DISCUSSION

Garlic extracts exhibited different inhibition levels against *H. pylori*. In the dose response study, the inhibition zone increased with increasing concentration of extracts. At high concentrations extracts showed remarkable inhibition against *H. pylori*.

In the screening of antibacterial activity using agar-well diffusion technique, methanol extracts of garlic was found to be high inhibition zones in other wordsa minimum zone diameter of 18 mm with distilled water and maximum zone diameter of 39 mm with methanol extracts are identified in the study (Table-1). Methanol extracts showed significant activity with the zone diameter between 32 and 39 mm against *H. pylori* in our study. On the other hand, similar results are observed from acetone and hexane extracts. Especially the concentrations of 100, 150 and 200 mg/mL were not significantly different for these extracts[8] observed similar strong inhibitory activity of different concentrations of *Allium sp*. at his study. Similarly distilled water showed at least inhibition levels. This may be affected by the solubility on agar plate.

Solvents of hexane, acetone, methanol and water were used in this study to determine the inhibition levels for *H. pylori*.

TABLE-1
ANTIBACTERIAL ACTIVITY OF SOLVENT EXTRACTS AND ESSENTIAL OILS AGAINST *H. pylori*

	50	100	150	200
DW	18 ± 0.4	20 ± 0.8	21 ± 0.6	24 ± 0.3
Acetone	27 ± 0.4	29 ± 0.2	30 ± 0.2	31 ± 0.2
Hexane	25 ± 0.7	28 ± 0.8	29 ± 0.5	29 ± 0.8
Methanol	32 ± 0.7	34 ± 0.8	38 ± 0.6	39 ± 0.4

These solvents may extract a wide range of active compounds. According to MIC results (Table-2) among the extracts tested in the study occured that methanol had the lowest MIC value with the concentration of 1:4096 dilution followed by acetone with a MIC of 1:2048, hexane with a MIC of 1:1024 and distilled water with a MIC of 1:1024 showed comparatively less sensitive. The low MIC values observed a good indication of high efficiency against *H. pylori* at the low concentrations. According to our study high inbition levels with MIC determinations and well diffusions are screened with the extracts.

Garlic extracted either in water or in organic solvents have biologically active compounds, which can be used in the synthesis of potent drugs. Thus garlic, which are normal ingredients of our routine food preparations, can provide protection to a certain extent against our natural enemies like bacterial pathogens.

All the extracts studied herein demonstrated considerable and efficient inhibition effects through *H. pylori*, related to the type of extracts used. Although evidence on efficacy of the tested extracts have been obtained, further investigations are necessary because the effectiveness of these inhibitions may be related to the type of garlicor may be affected by the solubility and rate of diffusion in medium and such examples may cause related results.

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