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In vitro Study of Anti-*Helicobacter pylori* Activity of Honey: A Systematic Review

(Kajian *In vitro* ke atas Aktiviti Anti-*Helicobacter pylori* oleh Madu: Ulasan Sistematik)

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ABSTRACT

This review explores the beneficial effects of honey on Helicobacter pylori*-induced gastric ulcer and duodenal ulcer. The research of the effectiveness of honey on* H. pylori *infection using electronic databases was done, these include Medline via Ovid Medline, Scopus and ScienceDirect published from the year 2000 to 2018. The articles were evaluated and selected based on the criteria which report on the effects of honey on gastric ulcer and duodenum ulcer caused by* H. pylori *infection. There are 53 articles were identified, which stated to the studies that relate to the criteria. Afterwards, nine articles on honey and its extracts were selected and assessed accordingly in this review. All studies reported positive effects of honey on* H. pylori*-induced gastric ulcer and duodenal ulcer. Most of the studies showed at minimum 10% of honey concentration were effective as anti-*H. pylori *activity. This systematic review emphasized the potential of honey used to inhibit the* H. pylori *infection* in-vitro*. Future extensive studies are required to find the active component and molecular mechanism of honey before animal and human observational studies could be conducted to deliver valid evidence.*

Keywords: Duodenal ulcer; gastric cancer; gastric ulcer; gastritis; Helicobacter pylori*; honey*

ABSTRAK

Ulasan ini adalah untuk mengkaji kesan manfaat madu pada Helicobacter pylori *teraruh ulser gaster dan ulser duodenal. Satu kajian terhadap keberkesanan madu pada jangkitan* H. pylori *menggunakan pangkalan data elektronik telah dilakukan, ini termasuklah Medline melalui Ovid Medline, Scopus dan ScienceDirect yang telah diterbitkan dari tahun 2000-2018. Artikel telah dinilai dan dipilih berdasarkan kriteria yang melaporkan mekanisme dan kesan madu pada ulser gaster dan ulser duodenum yang disebabkan oleh jangkitan* H. pylori*. Terdapat 53 artikel telah dikenal pasti yang merujuk kepada kajian yang sepadan dengan kriteria. Seterusnya, sembilan artikel mengenai madu dan ekstrak madu telah dipilih dan dinilai sewajarnya dalam kajian ini. Semua kajian melaporkan kesan positif pada madu terhadap* H. pylori *yang berpunca daripada ulser gastrik dan ulser duodenal. Kebanyakan kajian menunjukkan sekurang-kurangnya kepekatan madu sebanyak 10% berkesan sebagai aktiviti anti-*H. pylori*. Kajian sistematik ini menekankan potensi madu yang digunakan untuk merencatkan jangkitan* H. pylori *secara* in-vitro*. Kajian menyeluruh pada masa akan datang adalah perlu untuk mencari komponen aktif dan mekanisme molekul yang ada pada madu sebelum kajian pengamatan terhadap haiwan dan manusia dijalankan untuk memberi bukti yang sah.*

Kata kunci: Gastritis; Helicobacter pylori*; kanser gaster; madu; ulser duodenal; ulser gaster*

Introduction

Helicobacter pylori can be defined as a type of bacterium that is classified as gram-negative, flagellated, spiral-shaped and it produces large amounts of urease enzyme that neutralizes the pH inside the bacterial cells. *H. pylori* colonize within the gastrointestinal tract and mucosa layer of the stomach lining which causes inflammation and ulcers in the stomach or duodenum (Cui et al. 2012; De Monte et al. 2015; Eusebi et al. 2014). Chronic infections with *H. pylori* can lead to chronic gastritis, peptic ulcers, gastric cancer and mucosa-associated lymphoid tissue lymphoma. Although *H. pylori* infection prevalence had decreased, this bacterium still infects 30% to 50% of the general population (Altman et al. 2008; Malnick et al. 2014). In addition, patients with the severe gastroduodenal disease will have a high rate of *H. pylori* infection (Alfizah et al. 2010). Finding a good treatment for *H. pylori* infection has always been a very challenging course. The first-line regimen to destroy *H. pylori* comprises of a triple therapy which consists of a combination of two of the standard antibiotics (clarithromycin and amoxicillin or metronidazole) plus a proton pump inhibitor (Malfertheiner et al. 2002). However, the effectiveness of the standard triple therapy has their limitation, due to the rise in the occurrence of antibiotic resistance to *H. pylori* and the noncompliance of patients to treatment routine (Alfizah et al. 2014; Gisbert & Calvet 2012; Kanizaj & Kunac 2014).

On the other hand, a lot of *in-vitro* studies on honey have shown positive activities against *H. pylori* infection (Manyi-Loh et al. 2013; Matongo & Nwodo 2014; Ruckriemen et al. 2017). Honey has been used as a food product and traditional medicine for thousands of years to cure a variety of illness because of its healing properties (Allsop & Miller 1996; Estevinho et al. 2008). Nowadays,

its nutritional and medical values were reevaluated by most of the researchers and have been scientifically proven as antimicrobial, anti-inflammatory, antioxidant, anticancer and antidiabetic effects (Erejuwa et al. 2011, 2010; Othman 2012; Sherlock et al. 2010). Honey is also reported to be active in treating numerous form of microbes related disease, comprising gram-positive and negative organisms, aerobic and anaerobic bacteria (Ndip et al. 2007; Zaghloul et al. 2001). In addition, properties of honey such as pH, osmolarity, and mostly it's peroxidase activity that acted as antimicrobial effects (Ghazali 2009; Nasir et al. 2010). The existence of non-peroxidase substances such as phenolic acids, flavonoids, and lysozymes in honey also contribute to their antimicrobial activity (Alnaqdy et al. 2005; Tan et al. 2009). In summary, the purpose of this review is to summarize and to elucidate the importance of honey as an anti-*H. pylori* and to investigate the key components on honey against *H. pylori*-induced gastric ulcer and duodenal ulcer disease. In addition, since patients' prevalence is noncompliance to the treatment regimen is high (Samie et al. 2007; Tanih et al. 2010), it is vital for the world to find a natural and alternative treatment to conquer this matter.

Materials and Methods

Literature search strategy

This systematic review was designed and carried out to analyse related studies of honey as an alternative to *H. pylori* treatment. For the strategy search of health science journals; database from Medline *via* Ovid Medline, Scopus and ScienceDirect were used. The search plan involved in this research review is the combination of keywords: honey, and *Helicobacter pylori* OR *H. pylori*.

Inclusion and exclusion criteria

Every article must fit into the inclusion and exclusion criteria to be selected. Thus, there are some guidelines made specifically for this. The inclusion criteria are: Studies written in English and the title and abstracts were related to the keywords given. Articles were also included if the paper mentioned: Usage of honey as an alternative treatment; *H. pylori* bacteria; and recent articles from the year 2000 to 2018. On the other hand, articles will not be selected if they were review articles, articles written in other languages, combination compound on honey, not related to *in-vitro* study, and duplicate studies.

Data extraction and management

To complete this search review, three reviewers screened the papers and decided for articles selected before the data extraction phase. The data collection were systematized and all data extraction was performed individually. The following data were recorded in this review: name of authors; the type and origin of the honey sample; type of strain *H. pylori*; methods of anti-*H. pylori* activity used; and a brief description of the study results.

Results

Search results

There are 53 collected works found theoretically related to the articles after three self-reliant reviewers screened the articles' result according to the title and abstract. In the total of those 53 articles; 26 are from Ovid, 21 are from Scopus, and 6 from ScienceDirect were saved for further evaluation and data extraction. Then, 44 articles were excluded and the remaining of 9 articles were selected for this systematic review. A flowchart of the selected articles was created to represent the study selected as shown in Figure 1.

Study characteristics

Table 1 shows a summary of the characteristics of the overall study. Based on the types of *in-vitro* studies chosen, nine articles on the studies of honey were included in this review. To be specific, out of nine honey studies, four studies focused on raw natural honey (Abdel-Latif & Abouzied 2016; Kim et al. 2015; Ndip et al. 2007; Nzeako & Al-namaani 2006), two studies examined on extracted honey (Manyi-Loh et al. 2012; Matongo & Nwodo 2014) and three other studies demonstrated the effects of raw honey and its extraction (Kim et al. 2017; Manyi-Loh et al. 2013; 2010)

Effect of honey on *Helicobacter pylori*

Kim et al.(2017) assessed the anti-*H. pylori* activity of Korean Acacia honey extracted with n-hexane, dichloromethane, ethyl acetate, and n-butanol. Results showed inhibition zone diameters of 9.2 ± 5.8 mm at 20% (v/v) concentration of raw Acacia honey. The ethyl acetate extract showed the highest inhibitory activity, with an inhibition zone of 8.5 ± 3.1 mm while the rest of solvent extracts (n-hexane, dichloromethane, and butanol) failed to inhibit the *H. pylori* at 10 μg/mL concentration. Whereas in their previous study in 2015, the authors assessed the antimicrobial activity of selected Korean honey on *H. pylori* strain. Results of raw honey showed at 10% (v/v) concentration, Chestnut honey had the strongest inhibitory activity (3.5±0.7 mm), followed by Linden tree honey (2.5±0.4 mm), Snowbell honey (2.4±0.4 mm), Acacia honey (2.3±0.4 mm), and Mandarin orange honey (1.6±0.6 mm). However, when authors tested honey with catalase (by removing the hydrogen peroxide) using the same honey concentration, they failed to get *H. pylori* inhibitory effect.

Abdel-Latif and Abouzied (2016) investigated the effect of natural honey and its molecular mechanism thru inhibition of nuclear factor-kB (NF-kB) and activator protein-1 (AP-1) activation in gastric epithelial cells induced by *H. pylori*. Manuka honey and other commercial

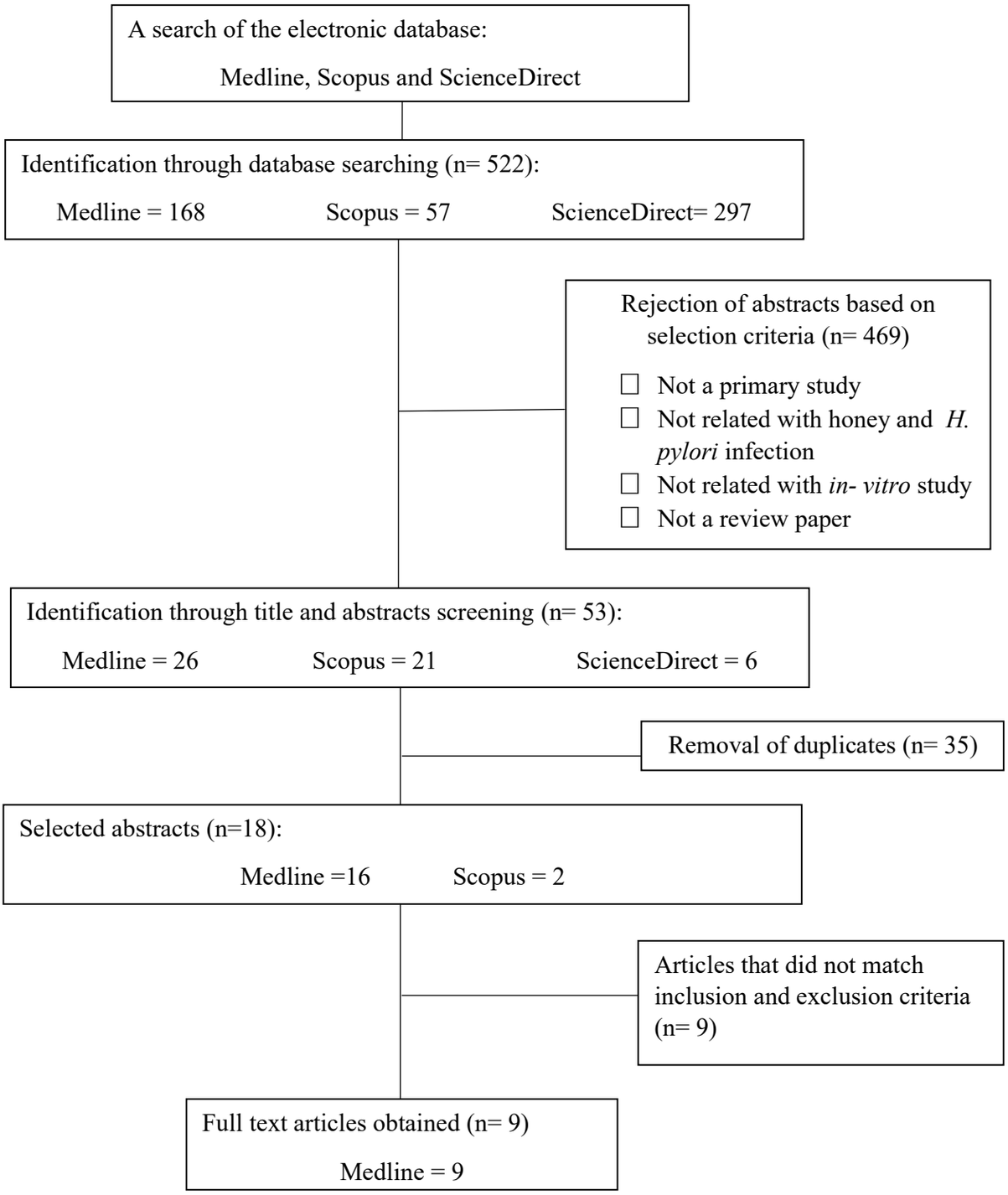

FIGURE 1. Flowchart of selected articles for this review

TABLE 1. Summary of *in-vitro* studies of honey in relations to the antibacterial activity against *Helicobacter pylori*

Reference	Honey sample	*H. pylori* strain	Anti-*H. pylori* activity method	Results
Kim et al. (2017)	Acacia honey from Seoul, Korea was extracted with; N-hexane Dichloromethane Ethyl acetate (EtOAc) N-butanol	*H. pylori* strain ATCC 43526 (OD540 = 0.5)	Agar well diffusion	•Raw honey Inhibition zone diameters of 9.2 ± 5.8 mm at 20% (v/v) concentration • Extract honey At 10 µg/mL concentration, the ethyl acetate extract has the highest inhibitory activity Hexane, dichloromethane, and butanol extracts did not exhibit any activity at this concentration
Abdel-Latif & Abouzie (2016)	20% solutions of different commercial honey brands from Irish: Pure Acacia Boyne Valley Healy's Natural Manuka (from New Zealand)	*H. pylori* strain NCTC 11638 (6×10^8 CFU mL^{-1})	Electrophoretic mobility shift assay (EMSA) Western blotting	• Raw honey Highest inhibition of NF-kB and AP-1 activation by 20% concentration of Manuka honey and other commercialised honey Honey blocked I kappa B alpha (IkB-α) protein degradation and downregulation of cyclooxygenase-2 (COX-2) protein levels at 20% concentration of tested raw honey
Kim et al. (2015)	Five types of honey from Seoul, Korea: Acacia Chestnut Linden tree Mandarin orange Snowbell	*H. pylori* strain ATCC 43526 (OD540 = 0.5)	Agar well diffusion	• Raw honey Chestnut honey showed the highest inhibition zone diameter at 3.5±0.7 mm, followed by Linden tree 2.5±0.4 mm, Snowbell 2.4±0.4 mm, Acacia 2.3±0.4 mm, and Mandarin orange 1.6±0.6 mm at a concentration of 10%
Matongo & Nwodo (2014)	The chloroform extract of Natural honey (HS) from South Africa Diethyl ether extract of Manuka honey (HM) from New Zealand	*H. pylori* strain 369C and ATCC 43526 as the control strain 2.0×108 CFU/mL	*H. pylori* ureases from sonication and measured spectrophotometrically. Calculation of urease inhibition by comparing the NADH oxidation rate	•Extract honey The HS extracted with chloroform shown inhibitory activities 48% and 42%, while HM extracted with diethyl ether shown 45% and 51% inhibitory activity for *H. pylori* urease 369C and ATCC 43526
Manyi-Loh et al. (2013)	Six types of honey from South Africa in concentrations 10, 20, 50 and 75 (%v/v): Pure Honey (PH) Citrus blossom (CB) Goldcrest (GC) Champagne Royal Train (CRT) Honeylene (HL) Heritage (HH)	30 clinical isolated *H. pylori* and ATCC 43526 as control strain (0.5 McFarland standard = 1.8×108 cfu/mL)	Hole plate diffusion	• Raw honey Highest inhibition by PH (16.0±7.5 mm), CB (15.5±8.5 mm) and GC (13.7±10.0 mm) honey at 75% (v/v) concentration CRT (12.7±8.6 mm, 56.7%), HL (13.0±9.2 mm, 63.3%) and HH (13.1±9.2 mm, 60.0%) honey produced the highest inhibition at 50% (v/v) concentration • Extract honey All honey extracts demonstrated inhibition range from (14.5-22.2 mm) mean zone diameter CRT diethyl ether extract (22.2±6.1 mm) was the most active honey extracts N-hexane extract (15.8±7.9 mm) in PH honey produced less antibacterial activity

Manyi-Loh et al. (2012)	N-hexane extract of Goldcrest honey from South Africa	*H. pylori* strain PE93A and ATCC 43526 as the control strain (108 CFU/ mL)	Broth microdilution test	• Extract honey 5 mg/mL was the lowest MIC_{50} (best activity) exhibited by GCF3 GCCL, GCF2 and GCF4 also produced the same inhibition at 7.5 mg/ mL
Manyi-Loh et al. (2010)	Three types of honey from South Africa was diluted with sterile distilled water at concentrations 10, 20, 50 and 75 (%v/v): Pure honey (PH) Citrus blossom (CB) Goldcrest (GC) honey extracts: N-hexane, Diethyl ether Chloroform Ethyl acetate	30 clinical strains *H. pylori* and NCTC11638 as the control strain (0.5 McFarland standard = 1.8×108 CFU/mL)	Agar well diffusion technique	• Raw honey All honey tested were most active at 75% v/v PH (16.0±7.5 mm) had the greatest inhibitory effect followed by CB (15.5±8.5 mm) and GC (13.7±10.0 mm) At 20% (v/v) and 10% (v/v) concentrations, Goldcrest and Pure had the weakest activity • Extract honey All honey extracts produced ranged 15.8-18.8 mm zone of inhibition Diethyl ether extract had the strongest inhibitory activity N-hexane extract had the weakest antibacterial effect
Ndip et al. (2007)	Four types of honey were diluted with sterile distilled water at different concentrations of 10, 20, 50 and 75% (v/v): Manuka from New Zealand Capillano from Australia Eco from Kenya Mountain from Cameroon	15 isolated *H. pylori* and NCTC 11638 as the control strain (0.5 McFarland standard = 1.5×108 CFU/mL)	Disk diffusion assay	• Raw honey At 75% (v/v) concentration, Mountain honey (82.22%) has the strongest inhibitory activity, followed by Capillano and Manuka honey (75.56%) and Eco-honey (73.36%)
Nzeako & Al-namaani (2006)	Eight commercial honey from Oman: Black Forest Langnese Forest Langnese Natural Bee Black Forest Blossom Bee Al-Shifa Natural Al-Nada Clove Al-Nada Chestnut	5 isolates of *H. pylori* and *Staphylococcus aureus* NCTC 6571 as the control organism (1×104 CFU/mL)	Agar well diffusion method	• Raw honey All honey samples produced growth inhibition zones but when diluted to 1:2-1:8 produced different zone sizes. Black Forest honey produced the highest growth inhibition zone followed by Langnese Forest honey and Langnese natural bee honey at 1:2 dilution

honey brands at 20% concentration showed the highest inhibition of NF-kB and AP-1 activation. Similarly, at 20% concentration of tested raw honey showed inhibition of I-kappa-B-alpha (IkB-α) protein degradation and downregulates *cyclooxygenase-2* (COX-2) protein levels in *H. pylori*-induced infected cells. Matongo and Nwodo (2014) evaluated *H. pylori* urease inhibition by honey fractions took from different geographical areas. They found that the inhibitory activities of Natural honey (HS) extracted by Chloroform against *H. pylori* urease (369C and ATCC 43526) were 42% and 48% whereas the inhibitory activities of Manuka honey (HM) extracted by diethyl ether were 45% and 51%, respectively.

Manyi-Loh et al. (2013) evaluated 30 clinical isolates of *H. pylori* on six local honey and its solvent extracts (n-hexane, diethyl ether, chloroform and ethyl acetate). The six local kinds of honey at the various concentration exhibited a variable level of antibacterial activity. The utmost inhibitory activities of raw honey when tested at 75% (v/v) concentration was PH (16.0±7.5 mm, 73.3%), followed by CB (15.5±8.5 mm, 70.0%) and GC (13.7±10.0 mm, 66.7%), whilst at the concentration of 50% (v/v), CRT (12.7±8.6 mm, 56.7%), HL (13.0±9.2 mm, 63.3%) and HH (13.1±9.2 mm, 60.0%) were utmost active. When the authors tested the anti-*H. pylori* activity with solvent extracts, all extracted honey (PH, GC, CRT and HL) demonstrated from 14.5-22.2 mm and 53.3-93.35% inhibitions. The highest activity of honey solvent extracts was diethyl ether in CRT honey (22.2±6.1 mm, 93.3%) while n-hexane extract in PH honey (15.8±7.9 mm, 53.3%) has the least antibacterial activity.

On the other hand, in 2012, the same authors did determine the fraction of GC n-hexane extract to find the minimum inhibitory concentrations (MIC) against *H. pylori* strain. Results of extracted honey showed that antibacterial assay of MIC_{50} values ranged from 5 -10 mg/mL depending on the fractions and the isolates. 5 mg/mL was the lowest MIC_{50} (best activity) shown by Goldcrest mobile phase fractions three (GCF3). However, Goldcrest chloroform fraction (GCCL), Goldcrest mobile phase fractions two (GCF2) and Goldcrest mobile phase fractions four (GCF4) showed same inhibitory against the test isolates at 7.5 mg/mL (Manyi-Loh et al. 2012). The same authors in 2010 also evaluated anti-*H. pylori* activity of three South African honey at four different concentrations and used organic solvents to extract the antimicrobial components. The raw honey showed the utmost active concentration at 75% (v/v), whereas PH (16.0±7.5 mm, 73.33%) has the greatest inhibitory activity, followed by CB (15.5±8.5 mm, 70%) and GC (13.7±10.0 mm, 66.7%). Whereas at 10% and 20% (v/v) concentrations, GC and PH have the least. The susceptibility testing of all solvent honey extracts inhibits the strains with the zone of inhibition 15.8-18.8 mm. Diethyl ether (18.8±8.3 mm, 70%) showed the highest extract, while n-hexane (15.8±7.9 mm, 53.3%) extract was the least. The chloroform (16.9 ± 6.6 mm, 63.3%) and ethyl acetate (16.3 ± 7.6 mm, 60%) extract also has great with inhibitory activities (Manyi-Loh et al. 2010).

Ndip et al. (2007) evaluated the antimicrobial potential of four various raw honey against clinical isolates of *H. pylori*. The findings showed that the four kinds of honey selected exhibited antibacterial activity at different concentrations were not significant. At the concentration of 75% v/v, Mountain honey produced the highest inhibition activity at 82.22%, followed by Capillano® and Manuka™ honey at 75.56%, and Eco honey at 73.36%. Nzeako and Al-namaani (2006) assessed the isolates *H. pylori* on the antibacterial potential of eight samples of commercial honey (Black Forest, Langnese Forest, Langnese Natural Bee, Black Forest, Blossom Bee, Al-Shifa Natural, Al-Nada Clove and Al-Nada Chestnut). All raw honey samples without dilution produced growth inhibition zones of *H. pylori* with different zone sizes when diluted to 1:2–1:8. Honey Black Forest produced the highest growth inhibition zone (19 mm), then the Langnese Forest honey (17 mm) and lastly the Langnese natural bee honey (11 mm) at 1:2 dilution. Overall, most of the authors used agar well diffusion method (Dastouri et al. 2008) as an antibacterial mechanism of honey. The *H. pylori* strain were spread plated onto Columbia Agar plates and were allowed to dry for 3-5 min. After that, using sterile cork borer of 6 mm diameter, five wells were punched in each agar plate. Lastly, each of 100 µL of honey solution at different concentrations was filled in the wells and incubated at 37ºC under microaerophilic conditions. The test was carried out in triplicate and observed after 2 to 5 days. The antimicrobial activity was measured in zone inhibition of *H. pylori*, obtained in mean diameter (mm) around each well.

Discussion

This systematic review shows advantageous *in-vitro* study findings on the effects of numerous honey samples as an alternative treatment on *H. pylori*-induced gastric ulcer. The differences in honey preparations and honey concentrations made it difficult to compare the results generated from the studies and reviews. In this review, six studies demonstrated the effect of raw honey from different sources and shown that they are efficacious in inhibiting the growth of *H. pylori* (Abdel-Latif & Abouzied 2016; Kim et al. 2017; 2015; Manyi-Loh et al. 2010; Ndip et al. 2007; Nzeako & Al-namaani 2006). There are various reports on the antibacterial activities of natural honey, that is ascribed to its acidity, osmolarity, hydrogen peroxide content, and phytochemical components (Mannina et al. 2015). Raw honey that exists in the beehive is a natural sweetener, which is being processed without adding heat. It has fructose and glucose as the main component, followed by water, ash, proteins and amino acids and trace amounts of enzymes, vitamins and other components such as phenolic compounds. However, there were companies who fraudulent the honey. They manufactured the syrups made to imitate the honey (Molan 1996).

Four studies showed the inhibition of *H. pylori* with crude honey at a minimum of 10% concentration. Both studies of Kim et al. (2017, 2015) documented anti-*H.*

pylori activity at a concentration as low as 10% (v/v) in their preliminary screening study of crude Korean acacia honey and five different floral types of crude honey. Authors suggested that the hydrogen peroxide content was responsible for the inhibitory effect of five different floral kinds of honey from Korea (Kim et al. 2015). Manyi-Loh et al. (2010) also documented that their three locally produced honey exhibit antibacterial action on local strains of *H. pylori* from concentrations as low as 10% (v/v). Similarly, Ndip et al. (2007) observed that honey at 10% concentration inhibited the growth of *H. pylori* isolates. This corroborates the previous *in-vitro* finding, Ali et al. (1991) documented at 10 - 20% of natural honey concentrations had an inhibitory effect against *H. pylori*. In another preliminary screening study done by Manyi-Loh et al. (2013) of six crude honey varieties obtained at various concentrations, it showed anti-*H. pylori* activity from 11.0 mm -16.0 mm inhibition zones. Nzeako and Al-namaani's (2006) experiment observed that the highest antibacterial activity was natural Black Forest honey, then Langnese Forest honey and Langnese natural bee honey at 1:2 dilution. These authors studies outcome agrees with many earlier researchers on observation of *in-vitro*, in which honey own the antibacterial activity against *H. pylori* (Ali et al. 1991; al Somal et al. 1994; McGovern et al. 1999; Molan 2001; Osato et al. 1999). Although all of the honey selected in this review demonstrated anti-*H. pylori* activity, there was a dissimilarity in the percentage of susceptibilities of the bacteria *H. pylori* to the variant types of honey tested. This dissimilarity has been ascribed to the floral sources and plant species scavenged by the bees to harvest the honey (Yao et al. 2003). Moreover, in view of the source of floral honey, its placement in various geographical distribution areas might expect to show a discrepancy and this will result in biological activities due to variable climatic environments in different regions or countries (Basson & Grobler 2008).

H. pylori inhabit on gastric cell causes mucosal inflammation and gastric cancer through many pathways by the transcription factors activation including nuclear factor-kB and activator protein-1 activity which modulates several cellular processes for the period of inflammation and carcinogenesis (Lamb & Chen 2010; Seo et al. 2004). Therefore, Abdel-Latif and Abouzied (2016) conducted a study which found that honey at 5% concentrations inhibits nuclear factor-kB and activator protein-1 with the maximum inhibition at 20% concentrations. Remarkably, blockage of nuclear factor-kB and activator protein-1 activities with Shaw's acacia, Boyne Valley, and Healy's natural honey are as effective as Manuka honey at similar concentrations of 10% - 20%. In corroboration with the authors' results, Hussein et al. (2013, 2012) stated that Gelam honey from Johor, Malaysia suppressed the nuclear translocation and activation of nuclear factor-kB and decreased the cytosolic degradation of IkB-α with a continuous decreasing of the inflammatory mediators COX-2 and TNF-α in both acute and severe inflammation rat model.

Honey comes from the plant source and is able to be extracted with organic solvents. The active component of honey is frequently low but compact after the extraction process which resulted in higher activities (Yaoa et al. 2005). This review also demonstrated five studies of the various types of extracted honey (Kim et al. 2017; Manyi-Loh et al. 2013, 2012, 2010; Matongo & Nwodo 2014), which showed noble potentials of inhibition of *H. pylori*. Kim et al. (2017) showed that among the organic solvent fractions tested on honey, those obtained from the ethyl acetate extract showed the highest activity against *H. pylori*. Meanwhile, Manyi-Loh et al. (2013) demonstrated that the CRT honey extracted by diethyl ether was the most potent against bacterial activity in the test isolates. In addition to the author's previous study in 2010 on the extraction of the most active honey (Pure honey), the highly active extract was diethyl ether extract. There was evidence of rising of inhibition zone diameter of the extracts that showed more activity compared to crude honey. Authors suggest that after extraction, the honey components of antimicrobial were concentrated (Manyi-Loh et al. 2010). These two studies done by Manyi-Loh et al. (2013, 2010) agrees with previous researchers that the solvent extracts of honey had significant antibacterial activity against gram-negative bacteria (Abhishek et al. 2010).

In comparison with the previous Manyi-Loh et al. (2012) study, they demonstrated anti-*H. pylori* activities of the extracted GC honey with n-hexane solvent fractions that gave the difference in antimicrobial activity, the best activity was demonstrated by GCF3 with the MIC50 value of 5 mg/mL against both isolates. On the other hand, the GC or MS analysis of this fraction found out many ingredients that are identified as the antioxidant and have the antimicrobial capability, which has been formally stated by means of another researcher (Jerkovic et al. 2009). This may suggest that because of the interaction of all the phytoconstituents in GCF3 that may lead to the antibacterial activity of the fraction and not acetic acid only. Moreover, Matongo and Nwodo (2014) evaluated the *H. pylori* urease inhibition by honey fractions showed the potential activity of the crude urease extract is higher than the purified form. Authors speculate that the crude urease might have rendered when associated with other molecules, the susceptibility of honey extracts will gain more inhibitory activity. Honey had plenty of mechanisms that can kill the microbes, its hydrogen peroxide content showed to be the main component that acts as an antibacterial effect. Additionally, the previous study also has shown that organic solvents can extract the non-peroxidase components of honey (Molan & Russell 1988). Apart from that, in the aspect of the clinical setting, honey might contribute to eliminating *H. pylori* infection. In a study by Boyanova et al. (2015), results showed reduced in prevalence of *H. pylori* infection in honey consumer of 150 dyspeptic patients. When patients consuming honey more than one day weekly, there were positivity result showed by 50.6% lower compared with 70.8% of the remainder.

STRENGTH AND LIMITATION OF THIS REVIEW

The increase of antibiotics resistance in the treatment of *H. pylori* has been one of the major worldwide community health issues. It is a challenging problem since the ideal treatment of *H. pylori* infection still stays a critical issue even though it has been studied widely. The natural product might be useful as an alternative treatment or as an adjuvant. Thus, studies on the effects of honey on *H. pylori* infection have shown some promising results in terms of their antimicrobial and antioxidant properties. To add, the benefit of this review is the focus on the effects of honey, which may be beneficial as alternative medications against *H. pylori* infection. Still, there are limitations identified when conducting research on honey and *H. pylori*. Honey preparations and sources used were not standardized. The honey was collected from different variable climate environments. The difference between preparations and sources may produce different effects on the inhibition of *H. pylori*, making the generalization of results and outcome difficult. Apart from that, since there was variants type of *H. pylori* strains, selection of *H. pylori* also need to be standardized, for instance, *H. pylori* strain ATCC 43526 (Kim et al. 2017) versus *H. pylori* strain NCTC 11638 (Abdel-Latif & Abouzied 2016). This is because these strains were usually collected based on their local preparation and availability. Lastly, the technique of examining the antimicrobial of *H. pylori* also was different from the selected studies (Manyi-Loh et al. 2012; Matongo & Nwodo 2014).

Recommendations

Honey might be among the antibacterial agents to treat *H. pylori* infection. Unlike refined sugars, honey is safe to consume for diabetic patients. It is advantageous in substituting honey for refined carbohydrates as these results were validated from the previous study (Johnson et al. 2007; Vallianou 2014). Although there are many promising numbers of research studies on natural treatment, further extensive research is needed to quicken the steps for the new nutraceutical drug development. Furthermore, honey usage needs to be standardized to allow direct evaluation and analysis of the studies. Thus, a more well-designed study should be conducted on animal and human which focuses on therapeutic effects related to honey as one of the future alternative treatment or prevention of *H. pylori* infection.

Conclusion

The evidence in this systematic review showed the validity of honey tested has antibacterial properties by inhibiting the growth of *H. pylori* infection. The extraction of a honey constituent with *in-vitro* anti-*H. pylori* activity deserves further exploration in animal and human trials, and may eventually become clinically useful. Therefore, this review can be a guideline or benchmark to continue the research of honey properties to achieve the dietary substance for the improvement of anti-*H. pylori* infection.

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