J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 48(3): 819-825, 2024
Doi: 10.33483/jfpau.1438607

ORIGINAL ARTICLE / ÖZGÜN MAKALE

ANTIMICROBIAL AND ANTIBIOFILM ACTIVITIES OF VARIOUS VEGETABLE OILS AGAINST *HELICOBACTER PYLORI*

ÇEŞİTLİ BİTKİSEL YAĞLARIN HELİCOBACTER PYLORİ'YE KARŞI ANTİMİKROBİYAL VE ANTİBİYOFİLM ETKİNLİKLERİ

Elif AYDIN[1]*

[1]Tavsanli Vocational School of Health Services, Kutahya Health Sciences University, 430200, Kutahya, Türkiye

ABSTRACT

Objective: *Vegetable oils have various biologically active components, including antibacterial, antioxidant, and anti-inflammatory properties. These oils help control nausea, vomiting, coughing and gas, as well as diarrhea and dyspepsia. It also helps to reduce stomach bloating and intestinal spasm pain. To sum up, the objective of this investigation was to assess the antibacterial and antibiofilm properties of twelve different vegetable oils against the reference strain of Helicobacter pylori, NTCC 11637.*
Material and Method: *For antibacterial activity, the minimum inhibitory concentration and the agar-well diffusion method were employed, and for antibiofilm activity, the microplate method.*
Result and Discussion: *Vegetable oils showed antimicrobial activity at concentrations of 62.5-15.625 µg/ml and antibiofilm activity at concentrations of 250-15.625 µg/ml. According to our findings, the vegetable oils we utilized may have the ability to form a novel class of Helicobacter pylori inhibitors with anti-H. pylori properties.*
Keywords: *Antimicrobial, antibiofilm, Helicobacter pylori, vegetable oils*

ÖZ

Amaç: *Bitkisel yağlar, antibakteriyel, antioksidan ve anti-enflamatuvar özellikler de dahil olmak üzere çeşitli biyolojik aktif bileşenlere sahiptir. Bu yağlar bulantı, kusma, öksürük ve gazın yanı sıra ishal ve dispepsiyi kontrol etmeye yardımcı olur. Ayrıca mide şişkinliğini ve bağırsak spazmı ağrısını azaltmaya yardımcı olur. Sonuç olarak, bu araştırmanın amacı 12 farklı bitkisel yağın Helicobacter pylori NTCC 11637 standart suşu üzerindeki antimikrobiyal ve antibiyofilm etkinliğini değerlendirmektir.*
Gereç ve Yöntem: *Antimikrobiyal etkinlik için minimum inhibitör konsantrasyon ve agar-kuyu difüzyon yöntemi, antibiyofilm etkinlik için ise mikropleyt yöntemi kullanıldı.*
Sonuç ve Tartışma: *Bitkisel yağlar 62.5-15.625 µg/ml konsantrasyonlarda antimikrobiyal, 250-15.625 µg/ml konsantrasyonlarda ise antibiyofilm etkinlik göstermiştir. Sonuçlarımız, kullandığımız bitkisel yağların, anti-Helicobacter pylori etkileri olan yeni bir Helicobacter pylori inhibitörleri sınıfının potansiyel bir bileşiği olabileceğini gösterdi.*
Anahtar Kelimeler: *Antimikrobiyal, antibiyofilm, bitkisel yağlar, Helicobacter pylori*

* **Corresponding Author / Sorumlu Yazar:** Elif Aydın
e-mail / e-posta: elif.aydin@ksbu.edu.tr, **Phone / Tel.:** +905053553846

Submitted / Gönderilme : 16.02.2024
Accepted / Kabul : 02.05.2024
Published / Yayınlanma : 10.09.2024

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral-shaped, urease-producing, gram-negative, single-flagellated bacterium that was originally identified from a human stomach in 1983. Its size ranges from 2-4 μm to 0.5-0.9 μm. This disease has the ability to infect over 50% of the global population and between 70 and 90% of people living in underdeveloped nations [2,3]. This bacterium causes gastrointestinal issues and is linked to a rapidly growing number of cases of peptic ulcer, gastritis, gastric cancer, and lymphoid tissue lymphoma connected with the gastric mucosa [4,5].

A combination antibiotic therapy including two types of antibiotics (amoxicillin, clarithromycin, levofloxacin, and metronidazole) with one proton pump inhibitor (omeprazole, lansoprazole, and pantoprazole) is used twice a day for seven days to treat *H. pylori*. This is a disease that has a low success rate despite combined treatment, and its treatment has side effects. Applied treatments fail due to age, smoking, increased bacterial load and virulence, adverse drug reactions, antibiotic resistance, and different pharmacokinetic and pharmacodynamic factors [6,7].

New therapeutic treatments or adjunctive therapies are needed to increase the rate of *H. pylori* eradication and reduce the side effects of treatment, and there is a growing interest in agents that can enhance *H. pylori* eradication, such as the use of herbal oils. At such a time, natural product sources such as plant extracts and vegetable oils provide alternative remedies to combat both resistant and non-resistant microbes [8]. The World Health Organization (WHO) states that traditional medicine is the primary source of basic healthcare for most people worldwide. Aromatic and medicinal plants are a valuable source of naturally occurring organic compounds that are frequently utilized in medicine [9]. Vegetable oils are natural, concentrated volatile aromatic compounds (VOCs) isolated from plants. Vegetable oils have some preventive/therapeutic properties such as anti-bacterial, anti-fungal, anti-viral, insecticidal and antioxidant properties. There are many plants that can be used as alternative treatments against antibiotic resistance [10]. The bioactive components of commonly used vegetable oils can have multiple antimicrobial effects, such as altering DNA and RNA synthesis, breaking down bacterial cell walls, disrupting the structure of the cell membrane, changing the levels of fatty acids, phospholipids, and protein translocation, so it is possible to use vegetable oils as antimicrobial agents against harmful microorganisms [11-13].

Studies on the use of plant organisms have accelerated recently due to the numerous adverse effects and treatment failures of synthetic medications used to treat *H. pylori* infection. Thus, this study set out to ascertain the antibacterial and antimicrobial activity of herbal oils derived from widely used medicinal plants on the gastrointestinal pathogen *H. pylori* bacteria.

MATERIAL AND METHOD

Preparation of Vegetable Oils

The 12 vegetable oils used in the study were commercially available (Biotama, Turkey) and are listed in Table 1. The initial concentration of all the different concentrations of oils used was dissolved in dimethyl sulfoxide (DMSO) (1 mg/ml) and filtered through 0.22 μM membrane filters.

H. pylori Culture

National Collection of Type Cultures *Helicobacter pylori* NTCC 11637 standard strain was used in this study. The strain was maintained in Mueller-Hinton Broth (MHB, Himedia, India) containing 20% glycerol and 10% bovine fetal serum (BFS) at -80', 10% bovine fetal serum (FBS, Serox GmbH, Mannheim, Germany), and incubated in a microaerophilic atmosphere with a ready-made kit (CampyGen, Thermo Fisher Diagnostics AG, Pratteln, Switzerland) for 72 hours at 36°C. Following incubation, 5% sheep blood was added to Columbia Agar (Himedia, India), and the mixture was incubated for 72 hours at 36°C in a microaerophilic environment [14].

Determination of Minimal Inhibitory Concentration (MIC) of Vegetable Oils on *H. pylori*

Vegetable oils' antibacterial activity was evaluated using the MIC research method described in the Clinical and Laboratory Standards Institute Manual (CLSI, 2022) [15]. The revived *H. pylori* strain

was adjusted to 1.0 with a McFarland turbidity standard. In a 96-well microplate, 80 µl of MHB supplemented with 10% BFS was applied to each well. Vegetable oil (100 µl) in each well (250-0.98 µg/ml) concentrations were transferred to the first well and diluted two-fold. Finally, 20 uL of a bacterial suspension previously standardized to 1.0 McFarland turbidity standard was dispensed into each well. One well contained only MHB supplemented with 10% BFS for negative control and one well contained bacterial suspension with MHB supplemented with 10% BFS for positive control. All micropellets were incubated for 72 h at 36°C under a 10% CO_2 atmosphere [14]. After incubation, absorbance was measured at 570 nm. The minimum inhibitory concentration (MIC) of H. pylori was determined by comparing its observable growth to that of the control.

Table 1. The names of plants and oils used in this study

	Oils	**Plants**
1	Jasmine oil	*Jasminum nudiflorum*
2	Cumin oil	*Cuminum cyminum*
3	Cinnamon oil	*Cinnamomum verum*
4	Rosemary oil	*Rosmarinus officinalis*
5	Rosehip oil	*Rosa canina*
6	Indian oil	*Ricinus communis*
7	Ylang ylang oil	*Cananga odorata*
8	Jojoba oil	*Simmondsia chinensis*
9	Safflower oil	*Carthamus tinctorius*
10	Cardamom oil	*Elettaria cardamomum*
11	Lily oil	*Lilium candidum*
12	Nettle oil	*Urtica dioica*

Evaluation of Antimicrobial Activity of Vegetable Oils on *H. pylori* by Agar-Well Diffusion Method

On Mueller-Hinton Agar (MHB, Himedia, India) with 5% sheep blood, a 1.0 McFarland turbid standardized bacterial solution was applied. Different oil concentrations ranging from 20, 40, 60, and 80 µl were applied to each well individually, and the wells were then incubated at 36°C for 72 hours in a microaerophilic environment [16]. After incubation, the presence or absence of zones was evaluated.

Evaluation of the Antibiofilm Activity of Vegetable Oils on *H. pylori*

Antibiofilm activity of vegetable oils was continued by optimizing the method used by Yu et al. [17]. In order to examine the potential suppressive impact of vegetable oil on *H. pylori* biofilm development, a 96-well microplate was filled with 80 µl of MHB supplemented with 10% BFS in each well. Two-fold serial dilutions of vegetable oil in the range of 250-0.98 µg/ml were added to the suspended *H. pylori* samples. After incubation under microaerobic conditions at 36°C for 48 h under 10% CO_2 atmosphere, bacterial suspensions were removed. To get rid of any leftover *H. pylori*, the plates were gently washed three times with 200 µl of phosphate-buffered saline (PBS). Subsequently, 200 µl of 1% crystal violet was transferred to the tube and staining was performed for 20 minutes at room temperature. Following staining, three sterile PBS washes were used to remove the stains from the crystal violet. Once the micropattern was flipped over to dry, 200 µl of a 33% glacial acetic acid solution was poured into the tube, dissolving the crystal violet rings for ten minutes. At 570 nm, the optical density of crystal violet was determined.

RESULT AND DISCUSSION

For many years, vegetable oils have been extensively utilized in a variety of industries, including natural treatment, medicine, food preservation, and medical. The scientific study of vegetable oils used in alternative medicine techniques is essential to improving the quality of care provided to patients. The U.S. Food and Drug Administration has approved the oil as safe since it often has no negative side

effects [18]. In addition, it becomes very difficult for microorganisms to gain resistance to oils. The creation of new antibacterial agents through vegetable oils holds great promise [19,20]. In light of all this information, we hypothesized that as an alternative natural treatment for *H. pylori* infection, the 12 known vegetable oils could be used.

Results of the Minimal Inhibitory Concentration (MIC)

The antimicrobial results of a total of 12 vegetable oils used in the study against *H. pylori* NTCC 11637 standard strains are given in Table 2. All oils were effective against *H. pylori* and their concentration ranges were 62.5-15.625 μg/ml. Among the oils, cumin, rosemary and coconut oil were effective at low concentrations (15.625 μg/ml).

Vegetable oils have been discovered to have varying degrees of antibacterial activity against *H. pylori* in the literature currently under publication. The vegetable oils *Eucalyptus globulus, Juniperus communis, Rosmarinus officinalis*, and *Thymus vulgaris* were discovered by Tanalp et al. to have inhibitory effects [21]. In another study, it was reported that five different essential oils extracted from plants showed inhibitory effects on drug-resistant clinical *H. pylori* isolates [22]. Likewise, Ohno et al. observed that thirteen essential oils totally prevented *H. pylori* from growing *in vitro* [23]. In our study, MIC analysis of 12 vegetable oils on *H. pylori* NTCC 11637 standard strain showed that all of them had inhibitory antibacterial effects.

Table 2. Antimicrobial values of vegetable oils on tested microorganisms

	Oils	Minimal Inhibitory Concentrations (μg/ml)	Agar-Well Diffusion (μl)
1	Jasmine oil	62.5	60
2	Cumin oil	15.625	20
3	Cinnamon oil	62.5	20
4	Rosemary oil	15.625	40
5	Rosehip oil	62.5	40
6	Indian oil	15.625	40
7	Ylang ylang oil	62.5	20
8	Jojoba oil	62.5	20
9	Safflower oil	62.5	20
10	Cardamom oil	31.25	60
11	Lily oil	62.5	40
12	Nettle oil	62.5	20

Antimicrobial Activity Results by Agar-Well Diffusion Method

With the agar well technique, like the MIC results, all oils were effective, with cumin, cinnamon, ylang ylang, jojoba, safflower oil and nettle oil being the least effective (20 μl) (Table 2). Jamine and cardamom oil were active at high concentrations (60 μl). Cumin oil was found to be more effective in both the MIC and agar well diffusion techniques.

In a study using the disk diffusion method to examine the antibacterial qualities of vegetable oils against *H. pylori*, researchers found that 30 oils affected growth with different zones of inhibition of 60 essential oils. [24]. Again, Esmaili et al. evaluated the anti-*H.pylori* activities of two different oils by the agar diffusion method and found that they were effective at low concentrations [25]. In our study, all the oils we used by agar well method showed anti-*H.pylori* activity.

Evaluation of the Antibiofilm Activity of Vegetable Oils on *H. pylori*

The inhibitory effect of vegetable oil on *H. pylori* biofilm is shown in Figure 1. Vegetable oil concentrations ranging from 250 μg/ml (2 MIC) to 15.625 μg/ml (MIC) markedly suppressed *H. pylori* biofilm formation. In the antibiofilm activity experiment, Cumin, Cinnamon, Rosemary, Ylang ylang, Jojoba and Nettle oils gave the lowest concentration of 15.625 μg/ml and Rosehip oil gave the highest concentration of 125 μg/ml.

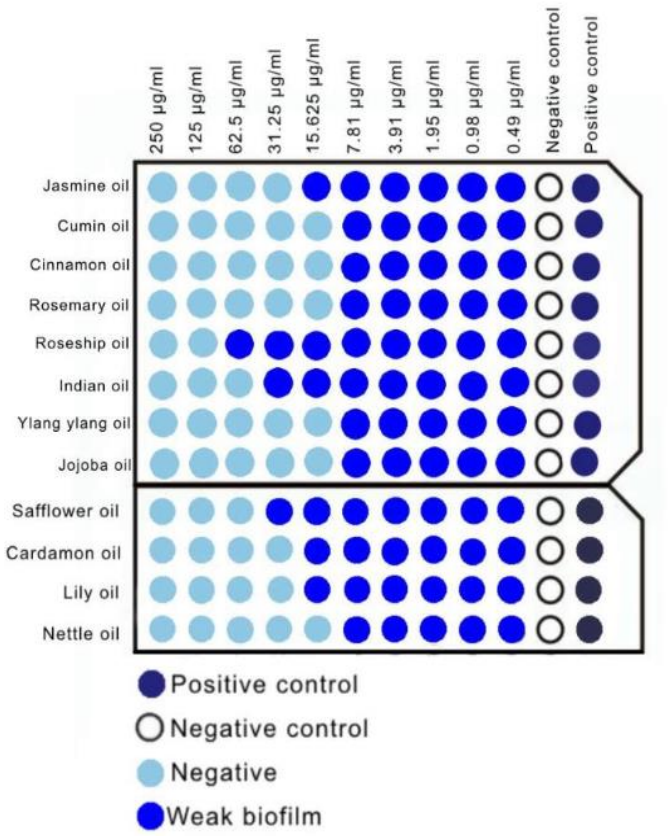

Figure 1. Antibiofilm results

Due to its capacity to create biofilms both within and outside of human hosts, *H. pylori* may offer better protection in arid environments. According to Yu et al., the *H. pylori* NCTC11637 strain's biofilm production was inhibited by Atractylodes lancea volatile oil [26]. Elbestawvy et al. also reported that Eugenol essential oil had an antibiofilm effect on *H. pylori* bacteria at 25 - 50 µg/ml [27]. In this study, we found that all the oils we examined for antibiofilm activity had a significant effect on *H. pylori*.

All these challenges to getting rid of *H. pylori* suggest that other treatments, such as traditional medicine, need to come into play. In this investigation, we investigated the anti-*H. pylori* efficacy of vegetable oils, which we think may be important for the treatment of *H. pylori* , which is difficult due to the fact that 50% of the population is infected and resistance to antimicrobial agents has increased significantly. Taken collectively, our findings imply that vegetable oils, a conventional pharmaceutical substance that is accessible, affordable, and low-cost, would be a potential option for a new class of *H. pylori* inhibitor drugs with anti-*H. pylori* efficacy. We think that more *in vitro*, *in vivo*, and molecular studies investigating antimicrobial mechanisms, especially for drug-resistant isolates, should be carried out in order to be used safely for treatment in humans. In addition, when the literature was reviewed, it was noted that there were not many studies examining the effects of vegetable oils on biofilm formation, and more studies are needed in this regard.

AUTHOR CONTRIBUTIONS

Concept: E.A.; Design: E.A.; Control: E.A.; Sources: E.A.; Materials: E.A.; Data Collection and/or Processing: E.A.; Analysis and/or Interpretation: E.A.; Literature Review: E.A.; Manuscript Writing: E.A.; Critical Review: E.A.; Other: -

CONFLICT OF INTEREST

The author declares that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The author declares that the ethics committee approval is not required for this study.

REFERENCES

1. Warren, J.R., Marshall, B. (1983). Unidentified curved bacilli on gastric epithelium in active chronic gastritis. The Lancet, 321(8336), 1273-1275. [CrossRef]
2. Lü, M., Yu, S., Deng, J., Yan, Q., Yang, C., Xia, G., Zhou, X. (2016). Efficacy of probiotic supplementation therapy for *Helicobacter pylori* eradication: A meta-analysis of randomized controlled trials. PloS One, 11(10), e0163743. [CrossRef]
3. Çekin, A.H., Şahintürk, Y., Akbay Harmandar, F.E.R.D.A., Uyar, S., Yolcular, B.O., Çekin, Y. (2017). Use of probiotics as an adjuvant to sequential *H. pylori* eradication therapy: Impact on eradication rates, treatment resistance, treatment-related side effects, and patient compliance. Turkish Journal of Gastroenterology, 28(1), 3-11. [CrossRef]
4. Rothenbacher, D., Brenner, H. (2003). Burden of *Helicobacter pylori* and *H. pylori*-related diseases in developed countries: Recent developments and future implications. Microbes and Infection, 5(8), 693-703. [CrossRef]
5. Lehours, P., Yılmaz, O. (2007). Epidemiology of *Helicobacter pylori* infection. Helicobacter, 12, 1-3. [CrossRef]
6. Boltin, D. (2016). Probiotics in *Helicobacter pylori*-induced peptic ulcer disease. Best Practice & Research Clinical Gastroenterology, 30(1), 99-109. [CrossRef]
7. Alvi, S., Javeed, A., Akhtar, B., Sharif, A., Akhtar, M.F. (2017). Probiotics for cure of *Helicobacter pylori* infection: A review. International Journal of Food Properties, 20(10), 2215-2222. [CrossRef]
8. Prabuseenivasan, S., Jayakumar, M., Ignacimuthu, S. (2006). *In vitro* antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine, 6(1), 1-8. [CrossRef]
9. Karameşe, M., Özgür, D. (2020). The antibacterial and antifungal activities of commonly used herbal oils. Journal of Experimental and Clinical Medicine, 37(2), 47-51.
10. Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods-A review. International Journal of Food Microbiology, 94(3), 223-253. [CrossRef]
11. Lambert, R.J.W., Skandamis, P.N., Coote, P.J., Nychas, G.J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. Journal of Applied Microbiology, 91(3), 453-462. [CrossRef]
12. Shan, B., Cai, Y.Z., Brooks, J.D., Corke, H. (2007). The *in vitro* antibacterial activity of dietary spice and medicinal herb extracts. International Journal of Food Microbiology, 117(1), 112-119. [CrossRef]
13. Witkowska, A.M., Hickey, D.K., Alonso-Gomez, M., Wilkinson, M. (2013). Evaluation of antimicrobial activities of commercial herb and spice extracts against selected food-borne bacteria. Journal of Food Research, 2(4), 37. [CrossRef]
14. Spósito, L., Oda, F.B., Vieira, J.H., Carvalho, F.A., dos Santos Ramos, M.A., de Castro, R.C., Bauab, T.M. (2019). *In vitro* and *in vivo* anti-*Helicobacter pylori* activity of Casearia sylvestris leaf derivatives. Journal of Ethnopharmacology, 233, 1-12. [CrossRef]
15. Clinical and Laboratory Standards Institute. 2022. Performance standards for antimicrobial susceptibility testing; Thirty-Second Informational Supplement., M100-S32. CLSI, Wayne, PA.
16. Boyanova, L., Gergova, G., Nikolov, R., Derejian, S., Lazarova, E., Katsarov, N., Krastev, Z. (2005). Activity of Bulgarian propolis against 94 *Helicobacter pylori* strains *in vitro* by agar-well diffusion, agar dilution and disc diffusion methods. Journal of Medical Microbiology, 54(5), 481-483. [CrossRef]
17. Yu, H.H., Song, Y.J., Yu, H.S., Lee, N.K., Paik, H.D. (2020). Investigating the antimicrobial and antibiofilm effects of cinnamaldehyde against *Campylobacter* spp. using cell surface characteristics. Journal of Food Science, 85(1), 157-164. [CrossRef]
18. U.S. Food and Drug Administration Web Site Substances Generally Recognised as Safe. Code of Federal Regulations 21 CFR 182.20. Retrieved February 15, 2024, from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=182.20 [Ref list]
19. Ohno, T., Kita, M., Yamaoka, Y., Imamura, S., Yamamoto, T., Mitsufuji, S., Imanishi, J. (2003). Antimicrobial activity of essential oils against *Helicobacter pylori*. Helicobacter, 8(3), 207-215. [CrossRef]
20. Preuss, H.G., Echard, B., Enig, M., Brook, I., Elliott, T.B. (2005). Minimum inhibitory concentrations of herbal essential oils and monolaurin for gram-positive and gram-negative bacteria. Molecular and Cellular Biochemistry, 272, 29-34. [CrossRef]
21. Karaca, T.D., Başak, A.R.U., Çağlar, K. (2023). Evaluation of antimicrobial activities of various herbal oils

against *Helicobacter pylori* and their cytotoxic effects on Huvec cell line. Hacettepe Journal of Biology and Chemistry, 51(1), 103-113. [CrossRef]

22. Ali, S.S., Abd Elnabi, M.K., Alkherkhisy, M.M., Hasan, A., Li, F., Khalil, M., El-Zawawy, N. (2022). Exploring the potential of Cinnamomum zeylanicum oil against drug resistant *Helicobacter pylori*-producing cytotoxic genes. Journal of Applied Biomedicine, 20(1). [CrossRef]
23. Ohno, T., Kita, M., Yamaoka, Y., Imamura, S., Yamamoto, T., Mitsufuji, S., Imanishi, J. (2003). Antimicrobial activity of essential oils against *Helicobacter pylori*. Helicobacter, 8(3), 207-215. [CrossRef]
24. Bergonzelli, G.E., Donnicola, D., Porta, N., Corthésy-Theulaz, I.E. (2003). Essential oils as components of a diet-based approach to management of Helicobacter infection. Antimicrobial Agents and Chemotherapy, 47(10), 3240-3246. [CrossRef]
25. Esmaeili, D., Mobarez, A.M., Tohidpour, A. (2012). Anti-*helicobacter pylori* activities of shoya powder and essential oils of *Thymus vulgaris* and *Eucalyptus globulus*. The Open Microbiology Journal, 6, 65. [CrossRef]
26. Yu, M., Wang, X., Ling, F., Wang, H., Zhang, P., Shao, S. (2019). *Atractylodes lancea* volatile oils attenuated *Helicobacter pylori* NCTC11637 growth and biofilm. Microbial Pathogenesis, 135, 103641. [CrossRef]
27. Elbestawy, M.K., El-Sherbiny, G.M., Moghannem, S.A. (2023). Antibacterial, antibiofilm and anti-inflammatory activities of eugenol clove essential oil against resistant *Helicobacter pylori*. Molecules, 28(6), 2448. [CrossRef]