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ORIGINAL ARTICLE

## Study Effectiveness of Some Herbal Extract and Honey on *Helicobacter pylori* Bacteria

Amer A. Hammadi

<sup>1</sup>Department of Clinical Laboratories, College of Applied medical sciences, University of Kerbala, Karbala, Iraq

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#### Corresponding author:

Amer A. Hammadi

Email: [amer.a@uokerbala.edu.iq](mailto:amer.a@uokerbala.edu.iq) Department of Clinical Laboratories, College of Applied medical sciences, University of Kerbala, Karbala, Iraq

#### ABSTRACT

**Objectives:** The aims of current research detection the inhibition efficiency of alcoholic extraction plants *Linum usitatissimum*, *Gcyrrhiza glabra*, *Punica granatum* and honey each one alone and mixed of these plants with honey to investigate their effectiveness antibacterial action against *Helicobacter pylori*.

**Methods:** the solvent utilized is ethanol to obtain of cured extract of plants *L. usitatissimum*, *G. glabra*, *P. granatum* extraction and dilution of honey, which tested the effectiveness on *H. pylori* bacteria to determine the most efficient concentration of solvent optimization, and then was determined minimum inhibitory concentration (MIC) of the extract more efficient.

**Results:** The extraction showed action in vitro against *H. pylori* comparison with the antibiotic this appear in inhibition zone diameter for these pathogenic bacteria in, *G. glabra* in concentration (50, 25 mg/mL) (6.23, 1.33 mm) in arrangement and there is effectiveness in treated with honey in (50, 25, 12.5 mg/mL) (13.93, 7.66, 4.03 mm) and mixed of all plants (50, 25, 12.5, 6, and 3 mg/mL) (40.36, 29.23, 18.66, 10.03, 4.56 mm) in diameter.

**Conclusion:** The current research would help isolate new products/drugs. Results of this work have shown that three plant extracts and honey possess compounds with antimicrobial and antioxidant properties.

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### INTRODUCTION

Steroids and nonsteroids chemical drugs are utilized in inflammation and severe pain. But, with side effects most of them had. Incurrent researches advise using medicinal plants to cure different diseases.<sup>1</sup> Most of these plants have shown favorable results in preventing and treating complexity and diseases, with minimum side effects and low cost. *Linum usitatissimum* L, with the regional name of "Gole Katan" is an annual herbal plant and an oily.<sup>2-3</sup> It is an antioxidant imbalance with disturbance in defens mucosal elements that caused

peptic ulceration. One famous element, peptic ulcer essential causative ones that initiation were alcohol misuse, acute and chronic overwork, the infected of *Helicobacter pylori* is long time utilized for NSAIDs.<sup>4</sup>

For centuries in, traditional Chinese routines utilized Licorice herb (*Glycyrrhiza glabra*). It is reported to be antioxidant two parts of these plant Roots and Rhizomes, antiviral, and antimicrobial. Otherwise, it has anti-cancer, anti-ulcer, and inflammatory activity. From the root of *G. glabra* plant extraction is named licorice and its one vigorous component is

glycyrrhizin acid, hypertension and edema, which result in its affinity for mineralocorticoid receptors might cause. Patients suffering from hypertension and/or cardiovascular disease must be utilized.<sup>5,6</sup>

Classical Iranian medicine *Punica granatum* L. (Lythraceae) for treating gastritis was utilized. It has antiparasitic, antiviral, antibacterial, anti-inflammatory and antioxidant effects recorded for pomegranate peel.<sup>7</sup> In addition, plant could reduce the problems for *H. pylori* antibiotic resistance through raised cell surface hydrophobicity for *H. pylori* bacteria and prevent attachment of the bacteria for the mucosa of gastric region. The evidences is substantially recommended for *H. pylori*-ability prevention effect produces gastric illness through increasing *H. pylori* as well as appeared anticancer and anti-inflammatory effects through *P. granatum*.<sup>8</sup> Reiterated oral administration of *P. granatum* 400 mg/kg reduced the intensity of ethanol-induced stomach deterioration. Because of tannin's presence and phenolic compounds in the pomegranate peel, it has anti-gastric bacteria action.<sup>9</sup>

There was increasing attention for using honey as a "natural" therapy for treating bacterial illness in the last years. Honey has shown effectiveness versus a wide range of microorganisms and records of its prevention effect of specific microbial organisms have been widely authenticated.<sup>10</sup> It is well known for honey positive effect in the wound environment. Honey controlled the moist wound environment that caused recuperation, and its highly stickiness assisted in supplying a protective septum to prevent infection. In Cameroon, it is commonly utilized by several people as antimicrobial properties because it is easily available and cheap. Honey is utilized as an antiseptic to burns and wounds and treated of gastrointestinal complications.<sup>11,12</sup>

## MATERIAL AND METHODS

### Plant substance

Karbala city regional emporium collected dried plants parts of *L. usitatissimum* seed, *G. glabra* root, *P. granatum* Peel, and honey. Then placed plants parts in the oven in 45°C to dry for weeks before transporting and crushing through manual or mechanical to get powder of these plants parts.

### Bacterial isolation

The specimens of dyspepsia patients were investigated for biopsy samples taken from a male and non-diarrheic adult male and female in a survey between 10–80 years old administered the Al- Hussany general hospital, gastrointestinal tract center (G.I.T) Karbala city, Iraq.

From each patient taken biopsy for bacterial culturing first in brain heart in fusion media and after incubation period the tubes growth culturing in selective media for *H. pylori* Columbia horse lysis blood agar, biochemical methods to confirmative identification *H. pylori* bacteria.

### Culture media Preparation

#### Brain Heart Infusion Media

Media prepared through dissolving 37 g of powder to 1 liter of distilled water, boiling to dissolve the media, adding *H.*

*pylori* supplement before sterilizing. This medium was used for preservation of isolates as oxoid company procedure.

#### Columbia Agar

It was prepared by dissolving 39 g of media powder to 1 liter of distilled water, boiling to dissolve the media, adding *H. pylori* supplement before sterilizing and adding 20-30 mL of lysed blood Sheep after cooling as oxoid company procedure for isolated pure bacteria colony.

#### Ethanol Extracted Method

A powder from each plant type was 50 mg taken and mixed with 250 mL of ethanol (70%) ratio 1/5 and placed in the shaker for one night. Through filter paper suspension was filtration and putted in the clean container to evaporation from alcoholic and formed it powder.<sup>13</sup>

#### Well Method Procedure

Columbia r media pouring in petri dish at deepness 4 mm. *H. pylori* spreading on plats for isolation bacteria through cotton swab. Then for 30 min. dry the plats at 37°C and make well in culture plate has (several concentricity of many well utilized of a specific antibiotic) through used a sterilization borer crock for convenient width 10 mm under sterile condition. Specific antibiotic filled the well. Incubation the plates at 37°C to 18–24 hours. The inhibition zone around well has been measured.<sup>14</sup>

## RESULT AND DISCUSSION

Ethanol e extracted in the current study utilized of action components from the Plants *L. usitatissimum*, *G. glabra*, *P. granatum*, and honey. Different dilutions had been used on *H. pylori* bacteria. The antimicrobial action of the plants was evaluated utilizing the agar well diffusion way through diameter measuring of growth inhibited region and its ensuing concentrated is tabulation.

Table 1 showed no effect of *L. usitatissimum* ethanolic plant extract on *H. pylori* bacteria, this appear through diameter of inhibition zone and p-value comparison with the effectiveness of antibiotic and these also appear in Figure 1 represented relation between mean and treatment.

Table 2 showed limited effect on *H. pylori* in plant extract in concentration 50 mg through diameter of inhibition zone and p-value, also the figure 2 showed this fact for *G. glabra* plant extract.

There is no effected of *P. granatum* plant extract shown in Table 3. This appear by diameter of inhibition zone and p-value, and in figure 3.

Honey have more effectiveness against *H. pylori* bacteria in Table 4 through diameter of inhibition zone and p-value has significant relation in concentration (50 mg, 25 mg) comparison with effectiveness of antibiotic for this bacteria, this appears also in Figure 4 in above concentration.

In table 5, the mix of plants extract with honey appeared more effective against *H. pylori* bacteria in all concentrations (50, 25, 12.5, 6 mg) through diameter on inhibition zone of these concentrations and also by p-value appear significance

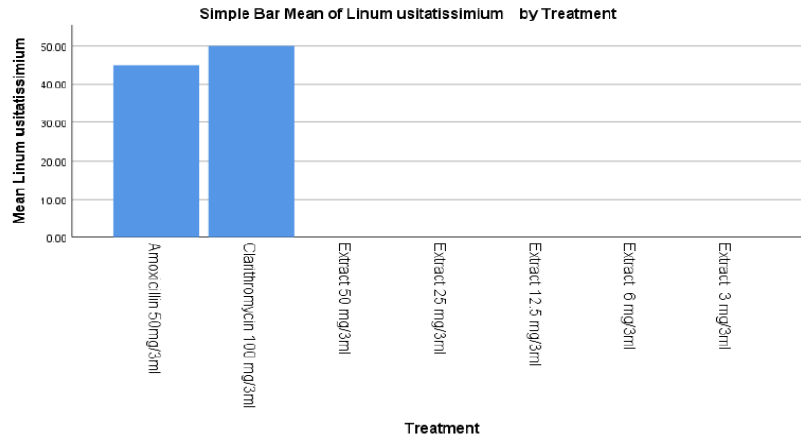


Figure 1: Relation between mean and treatment for *L. usitatissimum*.

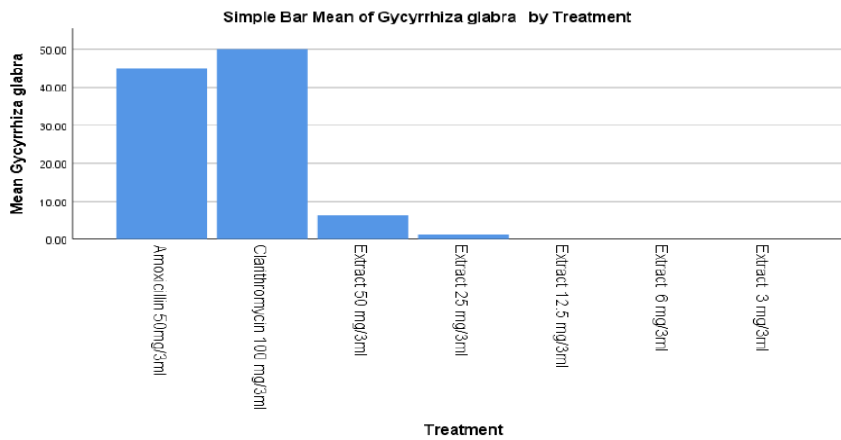


Figure 2: Relation between mean and treatment for *G. glabra*.

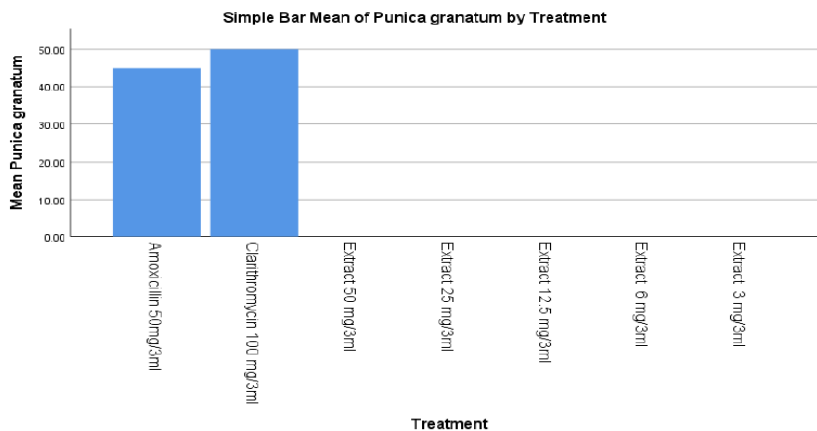


Figure 3: Relation between mean and treatment for *P. granatum*.

variation, and this clearly showed in figure<sup>5</sup> representative relation of mean of treatment and treatment with plant extract.

The unpurified extraction of flax seed lignans caused remarkable anti ulceration effectiveness through recuperation and protection against ulceration in the NSAID- creating

gastric ulceration model in Wistar ranges. Furthermore, anti ulceration possibility of flax seed lignans is compared with the  $\beta$ -carotene. Maintenance of decreased glutathione (GSH) was mechanism of action could be related to flax seed lignans' free radical sweeping action. This advantage of unpurified extract

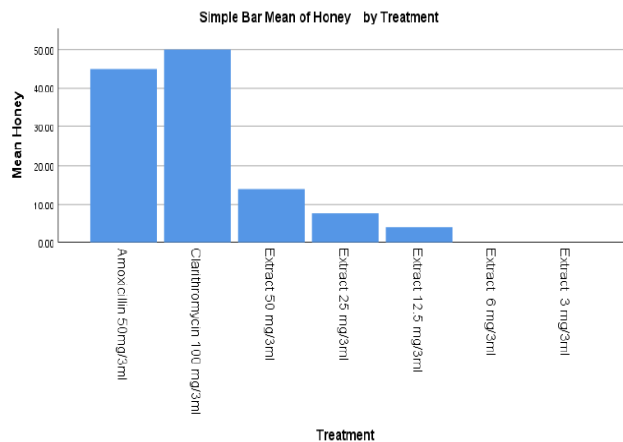


Figure 4: Relation between mean and treatment with honey.

Table 1: *L. usitatissimum* inhibition zone (mm) plant extract effect on *H. pylori* bacteria

Concentration of extract (mg/ml)	Inhibition zone			Mean of concentration	p- value
	1	2	3		
Amoxicillin 50 mg/ 3 mL	45 ± 0.0	42 ± 0.0	48 ± 0.0	45 ± 0.0	00.084
Clarithromycin 100 mg/ 3 mL	59 ± 0.0	43 ± 0.0	48 ± 0.0	50 ± 0.0	00.084
Extract 50 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	00.00
Extract 25 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 0.0	0 ± 0.0	00.00
Extract 12.5 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	00.00
Extract 6 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	00.00
Extract 3 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	00.00
Mean of extract Solvent	14.85 c	12.14 b	13.71 a	13.57 a	

Homogeneous subsets are displayed of means for groups. a. Harmonic Mean Sample Size = 3.000 is using.

Table 2: *G. glabra* inhibition zone (mm) plant extract effect on *H. pylori* bacteria

Concentration of extract (mg/ml)	Inhibition zone			Mean of concentration	p- value
	1	2	3		
Amoxicillin 50 mg/ 3 mL	45 ± 0.0	42 ± 0.0	48 ± 0.0	45 ± 0.0	00.096
Clarithromycin 100 mg/ 3 mL	59 ± 0.0	43 ± 0.0	48 ± 0.0	50 ± 0.0	00.096
Extract 50 mg/ 3 mL	6.6 ± 0.0	5 ± 0.0	7.1 ± 0.0	6.23 ± 0.0	00.062
Extract 25 mg/ 3 mL	0 ± 0.0	4 ± 0.0	0 ± 0.0	1.33 ± 0.0	00.032
Extract 12.5 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	00.00
Extract 6 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	00.00
Extract 3 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	00.00
Mean of extract Solvent	15.8 c	13.42 b	14.72 a	14.65 a	

Homogeneous subsets are displayed of means for groups. a. Harmonic Mean Sample Size = 3.000 is using

of flax seed lignans could be utilized as nutritional support through gastric ulceration.<sup>15</sup>

The influence of *G. glabra* on gastric bacteria extirpation was timing of treatment to beneficent patient's signs of dyspepsia. In clinical research of Raveendra *et al.* esteemed the influence of *G. glabra* versus placebo in function dyspepsia with determined patients' receive *G. glabra* to (1-month) decreased Index of Nepean Dyspepsia and there foreshown best refinement of symptoms comparison to patient's receive placebo.<sup>16</sup>

Sreenivasulu Puram *et al.* research patients' anguish with dyspepsia because of *H. pylori* infected are advised *G. glabra* at a dosage of 120 mg day to sixty days.<sup>17</sup> The responded range is comparison versus placebo utilizing HPSA examine with in days30 and 60days; showed that HPSA examines for day 60 is negative in 56% of licorice population versus 4% with placebo indicating a statistically remarkable differentiation. Licorice antibacterial seems that in dyspepsia appeared in above two research's, in addition its infection nature of *H. pylori*, also has anti-inflammatory influence.<sup>18</sup>

**Table 3:** Punica granatum inhibition zone (mm) plant extract effect on *H. pylori* bacteria

Concentration of extract (mg/ml)	Inhibition zone			Mean of concentration	P-Value
	1	3	3		
Amoxicillin 50 mg/ 3 mL	45 ± 0.0	45 ± 0.0	48 ± 0.0	45 ± 0.0 a	00.084
Clarithromycin 100 mg/ 3 mL	59 ± 0.0	50 ± 0.0	48 ± 0.0	50 ± 0.0 a	00.084
Extract 50 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0 b	00.00
Extract 25 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 0.0	0 ± 0.0 b	00.00
Extract 12.5 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0 b	00.00
Extract 6 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0 b	00.00
Extract 3 mg/ 3 mL	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0 b	00.00
Mean of extract Solvent	14.85 c	12.14 b	13.71 a	13.57 a	

Homogeneous subsets are displayed of means for groups. a. Harmonic Mean Sample Size = 3.000 is using

**Table 4 :** Inhibition zone (mm)of Honey against *H. pylori* bacteria

Concentration of extract (mg/ml)	Inhibition zone			Mean of concentration	P-Value
	1	2	2		
Amoxicillin 50 mg/ 3 mL	45 ± 0.0	42 ± 0.0	45.00 A	45.00	00.109
Clarithromycin 100 mg/ 3 mL	59 ± 0.0	43 ± 0.0	50.00	50.53 B	00.109
Extract 50 mg/ 3 mL	0.2 ± 0.0	16 ± 0.0	00.00	13.93 C	00.06
Extract 25 mg/ 3 mL	7.4 ± 0.0	9.2 ± 0.0	00.00	7.66 C	00.05
Extract 12.5 mg/ 3 mL	5 ± 0.0	2.8 ± 0.0	00.00	4.03 C	00.23
Extract 6 mg/ 3 mL	0 ± 0.0	0 ± 0.0	00.00	0.00 C	00.211
Extract 3 mg/ 3 mL	0 ± 0.0	0 ± 0.0	00.00	0.00 C	00.211
Mean of extract Solvent	16.8 c	16.14 c	13.57 b	17.23 a	

Homogeneous subsets are displayed of means for groups. a. Harmonic Mean Sample Size = 3.000 is using

Pomegranate peel There is no related study detecting anti-ulceration influence but in other researches fruit parts are assessment. Although the precise method of the anti-ulcer action of pomegranate peel has not been clearly delineated, it have some action substance which ulcer protection characters had been detected based on their anti oxidant action. Pomegranate is a rich exporter for polyphenols. It has anti oxidants such as anthocyanins, soluble polyphenols, and tannins which contain antiatherosclerotic characters. Evaluation in vitro of the pomegranate extraction and some choice medicine herbals on *H. pylori* action elucidate that the extraction of pomegranate had anti *H. pylori* action.<sup>19</sup>

The differences in action of the diverse honey at the diverse concentricity have been observed, might also propose that there can be provincial differences in addition, the nature of honey produced to effect the inhibition action as formerly suggested.<sup>20</sup>

Farther for, the differences can be assigned to the existence of additional substances

According to the type of honey produced, bees were able to collect nectar to whatever origin that is obtainable for insect at the time.<sup>21</sup> Proteinacious and Flavonoid substances with high sugar content have also been reported to function in their antibacterial action. In addition, increased range of hydrogen peroxide in honey, or non-peroxide elements which had authenticated to main antimicrobial elements for honey.<sup>22,23</sup>

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