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Impact of Garlic Extract on H.pylori Isolated from Chicken Meat Markets

Dhary Alewy Almashhadany[1]*, Sara Mohammed Mayas[2], Fawzia Jassim Shalsh[3]

[1]Knowledge University, College of Science, Department of Medical Laboratory Science, Erbil 44001, Kurdistan Region, Iraq. [2]Microbiology Section, Department of Biology, Faculty of Applied Science, Thamar University, Dhamar, Yemen. [3]Ministry of Science and Technology Industrial Microbiology Dept. Directorate of Agricultural Research.

Abstract

This study was designed to estimate the effect of garlic extract on *H. pylori* isolated from poultry meat at Dhamar Governorate, Yemen. In the laboratory, the isolation of *H. pylori* was done under aseptic conditions using modified Campy-blood agar and Belohorizonte agar. Purification isolates were done on the same agar media-biochemical examinations employed for the identification comprised essential biochemical tests. In vitro studies, show that *H.pylori* was survived at 2% of garlic extract, whereas at 4% and 6%, it was observed that the inhibition zone 10mm and 20mm respectively. In an experimental study, the count number of *H. pylori* was reduced with 2% and 4% of garlic extract, while at 6% no growth occurred. We concluded that treating chicken meat with garlic extract reduces the number of *H.pylori* cells, and garlic extract plays an important role in food preservation. Due to the significance of this study, we recommend further research and studies on *H. pylori* in different governorates of Yemen, particularly on the prevalence of *H. pylori* in various types of food, mainly food of animal origin, and study the impact of some medicinal plants such as garlic.

Keywords: *H.pylori*, Garlic extract, Chicken meat, In vitro study, Yemen

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*Corresponding Author: Dhary Alewy Almashhadany E-mail: dhary.alewy@knu.edu.iq

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Introduction

Helicobacter pylori (*H. pylori*) is one of the most common human bacterial pathogens worldwide, with 4.4 billion infected persons in 2015 [Almashhadany et al., 2022a]. A greater frequency was indicated in unsanitary and economically poor regions; the rate of *H. pylori* infection in Asia, Africa, and South America was considerably higher than that in Australia, North America, and Western Europe [Tonkic et al., 2012]. *H. pylori* is a Gram-negative rod microaerophilic, motile, and has a specific spiral shape. Although more than 80% of the infected individuals are without symptoms [Almashhadany and Mayass,2018; Khoder et al., 2019], *H. pylori* infection may play a vital role in many gastric diseases according to several works that have reported its contributions to gastritis and peptic ulcers[Cover and Blaser,2009]. Several researches have shown that the eradication of *H. pylori* in infected persons of all ages can decrease the frequency of gastric cancers [Varon *et al*., 2022, Aumpan *et al.*, 2023], and it is one of the risk factors of adenocarcinoma (Sala *et al*., 2023). Nowadays, *H.pylori* has been well-established that infection of the human host with this microorganism is a risk factor for the development of gastric-related diseases (Mulat Erkihun, 2022, Aumpan *et al*., 2023). Thus, it was categorized as a carcinogen in 1994 by the World Health Organization (WHO) (Graham et al.,2023).

Chicken meat is a popular protein source in many nations due to its source of high-quality protein, essential amino acids, vitamins, and minerals. On another hand, its contamination can take place at many phases of slaughter and manufacturing [Alagawany et al., 2020; Tagar and Qambrani,2023). However, several studies reported that *H. pylori* has survived in the gastrointestinal tracts of chickens, and the infected chickens can shed *H. pylori* in their feces, leading to contamination of the environment. These bacteria can contaminate chicken meat if basic hygiene and sanitation practices are not followed during slaughter and processing (Pius *et al*., 2021). Consumption of *H. pylori*-contaminated chicken meat may raise the risk of gastrointestinal illnesses in humans. Helicobacter has been classified as a zoonotic bacterium (Asadi *et al*.,2023). This is a significant concern, as contaminated poultry and poultry products can potentially serve as a source of human infection. Generally, the presence of *H. pylori* in the gastrointestinal tracts and feces of chickens highlights the possibility of zoonotic transmission, highlighting the significance of food safety measures and good hygiene practices in preparing and handling poultry products to minimize the risk of infection.

Anyway, medicinal plants are considered eco-friendly, simple, more secure, easy, quick, and less toxic than traditional treatment techniques (Abou Baker,2020). Garlic (Allium sativum), is a member of the family Liliaceae and it is one of the medicinal plants that has been researched the most (Cardos et al., 2021). Garlic and its preparations have long been recognized as effective preventative and therapeutic agents and have been demonstrated to have positive effects on different diseases including cardiovascular troubles, hypertension, diabetes, wound healing, osteoporosis, stress, and others (Sala et al.,2023; Sarswat et al., 2023).

In vitro anti-*H. pylori* activity was also demonstrated for garlic extract (Bouhenni *et al*., 2023). Garlic is high in carbohydrates, minerals, fat, vitamins, fiber, and phenolic compounds, all of which

support excellent health. Several in vitro and in vivo investigations have found that garlic and its bioactive compounds have anticancer, anti-inflammatory, antibacterial, antioxidant, antidiabetic, antihypertensive, antiobesity, and antithrombotic (Bazaraliyeva *et al*., 2022; Okoro *et al*., 2023). In addition, garlic exhibits broad antibiotic activity against both Gram-negative and Gram-positive bacteria due to the vital role of thiosulfates (allicin), and in vitro activity of anti-*H. pylori* for garlic extract was confirmed by some authors (Si XB et al., 2019; Bouhenni *et al*., 2023).

In a recent study conducted by Speciani et al., 2023, who monitored the association between garlic intake and colorectal cancers, the results showed that garlic reduced cancer risk with a statistical significance. Also, Vipula et al., 2023, illustrated that when a mix of plant extracts of Allium sativum and zingiber officinale extracts at dosages ranging from 200 to 400mg/kg were supplied to wistar rats' stomachs, all ulcers and perforations were healed. The prevalence of *H. pylori* infection has been considered one of the most common infections in both developed and developing countries including Yemen, and garlic has long been recognized as an effective preventative and therapeutic agent (Almashhadany et al.,2022b). Therefore, this study was conducted to understand the effect of garlic extract on *H. pylori isolated from chicken meat.*

Materials and methods

Isolation of *H.pylori* from Poultry Meat

In the laboratory, the isolation of *H. pylori* was done under aseptic conditions as formerly published (Al-Mashhadany and Mayass, 2017; Almashhadany, 2020). In brief, samples were cut into small slices using sterile blades for the release of adherent bacteria. From the breast and thigh, the optimal sample size (about 25 gm) was soaked in 250 ml of normal saline. After that, for enrichment, 0.5 ml of the suspension was then placed in a 4.5-ml Brain-Heart Infusion broth with 7% horse serum lacking antibiotics and incubated in a microaerophilic atmosphere (GasPack; Oxoid, Basingstoke, England) at 37°C for 3 to 7 days. Subsequently, modified Campyblood agar and EYE agar plates were inoculated with 100 µl of the enriched suspension and incubated at 37°C in the microaerobic condition in a candle jar and Campy Gen (2.5 L) in the incubator for 4-10 days (Coldham *et al.,* 2011; Lawson, 2015).

Purification of *H.pylori* isolates

For purification, progressing colonies were subcultured on the same agar media and incubated at 37°C for 48–72 hrs (Yin, 2009).

Identification of *H.pylori* Isolates

Concisely, after incubation, all cultural plates were inspected for expected colonies of *H. pylori.* Gram staining was done according to the standard technique with exposure of smears to safranin for 3 minutes. Biochemical examinations employed for the identification comprised essential biochemical tests (Table 1). Isolates that met the reference properties were considered *H. pylori* (Lawson, 2015; Al-Mashhadany et al., 2018).

Impact of Garlic Extract on *H. pylori*

Preparation of *H.pylori* Isolates

The strain of *H.pylori* was cultured on selective media under microaerobic conditions, and incubated at 37°C for 48 hr then examined to make sure of purity. Then 10 ml of sterile phosphate buffer saline (PBS) was added to each dish and colony by sterilized loop, collected, and mixed well by the vortex – Gein. After that series of

dilutions were worked. The number of bacterial cells was calculated by comparing with 0.5 McFarland tube and using a spectrophotometer in optical wavelength 480nm.

Preparation of Chicken meat samples

For this experiment, soft chicken meat pieces didn't give any positive growth for the *H.pylori* when planted on selective media and cut at 25 gm for each piece. Then 1 ml of broth containing 10^8 cells/ml on the surface and inside each piece of meat by sterile disposable syringe. All the pieces were put in sterile polyethylene bags subject to the garlic factor (Al-Mashhadany and Mayass, 2017).

Preparation of Garlic extract

Garlic extract was prepared as described by (Mouffok *et al*., 2023). The bulbs were peeled, and 100 gm were mixed with 100 ml of sterile distilled water. The mixture is then blended in a clean sterile blender for 2-3 min, before filtration through a sterile Buchner and filter paper. The supernatant is considered as water garlic extract with a concentration of 1:1 (w /v), then the garlic extract was stored at 2-8°C until used.

Study the effect of garlic extract on *H. pylori* using Disc Diffusion Agar

The susceptibility test was based on the agar diffusion method (Asadi et al., 2023). An inoculum of strain *H. pylori* was prepared by transferring fresh colonies in tubes containing sterile saline the adjusting the turbidity to 2.0 McFarland units (approximately $6x10^8$ CFU/mL). Half a milliliter of a suspension of bacteria was spread on the surface of a Muller Hinton agar medium supplemented with 10% blood-defibrinated sheep. The agar was incubated for 15 min at 37 °C to ensure that the agar would absorb the bacterial suspension. Finally, the disks of 10 µl of each garlic extract were placed on the agar using sterile forceps. A total of 5 replicates have been used in this study. After that, were incubated at 37°C under microaerophilic conditions for 3-5 days. The antibacterial activity was expressed as the mean diameter of the growth inhibition zone around the disks.

Study the effect of garlic extract on *H. pylori* using experimental inoculation

The garlic extract was added to the chicken meat samples in a concentration of 2%, 4%, and 6% by sterile pipette, then the meat samples were inoculated with the *H. pylori* bacteria10^8 cell/ ml and stored in the refrigerator. The samples were examined for the recovery of *H. pylori* as CFU /g at the zero time and after 1, 2, and 3 days using selective agar (Maślanka, et al., 2023).

Statistical analysis

Statistical analysis was done with the chi-square test, repetitions, Percentage, and Pearson correlation (IPM SPSS statistics 20).

Result

Isolation and identification of H. pylori

H. pylori appeared in different shapes on media (Figure 1). On modified Campy-blood agar media small, round, and translucent colonies. Golden colonies appeared on Belohorizonte agar (BHI agar with 2,3,5-triphenyltetrazoliumchloride).

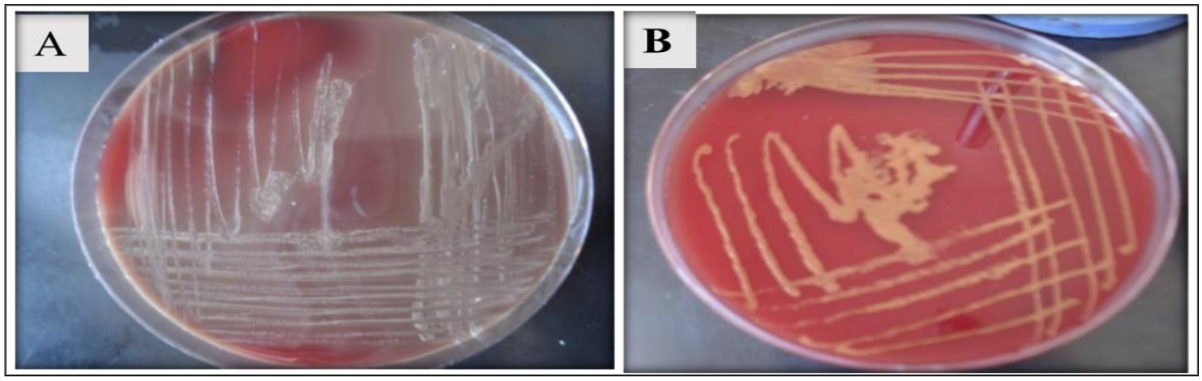

Figure (1): -*H. pylori* colonies on (A) modified Campy-blood agar and (B) Belo Horizonte agar

Purification of *H.pylori* Isolates

Pure culture of suspected *H. pylori* colony, appeared with modified gram stain as Gram-negative bacteria, rods or curved shaped (Figure 2).

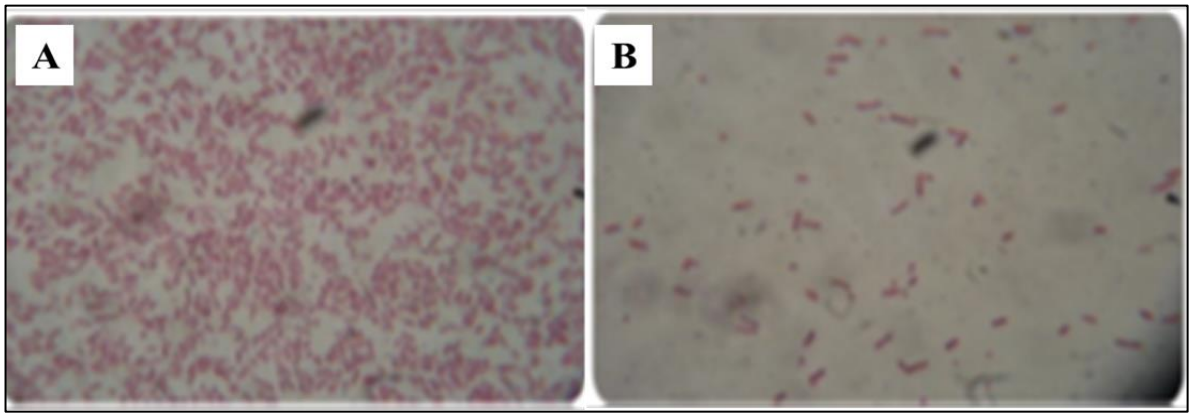

Figure (2): - H. *pylori* bacteria (negative Gram stain, rod or curved shaped).
(A): 40X magnification view (B): 1000X magnification view

Identification of H.pylori Isolates

Biochemical examinations employed for the identification comprised essential biochemical tests (Table 1).

Table (1): -Biochemical tests used for the confirming of *H. pylori*

Biochemical Tests	Results
Catalase	+
Oxidase	+
Urease	+
Indole	–
Growth in 1% Glycine	–
Growth in 3.5% NaCl	–
H2S Production in (TSI)	–
TSI with lead acetate paper	+
Nalidixic acid	**Resistance**
Cephalothin	**Sensitive**
Hippurate Hydrolysis	–

Impact of Different Concentrations of Garlic Extract on *H. pylori*

Study the effect of garlic extract on *H. pylori* using Disc Diffusion Agar

As shown in (Table 2 and Figure 3) no inhibition zone formed around the disc of garlic extract with 2% concentration, whereas the inhibition zone around the disc of garlic extract with 4% and 6% concentrations was 10 mm and 20 mm respectively.

Table (2): - Effect of garlic extract on *H. pylori* using Disc Diffusion Agar

Concentration of Garlic Extract	Inhibition Zone
2%	**0 mm**
4%	**10 mm**
6%	**20 mm**
R=1	**R^2=1 ***

*= There is a strong relationship between the different concentrations and inhibition zones in equal ratios.

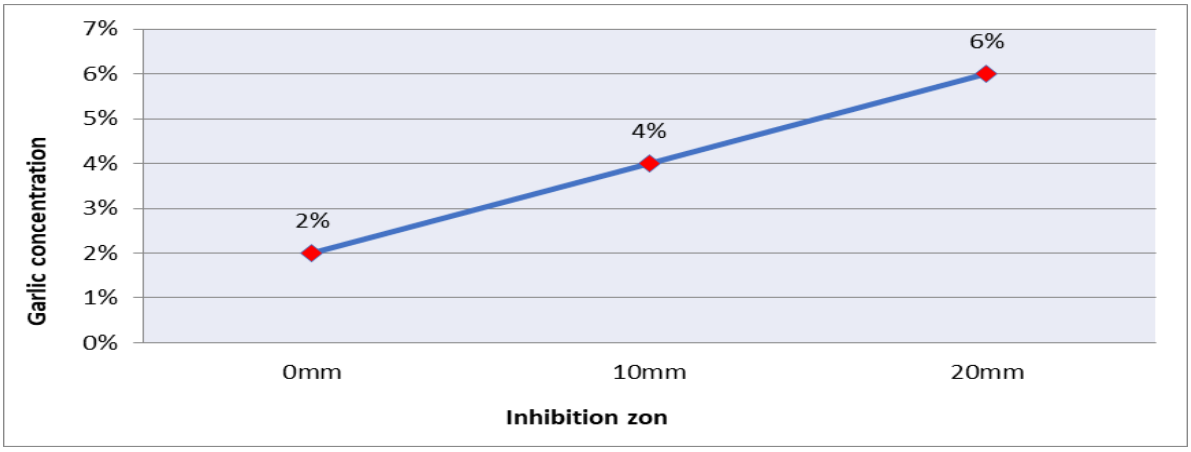

Figure (3): Effect of garlic extract on *H. pylori* using Disc Diffusion Agar

Study the effect of garlic extract on *H. pylori* using experimental inoculation

Count number of *H. pylori* was reduced with (2%) concentration of garlic extract from the moment the experiment started, and in (4%; 6%) concentrations, no growth occurred since the experiment was started (Figure 4).

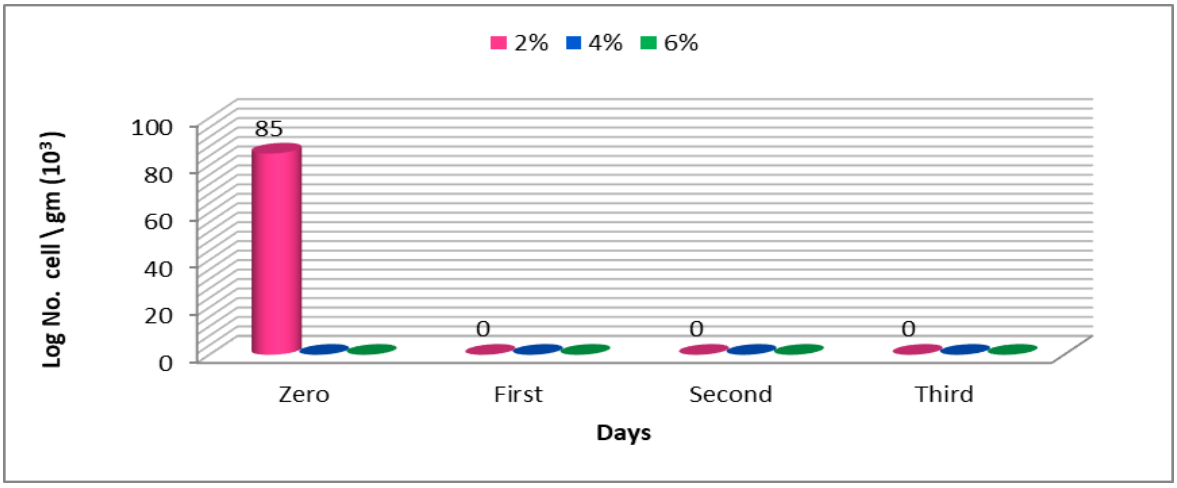

Figure 4. Effect of garlic extract on *H. pylori* using experimental study in chicken meat

Discussion

Helicobacter pylori is one of the highest-frequency human bacterial pathogens worldwide. It is expected that about two-thirds of the world community are infected with *H. pylori*, mainly in developing nations with greater incidence in poor and unsanitary regions. The prevalence and persistence of *H.pylori* in different foods, particularly food of animal origin have been a hot area of investigation during the past decades(Almashhadany,2018; Quaglia and Dambrosio 2018; Sjomina *et al.*, 2018).

Chicken meat is one of the most extensively consumed types of meat everywhere, and it is very important to humans for several reasons. Chicken meat is highly versatile in cooking, making it a popular choice for a wide range of dishes. Also, it is a rich source of high-quality protein, essential amino acids, vitamins, and minerals, such as zinc, iron, and selenium. As well as chicken meat is relatively more affordable compared to other types of meat, besides many other healthy and economic benefits (Marangoni et al., 2015). In another hand, garlic extract has been studied for its probable antimicrobial properties, comprising its ability to inhibit the growth of *H. pylori*. Several components found in garlic, such as allicin, ajoene, and diallyl sulfide, have confirmed antimicrobial activity against *H. pylori* in laboratory studies. However, the confirmation of morphological properties of *H.pylori* is a vital issue, because these properties help the researchers to examine the food regarding the presence or absence of this pathogenic bacteria.

H. pylori is a spiral-shaped, gram-negative bacterium with multiple flagella, which helps it move through the mucus lining of the stomach. Biochemical tests are laboratory procedures that assess the metabolic properties of the bacterium. These tests help differentiate *H. pylori* from other bacteria and confirm its presence (Table 1).

Several studies have reported the survival and presence of *H. pylori* in foods and water, particularly in ready-to-eat products and milk, proposing that they can be sources of infection (Quaglia & Dambrosio, 2018). Food intrinsic factors, such as pH ranging (from 4.9 to 6.0) and water activity (>0.97) could theoretically provide good conditions for *H. pylori* survival. Therefore, data on survival ability may be more significant than concerns about the growth of the bacteria in foods when determining the role of different types of food in *H. pylori* transmission to humans (Quaglia *et al.,* 2007; Quaglia & Dambrosio, 2018; Almashhadany 2018).

Molecular epidemiology studies have detected *H. pylori* DNA in different foodstuffs, water, and animals which suggests the existence of reservoirs for *H. pylori* outside the human gastrointestinal tract (Momtaz *et al.*, 2014; Mousavi *et al.,* 2015). Milk, meat, and vegetables are a potential source of *H. pylori* infections (Herrera, 2004). Milk products are the most studied, probably because the infection is mainly acquired during childhood, and milk is mostly consumed during this period (Al-Mashhadany and Mayass, 2017; Talimkhani & Mashak, 2017).

H. pylori's resistance to antibacterial therapies has become a global concern, with several published research demonstrating that both clinical and foodborne strains of *H. pylori* demonstrated a significant incidence of resistance to different antibacterial treatments (Alexander et al., 2021).

According to Table 2 and Figure 3, no inhibition zone formed around the disc of garlic extract at 2% concentration whereas the inhibition zone around the disc of garlic extract at 4% and 6% concentration was (10 mm and 20 mm) respectively, and in statics when we counted the Pearson correlation, we found it equals 1 this means that there is a strong relationship between the different concentrations and inhibition zone in equal ratios.

In vitro, *H. pylori* is susceptible to garlic extract at a fairly moderate concentration. Garlic (Allium sativum) contains about 2000 biologically active components including volatile, water-soluble, and oil-soluble organosulfur compounds (e.g., Diallyl sulfide (DAS), DADS, Diallyl trisulfide (DATS)), essential oils, dietary fiber, sugars (32%), flavonoids, and pectin (Cerny 2013). According to Shang et al.,2019, garlic contains two antibacterial enzymes: heat-resistant allicin and heat-resistant sulfur compounds, which act against bacteria *Staph aureus*, *E. coli*, and *B.subtilis*, fungus *Penicillium funiculus*, *Candida albicans,* and *H. pylori.* Nikolovski,2018, reports that water garlic extraction can be used to obtain Bioactive compounds such as Allicin (42–50 µg/ml), Allyl sulfide, DADS, Methanethiosulfonik acid S-methyl ester (MMTS).

Our result was in nonagreement with Weerasekera et al.,2008, who found that the 21 plant extracts tested for bactericidal activity against *H. pylori*, (fenugreek, coriander, Bengal quince, nightshade, garlic, black pepper, fennel) did not show inhibition after 60 minutes of incubation under microaerophilic condition. Also inconsistent with Thong-Ngam and Chatsuwan, 2007, who confirmed that there are no inhibition zones found when testing with *Aloe vera*, garlic, and plau-noi, while the inhibition zone diameters of 6.5-8 mm and 10-12 mm were detected at 200 and 400 micrograms curcumin disk, respectively.

Finally, Figure 4 illustrates the effect of garlic extract on *H. pylori* growth and survival in poultry meat polluted experimentally. The count numbers of *H. pylori* were reduced with (2%) concentration of garlic extract from the first moment of the start of the experiment, but in (4%,6%) concentration of garlic no growth occurred since the experiment was started. However, in the study conducted by Al-Mansuri,2011, in Basra governorate, Iraq, they reported the antimicrobial effect of aqueous, alcoholic extract, fresh juice, and powder solution of garlic against six isolates of *H. pylori.* All the extracts gave activity against the tested isolates under a concentration of 400 mg/ml. An aqueous extract gave activity higher than alcoholic extract while the fresh juice gave the highest effect on the tested isolates.

Conclusions

H. pylori occurs in different types of foods and maybe a significant circumstance for its survival and transmission. The frequency of *H. pylori* in raw chicken meat in Dhamar Governorate, Yemen, seems to be high, usually due to socioeconomic status, sanitary habits, poor living conditions, or other risk factors. In contrast, garlic is not only a prevalent component in cooking, but it's also vital in the "food as medicine" holistic health movement because of its supposed wellness benefits. We concluded that garlic extracts gave activity against the tested isolates of H.pylori, particularly an aqueous extract gave activity higher than alcoholic extract.

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