Key lime (*Citrus aurantifolia*) inhibits the growth of triple drug resistant *Helicobacter pylori*

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**Abstract**

**Background:** Eradication rate for *Helicobacter pylori* (*H. pylori*) has decreased due to antibiotic resistance. Therefore, new strategies are needed to enhance *H. pylori* eradication, especially for *H. pylori* with high antibiotic resistance. The objective of this study was to evaluate anti-*H. pylori* activities of constituents from key lime (*Citrus aurantifolia*) and their possible inhibitory effects on urease activity of *H. pylori*.

**Methods:** *Helicobacter pylori* strain ATCC 43526 and triple drug resistant (TDR) *H. pylori* strains were used in this study. Urease activities of *H. pylori* strains were measured by ammonia colorimetric quantification using ELISA reader. Minimum inhibitory concentrations were determined by agar dilution method for antibiotics and by modified media dilution method for each constituent of *Citrus aurantifolia* (*C. aurantifolia*).

**Results:** *Citrus aurantifolia* extract decreased the number of colonies of *H. pylori* strain ATCC 43526 and TDR *H. pylori* stains. An increasing concentration of *C. aurantifolia* extract attenuated urease activities of *H. pylori* strain ATCC 43526 and TDR *H. pylori* strains. Among constituents of *C. aurantifolia*, citral and 4-hexen-3-one were found to be able to inhibit the growth of *H. pylori* strain ATCC 43526 and TDR *H. pylori* strains. Furthermore, citral and 4-hexen-3-one inhibited urease activities of *H. pylori* strain ATCC 43526 and TDR *H. pylori* strains in a dose-dependent manner.

**Conclusion:** *Citrus aurantifolia* has antimicrobial effect on TDR *H. pylori* strains, suggesting that *C. aurantifolia* might have therapeutic potential to control antibiotic-resistant *H. pylori* strains that cause eradication failure using other antibiotics.
report has shown that phytochemical constituents of citrus peels possess biological activities, including anticancer, immunostimulation, and antigenotoxic effects [13]. Oranges, lemons, limes, grapefruit, and tangerines are well-known examples of citrus fruits. *Citrus aurantifolia* (*C. aurantifolia*), also known as key lime, is one of widely consumed citrus fruits in many cultural cuisines and juice production. It has antibacterial activities against *Mycobacterium tuberculosis*, *Staphylococcus aureus*, and others. Among various constituents of *C. aurantifolia*, citral, 4-Hexen-3-one, oleic acid, and palmitic acid have been found to possess antibacterial activities [14–19]. However, it is currently unclear whether *C. aurantifolia* and its constituents have anti-*H. pylori* activities. Therefore, the objective of this study was to evaluate anti-*H. pylori* activities of *C. aurantifolia* and its constituents and their possible inhibitory effects on urease activity of *H. pylori*.

**Methods**

**Key lime (*C. aurantifolia*) extraction**

Slices of *C. aurantifolia* were dried in a constant drying oven (VS-4150ND, VISION SCIENTIFIC, Daejeon, Korea) at temperature of 50 °C. Dried *C. aurantifolia* slices were mixed with liquid nitrogen and ground into fine powders using a mortar and pestle. Powders of *C. aurantifolia* (1 g) were then dissolved in 30 ml of sterile distilled water and incubated at room temperature for 24 h. Dissolved *C. aurantifolia* was filtered using a 0.45 µm pore syringe filter (Corning, NY 14831-001, USA). Twofold serial dilutions of *C. aurantifolia* extract (original concentration, 34 mg/ml) were made with distilled water (1:1 to 1:1024).

We used 4-hexen-3-one, oleic acid, and palmitic acid as constituents of *C. aurantifolia* to determine their antimicrobial activities and inhibitory effects on urease activity of *H. pylori* [14, 19]. For each constituent [citral (Sigma-Aldrich #W230316, USA), 4-hexen-3-one (Sigma-Aldrich #H13001, USA), oleic acid (Sigma-Aldrich #O1008, USA), and palmitic acid (Sigma-Aldrich #P0500, USA)], we prepared the following concentrations: 1, 2, 5, 10, 50, 100, 200, 400, 500, and 1000 µg/ml.

**Helicobacter pylori** strain ATCC 43526 and triple drug resistant (TDR) *H. pylori* strains

We used standard *H. pylori* strain (ATCC® CRL-43526™, USA) and TDR *H. pylori* strains isolated from gastric antrum and body from 18 patients with gastric epithelial neoplasm. Methods of isolation and culture for *H. pylori* were the same as those described in our previous study [20].

**Antimicrobial susceptibility testing**

We stored *H. pylori* strains at −80 °C. After thawing and culture of standard *H. pylori* strain and 18 TDR *H. pylori* strains, we measured minimum inhibitory concentrations (MICs) by agar dilution method for antibiotics and by modified media dilution method for *C. aurantifolia* extract and each constituent of *C. aurantifolia*. We made agar plates using Muller Hinton agar containing 5% sheep blood (Hanilcomed, Korea), 1% IsoVitalex (BD Biosciences), and one of the following drug concentrations for MIC assay: 2–32 µg/ml of metronidazole, 0.25–4 µg/ml of clarithromycin, 0.125–2 µg/ml of amoxicillin and levofloxacin, and 1–16 µg/ml of tetracycline. All antibiotics used in this investigation were purchased from Sigma (St. Louis, MO, USA) except clarithromycin which was obtained from Abbott Laboratories (Abbott Park, IL, USA). We added 10 ml of agar solution into 100 π plate and then cooled down. *H. pylori* strain ATCC 43526 (Manassas, VA USA,) was used as a quality control organism. Antibiotic concentrations used in this study were based on cutoff levels related to Laboratory Standards Institute (CLSI) clinical breakpoints for resistance. All MICs were interpreted using CLSI breakpoints. Antibiotic resistance was defined as follows: amoxicillin, MIC ≥ 0.5 µg/ml; clarithromycin, MIC > 1.0 µg/ml; metronidazole, MIC > 8 µg/ml; tetracycline, MIC > 4 µg/ml; and levofloxacin, MIC > 1 µg/ml.

We tested MIC for *C. aurantifolia* and four constituents of *C. aurantifolia*. We mixed 6 × 10⁸ CFU/ml *H. pylori* in twofold serial dilutions of *C. aurantifolia* extract (34 mg/ml–33.2 µg/ml, 1:1 to 1:1024) or in serial concentrations of its four constituents (1–1000 µg/ml), respectively. These mixtures of *H. pylori* with *C. aurantifolia* extract or its four constituents (5 µl each) were dropped immediately onto agar plates. We determined MIC levels of *C. aurantifolia* extract and each constituent based on invisible *H. pylori* colony on the agar plate after 7 days of incubation.

**Urease activity inhibition test**

We harvested *H. pylori* in 0.9% saline and then prepared mixtures of 6 × 10⁸ colony forming units (CFU)/ml of *H. pylori* with two-fold serially diluted solution of *C. aurantifolia* extract (1:1 to 1:1024). We prepared 6 × 10⁸ CFU/ml of *H. pylori* with citral, 4-hexen-3-one, oleic acid, or palmitic acid in the following concentrations: 10, 50, 100, 200, 400, 500, and 1000 µg/ml. *H. pylori* strains with each constituent were incubated at room temperature for 10 min. We used 0.9% saline as a control. We added each *H. pylori* strain (6 × 10⁸ CFU/ml) in 5 into 200 µl of the following mixture: 1.5% urea (Bioshop, Canada Inc.) and 0.1% EDTA with 0.02% cresol red solution (Bioshop,
Canada Inc.). The mixture ratio was 2:1. The reaction was incubated at room temperature for 20 min. After that, we measured urease activity at absorbance of 590 nm using a VersaMax™ ELISA reader (MOLECULAR DEVICES, Silicon Valley, CA, USA) [21, 22]. Urease inhibition test for each *H. pylori* strain was repeated three times.

**Statistical analysis**
Analysis of variance (ANOVA) was used to determine whether there were any statistically significant differences in urease activity depending on the concentration of *C. aurantifolia* extract and its constituents. Urease activities were shown as mean ± standard deviation (SD). All reported *P* values were two-sided and *P* < 0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS software, version 23 (IBM Corp, Armonk, NY, USA).

**Results**

**TDR *H. pylori* strains**
According to MIC data of clarithromycin, metronidazole, and levofloxacin for *H. pylori* in our previous study [20], TDR *H. pylori* strains were all resistant to clarithromycin, metronidazole, and levofloxacin. Results are summarized in Table 1.

**Effect of *C. aurantifolia* extract on growth and urease activities of *H. pylori* strain ATCC 43526 and TDR *H. pylori* strains**
First, we evaluated the effect of *C. aurantifolia* extract on the growth of standard *H. pylori* strain ATCC 43526 and TDR *H. pylori* strains. We observed visible growth of *H. pylori* mixed with *C. aurantifolia* after twofold serial dilution (1:1 to 1:1024) on agar plate after 7 days of inoculation. The number of visible colonies of *H. pylori* strain ATCC 43526 and TDR *H. pylori* strains was decreased in the presence of *C. aurantifolia* extract compared to that in the control without the presence of *C. aurantifolia* extract (Fig. 1a). We measured urease activities of *H. pylori* strain ATCC 43526 and TDR *H. pylori* strains mixed with *C. aurantifolia* extract at each dilution. Results for their inhibitory effects on urease activities of *H. pylori* strain ATCC 43526 and TDR *H. pylori* strains at each concentration of *C. aurantifolia* extract are shown in Fig. 1b. With increasing concentration of *C. aurantifolia* extract, higher attenuation of urease activity of *H. pylori* was observed (*P* < 0.001, Fig. 1c, Table 2). *H. pylori* strains ATCC 43526 treated with *C. aurantifolia* extract at dilution of 1:64 showed 18.77 ± 1.74% of urease activity compared to that of the control whereas TDR *H. pylori* strains treated with *C. aurantifolia* extract at dilution of 1:128 showed 2.62 ± 0.05% of urease activity compared to that of the control (Table 2).

### Table 1 Triple drug resistant *Helicobacter pylori* and antimicrobial activities of four components of *Citrus aurantifolia*

| Strain no. | MIC (μg/ml) |
|------------|-------------|
|            | Citral | 4-Hexen-3-one | Oleic acid | Palmitic acid | Clarithromycin | Metronidazole | Levofloxacin |
| TDR 1      | 5–10  | 20–50       | R         | R         | R             | R             | R           |
| TDR 2      | 10–20 | 50–100      | R         | R         | R             | R             | R           |
| TDR 3      | 5–10  | 20–50       | R         | R         | R             | R             | R           |
| TDR 4      | 10–20 | 100–200     | R         | R         | R             | R             | R           |
| TDR 5      | 10–20 | 50–100      | R         | R         | R             | R             | R           |
| TDR 6      | 10–20 | 100–200     | R         | R         | R             | R             | R           |
| TDR 7      | 100–200 | 100–200 | R       | R         | R             | R             | R           |
| TDR 8      | 5–10  | 20–50       | R         | R         | R             | R             | R           |
| TDR 9      | 10–20 | 20–50       | R         | R         | R             | R             | R           |
| TDR 10     | 2–5   | 5–10        | R         | R         | R             | R             | R           |
| TDR 11     | 5–10  | 50–100      | R         | R         | R             | R             | R           |
| TDR 12     | 2–5   | 20–50       | R         | R         | R             | R             | R           |
| TDR 13     | 10–20 | 20–50       | R         | R         | R             | R             | R           |
| TDR 14     | 5–10  | 20–50       | R         | R         | R             | R             | R           |
| TDR 15     | 10–20 | 20–50       | R         | R         | R             | R             | R           |
| TDR 16     | 5–10  | 20–50       | R         | R         | R             | R             | R           |
| TDR 17     | 2–5   | 20–50       | R         | R         | R             | R             | R           |
| TDR 18     | 2–5   | 20–50       | R         | R         | R             | R             | R           |
| ATCC 43526 | 2–5   | 20–50   | R         | R         | S             | R             | S           |

*No.* number, *MIC* minimum inhibitory concentration, TDR triple drug resistant, *R* resistant, *S* sensitive, ATCC 43526 *Helicobacter pylori* strains ATCC 43526
Effect of citral, 4-hexen-3-one, oleic acid, and palmitic acid on growth and urease activities of \( H. pylori \) strain ATCC 43526 and TDR \( H. pylori \) strains

We evaluated effects of constituents from \( C. aurantifolia \) on the growth of standard \( H. pylori \) strain ATCC 43526. We found visible growth of \( H. pylori \) colony treated with low dose of citral (\( \leq 2 \mu g/ml \)) and low dose of 4-hexene-3-one (\( \leq 20 \mu g/ml \)) on agar plate after 7 days of inoculation. Citral above concentration of 5 \( \mu g/ml \) persistently stopped the growth of \( H. pylori \) (MIC, 2–5 \( \mu g/ml \), Fig. 2a). 4-hexen-3-one above concentration of 50 \( \mu g/ml \) persistently stopped the growth of \( H. pylori \) (MIC, 20–50 \( \mu g/ml \), Fig. 2b). However, oleic acid or palmitic acid showed no effect on the growth of \( H. pylori \) strain ATCC 43526.

We measured effects of citral, 4-hexen-3-one, oleic acid, and palmitic acid on urease activities of standard \( H. pylori \) strain ATCC 43526. With increasing concentration, 4-hexen-3-one showed higher attenuation effects on urease activity of \( H. pylori \) strain ATCC 43526 (\( P<0.001 \)). \( H. pylori \) strain ATCC 43526 treated with 4-hexen-3-one at concentration of 10 \( \mu g/ml \) had urease activity of 37.11% compared to the control (\( P=0.006 \), Fig. 3a, Table 3). With increasing concentration of citral, higher attenuation of urease activity of \( H. pylori \) strain ATCC 43526 was achieved (\( P<0.001 \)). \( H. pylori \) strain ATCC 43526 treated with citral showed decreased urease activity depending on the concentration used (\( P<0.001 \)). \( H. pylori \) strain ATCC 43526 treated with citral at 100 \( \mu g/ml \) showed urease activity of 52.67% compared to the control (\( P=0.002 \), Fig. 3a, Table 3). However, palmitic acid or oleic acid showed no inhibitory effect on urease activity of \( H. pylori \) strain ATCC 43526 (Fig. 3a, Table 3).
Antimicrobial activities of four constituents from *C. aurantifolia* against TDR *H. pylori* strains are shown in Table 1. Oleic acid or palmitic acid showed no antimicrobial effect on TDR *H. pylori* strains. However, citral and 4-hexen-3-one inhibited the growth of TDR *H. pylori* strains.

We measured effects of citral, 4-hexen-3-one, oleic acid, and palmitic acid on urease activities of TDR *H. pylori* strains. With increasing concentration, 4-hexen-3-one showed higher attenuation effects on urease activity of TDR *H. pylori* strains (*P* < 0.001). TDR *H. pylori* strains treated with 4-hexen-3-one at concentration of 10 µg/ml had urease activity of 40.48% compared to the control (*P* = 0.007, Fig. 3b, Table 3). With increasing concentration of citral, higher attenuation of urease activity of TDR *H. pylori* strains was achieved (*P* < 0.001). TDR *H. pylori* strains treated with citral showed decreased urease activity depending on the concentration used (*P* < 0.001). TDR *H. pylori* strains treated with citral at concentration of 100 µg/ml showed urease activity of 17.74% compared to the control (*P* < 0.001, Fig. 3b, Table 3). However, palmitic acid or oleic acid showed no inhibitory effect on urease activities of TDR *H. pylori* strains (Fig. 3b, Table 3).

**Discussion**

Our present study showed that *C. aurantifolia* extracts could inhibit urease activity of antibiotic-susceptible *H. pylori* strain and TDR *H. pylori* strains in vitro in a dose-dependent manner. Among constituents of *C. aurantifolia*, citral and 4-hexen-3-one showed dose-dependent inhibition of urease activities of antibiotic-susceptible *H. pylori* strain and TDR *H. pylori* strains. Furthermore, citral and 4-hexen-3-one showed inhibitory effects on the growth of antibiotic-susceptible *H. pylori* strain and TDR *H. pylori* strains.

*Helicobacter pylori* eradication rates have decreased while their resistance rates to antibiotics have increased. To improve eradication rates of *H. pylori*, alternative treatments such as antibiotics combined with plant extracts, probiotics, curcumin, honey, and antioxidants

### Table 2 Inhibition effect of *Citrus aurantifolia* extract on urease activities of *Helicobacter pylori* strains ATCC 43526 and triple drug resistant *Helicobacter pylori*

| Dilution titer of *citrus aurantifolia* | ATCC 43526 Urease activity (%) | TDR Urease activity (%) |
|----------------------------------------|---------------------------------|-------------------------|
| Control                                | 100                             | 100                     |
| 1/1024                                 | 98.3                            | 67.6                    |
| 1/512                                  | 103.0                           | 28.0                    |
| 1/256                                  | 71.8                            | 31.5                    |
| 1/128                                  | 36.6                            | 2.6                     |
| 1/64                                   | 18.8                            | 2.7                     |
| 1/32                                   | 17.0                            | 2.7                     |
| 1/16                                   | 17.6                            | 2.6                     |
| 1/8                                    | 16.9                            | 2.6                     |
| 1/4                                    | 16.1                            | 2.7                     |
| 1/2                                    | 15.2                            | 2.7                     |
| 1                                      | 14.9                            | 2.8                     |

ATCC 43526 *Helicobacter pylori* strains ATCC 43526, TDR triple drug resistant

**Fig. 2** Effect of citral (a) and 4-hexen-3-one (b) on the growth of standard *H. pylori* strain ATCC 43526. Small spot without convexity and translucency is only trace of drop. Citral had inhibitory effect on standard *H. pylori* strain ATCC 43526, with MIC of 2–5 µg/ml. 4-Hexen-3-one also had inhibitory effect on standard *H. pylori* strain, with MIC of 20–50 µg/ml.
have been suggested [8]. Previous study has shown that lime juice concentrates have good inhibitory effects on both Gram-negative and Gram-positive bacterial strains, with MIC in the range of 12.5–50 μg/ml [23]. Another study has demonstrated that hexane extract of fruit peels of C. aurantifolia exhibits inhibitory effect against antimicrobial resistant M. tuberculosis strains, with MIC in the range of 25–50 μg/ml [24]. Among constituents from C. aurantifolia, palmitic acid, linoleic acid, oleic acid, 4-hexen-3-one, and citral are active against M. tuberculosis strains [14, 25]. We selected four available constituents (palmitic acid, oleic acid, 4-hexen-3-one, and citral) from C. aurantifolia that showed antimycobacterial activity. In our study, C. aurantifolia extract decreased the number of H. pylori ATCC 43526 colonies and TDR H. pylori colonies. Constituents of C. aurantifolia also showed inhibitory effects against H. pylori strain ATCC 43526, with MIC of citral at 5–10 μg/ml and MIC of 4-hexen-3-one at 20–50 μg/ml. Furthermore, citral showed inhibitory effects against 18 H. pylori strains with triple drug resistance. Its MIC ranged from 2 to 100 μg/ml. In addition, 4-hexen-3-one showed inhibitory effects against 18 H. pylori strains with triple drug resistance. Its MIC was in the range of 20–200 μg/ml.

Biglar et al. have shown that C. aurantifolia can inhibit the activity of Jack-bean urease (IC_{50} = 28 μg/ml) [26]. Our study also showed that C. aurantifolia extract could inhibit urease activity of H. pylori at dilution of 1:64 to 1:1. Among constituents from C. aurantifolia, citral and 4-hexen-3-one showed dose-dependent inhibitory effects on urease activity of H. pylori. It is known that H. pylori can neutralize acid in its environment by producing urease which breaks down urea in the stomach to carbon dioxide and ammonia. These chemicals then react with strong acids in the gastric environment to produce a neutralized area around H. pylori [27]. Previous animal study has shown that H. pylori is unable to colonize at gastric

**Table 3 Inhibition effect of citral, 4-hexen-3-one, oleic acid, and palmitic acid on urease activities of Helicobacter pylori strain ATCC 43526 and triple drug resistant Helicobacter pylori**

| Concentration (μg/ml) | Citral Urease activity (%) | 4-Hexen-3-one Urease activity (%) | Oleic acid Urease activity (%) | Palmitic acid Urease activity (%) |
|-----------------------|-----------------------------|----------------------------------|------------------------------|----------------------------------|
|                       | ATCC 43526                  | TDR                              | ATCC 43526                   | TDR                              | ATCC 43526                   | TDR                              |
| 0                     | 100                         | 100                              | 100                          | 100                              | 100                          | 100                              |
| 10                    | 62.48                       | 105.09                           | 37.11                        | 40.48                            | 99.29                        | 109.25                           | 100.83                           | 98.38                            |
| 50                    | 52.67                       | 120.07                           | 25.74                        | 4.97                             | 99.27                        | 116.37                           | 104.65                           | 80.02                            |
| 100                   | 45.04                       | 17.74                            | 18.47                        | 4.27                             | 98.58                        | 109.69                           | 104.51                           | 85.17                            |
| 200                   | 35.23                       | 17.29                            | 7.35                         | 4.44                             | 98.08                        | 121.82                           | 103.16                           | 84.76                            |
| 400                   | 29.70                       | 17.86                            | 6.67                         | 4.45                             | 99.61                        | 111.19                           | 105.19                           | 81.76                            |
| 500                   | 29.65                       | 16.39                            | 6.53                         | 4.92                             | 99.30                        | 78.19                            | 105.11                           | 87.00                            |
| 1000                  | 14.75                       | 17.26                            | 6.38                         | 5.14                             | 98.90                        | 72.97                            | 84.51                            | 47.34                            |
mucosa with normal physiological pH in urease-negative mutant piglet [28]. Recently, another study has demonstrated that bacterial load is decreased within 5–7 days in a urease knockout infection mouse model [29]. Urease expression is required for establishing initial colonization and maintaining chronic infection [2, 29]. In the present study, *C. aurantifolia* extract and its constituents showed inhibitory effects on urease activity of *H. pylori*, suggesting that they might have potential as adjuvants to enhance *H. pylori* eradication.

In this study, we did not show the association between antibacterial effect and inhibition of urease activity. Bactericidal effect of *C. aurantifolia* may affect the growth of *H. pylori* colonies, leading to inhibition of urease activity and vice versa. Although low dose of *C. aurantifolia* extract showed no obvious effect on the growth of *H. pylori*, it showed inhibitory effect on urease activity of *H. pylori*. Further studies are needed to evaluate the mechanism involved in the antibacterial effect of *C. aurantifolia* and the causal association between its inhibition of urease activity and bactericidal effects.

In conclusion, *C. aurantifolia* and its constituents attenuated urease activities of *H. pylori* strains. Citral and 4-hexen-3-one had antimicrobial effects on *H. pylori* strains with triple drug resistance, suggesting that *C. aurantifolia* might have potential as a therapeutic agent to control *H. pylori* strains that cause eradication failure with other antibiotics. Future studies are needed to evaluate the efficacy and toxicity of *C. aurantifolia* in vivo.

**Authors’ contributions**

CHP: study concept and design; analysis and interpretation of data; drafting and finalizing the manuscript; study supervision. SML: Carrying out the experiment; analysis and interpretation of data; drafting the manuscript. SYP: Carrying out the experiment; EAC, CHU, HSK, SKC, and JSR: Patient recruitment and care. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

**Availability of data and materials**

We allow the use of data and materials.

**Consent for publication**

All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

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**References**

1. Backert S, et al. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2016;21(Suppl 1):19–25.
2. Periti P, et al. Managing Helicobacter pylori infection in the new millennium: a review. J Chemother. 1999;11(sup4):3–53.
3. De Francesco V, et al. Worldwide *H. pylori* antibiotic resistance: a systematic J Gastroenterin Liver Dis. 2010;19(4):409–14.
4. Kolek运z S, et al. Prospective multicentre study on antibiotic resistance of Helicobacter pylori strains obtained from children living in Europe. Gut. 2006;55(12):1711–6.
5. Su P, et al. Antibiotic resistance of Helicobacter pylori isolated in the Southeast Coastal Region of China. Helicobacter. 2013;18(4):274–9.
6. Hunt R, et al. Helicobacter pylori in developing countries. World gastroenterology organisation global guideline. J Gastroenterin Liver Dis. 2011;20(3):299–304.
7. Lee JW, et al. Prevalence of primary and secondary antimicrobial resistance of Helicobacter pylori in Korea from 2003 through 2012. Helicobacter. 2013;18(3):206–14.
8. Takeuchi H, et al. Natural products and food components with anti-Helicobacter pylori activities. World J Gastroenterol. 2014;20(27):8971–8.
9. Krishnamurthy P, et al. Helicobacter pylori containing only cytoplasmic urease is susceptible to acid. Infect Immun. 1998;66(11):5060–6.
10. Smoot DT, et al. Helicobacter pylori urease activity is toxic to human gastric epithelial cells. Infect Immun. 1990;58(8):1992–4.
11. Weeks DL, et al. A H+-gated urea channel: the link between Helicobacter pylori urease and gastric colonization. Science. 2000;287(5452):482–5.
12. Bae JM, Kim EH. Dietary intakes of citrus fruit and risk of gastric cancer incidence: an adaptive meta-analysis of cohort studies. Epidemiol. 2013;38:e2016034 (eCollection 2016).
13. Diab KA. In vitro studies on phytochemical content, antioxidant, anticancer, immunomodulatory, and antigenotoxic activities of lemon, grapefruit, and mandarin citrus peels. Asian Pac J Cancer Prev. 2016;17(7):3559–67.
14. Sandoval-Montemayor NE, et al. Chemical composition of hexane extract of Citrus aurantifolia and anti-Mycobacterium tuberculosis activity of some of its constituents. Molecules. 2012;17(9):11173–84.
15. Mahadwar G, et al. Swarm motility of Salmonella enterica serovar Typhimurium is inhibited by compounds from fruit peel extracts. Lett Appl Microbiol. 2015;60(4):334–40.
16. Matewele P. The effect of electromagnetic field on antimicrobial activity of lime oil. J Microbiol Methods. 2010;83(2):275–6.
17. Surnier Z, et al. Cytotoxic and antibacterial activity of the mixture of olive oil and lime cream in vitro conditions. Afr J Tradit Complement Altern Med. 2013;10(4):137–43.
18. Liu Y, Huying E, Tanumihardjo SA. History, global distribution, and nutritional importance of citrus fruits. Compr Rev Food Sci Food Saf. 2012;11(6):530–45.
19. Park CS, et al. Pretreatment antimicrobial susceptibility-guided vs. clarithromycin-based triple therapy for Helicobacter pylori eradication in a region with high rates of multiple drug resistance. Am J Gastroenterol. 2014;109(10):1595–602. https://doi.org/10.1038/ajg.2014.222 Epub 2014 Aug 5
20. Minshahi F, et al. Omeprazole may exert both a bacteriostatic and a bactericidal effect on the growth of Helicobacter pylori (NCTC 11637) in vitro by inhibiting bacterial urease activity. J Clin Pathol. 1998;51:220–4.
21. David J, et al. Helicobacter pylori rof is required for arginine usage and acid protection in vitro but is not essential for colonization of mice or for urease activity. J Bacteriol. 1999;181:7314–22.
22. Bang CS, Baik GH. Attempts to enhance the eradication rate of Helicobacter pylori infection. World J Gastroenterol. 2014;20(18):5252–62.
23. Olkeh EI, et al. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. Food Sci Nutr. 2015;4(1):103–9. https://doi.org/10.1002/fsn3.268 (eCollection 2016 Jan).
25. Camacho-Corona Mdél R, et al. Activity against drug resistant-tuberculosis strains of plants used in Mexican traditional medicine to treat tuberculosis and other respiratory diseases. Phytother Res. 2008;22(1):82–5.

26. Biglar M, et al. A preliminary investigation of the jack-bean urease inhibition by randomly selected traditionally used herbal medicine. Iran J Pharm Res. 2012;11(3):831–7.

27. Eaton KA, et al. Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. Infect Immun. 1991;59(7):2470–5.

28. Eaton KA, Krakowka S. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by *Helicobacter pylori*. Infect Immun. 1994;62(9):3604–7.

29. Debowski AW, et al. Helicobacter pylori gene silencing in vivo demonstrates urease is essential for chronic infection. PLoS Pathog. 2017;13(6):e1006464. https://doi.org/10.1371/journal.ppat.1006464 (eCollection 2017 Jun).