Advanced trends in controlling *Helicobacter pylori* infections using functional and therapeutically supplements in baby milk

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Abstract *Helicobacter pylori* is a common human pathogen infecting about 30 % of children and 60 % of adults worldwide. It is responsible for diseases such as gastritis, peptic ulcer and gastric cancer. *H. pylori* treatment based on antibiotics with proton pump inhibitor, but therapy failure is shown to be higher than 20 % and is essentially due to an increasing in prevalence of antibiotic-resistant bacteria, which has led to the search for alternative therapies. In this study, we discuss the usage of natural extracts mixture as alternative or complementary agents in controlling *H. pylori* infection so here, we focused on the plant extracts of (Cloves, Pepper, Cumin, Sage, Pomegranate peel, Ginger, Myrrh and Licorice). To that end,

Phytochemical constituents detection like Tannins, Glycosides, Alkaloids, Flavonoids, Terpenoids, Saponins, Phenolic compounds, Reducing sugars, Volatile oils, Amino acids and Proteins was demonstrated. Each plant extract was examined individually or in combination for its antimicrobial activity against *H. pylori*. Out of the used extracts, four mixes were prepared and tested against *H. pylori*. The antibacterial activities of the four mixes, represented by the diameter of inhibition clear zone, recorded 21, 39, 23 and 28 mm. The most potent mix (mix2) was chosen and mixed with baby milk as a new combination for *H. pylori* infections treatment in babies.

Keyword *Helicobacter pylori* · Herbal plants · Phytochemical · Antimicrobial · Cytotoxicity · Baby milk

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Introduction

Helicobacter pylori (*H. pylori*) is a gram negative curved rod with a tuft of 4–7 polar flagella that permit it to penetrate the mucous layer and so has a good ability to induce gastric mucosal inflammation, which may progress into peptic ulcers (Dunn et al. 1997). It also has been associated with gastritis, gastric adenocarcinoma, and gastric mucosa-associated lymphoid type (MALT) B-cell lymphomas (Bakri 2013). *H. pylori* is a common unique bacterium among pathogens that can persist in the acidic environment of the human stomach. The human stomach can offer an acidic medium that help approximately 7 % of *H. pylori* genome to be expressed (Merrell et al. 2003). A large number of reports have been produced on *H. pylori* and showed that it is able to infect at least 50 % of the world's population (Perry et al. 2006) especially in their early life (Sabbi et al. 2008) and persists throughout the rest of their lifetime. According to (Melake et al. 2012; De Giacomo

et al. 2002) 63.3–70 % of children were infected by *H. pylori* at/before 14 and 15 of age revealing high rate of infection at the first and second decades. The most common route of transmission is person-person contact, by either the oral-oral route or the fecal-oral route (Brown 2000). The other routs include the water and food as a probable vehicles and sources of infection (Vale and Vitor 2010). The treatment of *H. pylori* passed through primary followed by secondary eradication therapy. The primary one based on triple therapy using amoxicillin, clarithromycin and proton pump inhibitor (Kubodera et al. 2012). After a while, it was discovered that this kind of treatment is incomplete and so the era of second eradication therapy was started. In this one, metronidazole (MTZ) is used in the place of clarithromycin where the resistance of *H. pylori* to clarithromycin increased more and more (Kubodera et al. 2012). This why there is strong recommendations for using nutritional therapy and phyto-therapy to improve health and treat diseases depending on their presumed safety and their potential nutritional and therapeutic effects (Pandey et al. 2011).

Our study aimed to using combinations of medicinal plant extracts with safety doses for controlling *H. pylori* infection. The aim also extended to the improvement of baby milk nutritional value using newly modified baby milk powder-plant extract combinations.

Material and methods

Bacteria and culture conditions

Helicobacter pylori with accession number KF233428, kindly provided from city of scientific research and technology applications, was cultivated on Columbia blood agar media (Oxoid, England) supplemented with 10 % sterile calf serum. The plates were incubated under micro-aerobic conditions with 5 % CO₂ at 37 °C for 72 h.

Plant materials and extraction method

The herbal plants (clove, pepper, cumin, sage, pomegranate peel, ginger, myrrh and licorice) were purchased from spices shop at Alexandria, Egypt. Plant materials were washed, air dried, ground and extracted as following: the hot water extract was prepared via boiling of 5 g of each plant individually in 100 ml distilled water with occasional shaking at 100 rpm. After 2 days of incubation, the obtained extracts were centrifuged at 2.147×g for 30 min followed by filtration using filter papers. The extracts were then lyophilized at -50 °C (Telstar Model 50, Spain) and the obtained powder was weighed and dissolved in d. H₂O with certain known concentrations in mg/ml as follows (clove 50.6, pepper 60, cumin 104.4, sage

102.4, pomegranate peel 120, ginger 66, myrrh 100 and licorice 72.2).

The capability of each individual extract to inhibit *H. Pylori* growth was tested by agar diffusion assay according to (Hosseinejad et al. 2011) with some modifications. Briefly, about 100 µl of *H. pylori* overnight culture (10⁶ CFU/ml) was spread over Colombia agar media plates supplemented with 10 % calf serum using sterile cotton swabs. The agar was perforated to form 9 mm well using sterile cork porer then, 100 µl of each plant extract was poured into the formed wells. The plates were then incubated under specific conditions of 5 % CO₂ and 37 °C for 72 h. Finally, the diameter of the formed clear zones was measured and recorded in millimeter.

Preparation and antibacterial activity of mixes

To figure out the activity of different extracts combinations against *H. pylori*; four different mixes of 4, 5 and 8 plants were prepared and tested as shown in Table 1. Each individual lyophilized extract was weighed in milligrams, dissolved in 50 ml d. H₂O and mixed randomly with another chosen extracts with a final concentration of mg/ml for each plant. The finally obtained mixes were tested against *H. pylori* using agar diffusion method according to (Hosseinejad et al. 2011). In order to select the most potent mix that inhibits *H. pylori* growth, the formed clear zones diameters were measured in mm. In order to determine minimum inhibitory concentration (MIC); different concentrations of mix2 in mg/ml were tested against the bacteria using the agar well diffusion method as previously described. After incubation, the clear zones were measured and the MIC of mix2 was recorded.

Antibacterial activity of mix2-baby milk combination

To investigate the antibacterial activity of mix2 with baby milk against *H. pylori*, different concentrations of mix2 were added to baby milk (Nactalia 2, France, purchased from local

Table 1 Preparation of different mixes of random combinations of the eight tested plant extracts

Plants	Concentration (mg/ml)			
	Mix1	Mix2	Mix3	Mix4
Cloves	12.65	10.12	6.325	10.12
Pepper	15	–	7.5	12
Cumin	26.1	–	13.05	20.88
Sage	25.6	–	12.8	20.48
Pomegranate peel	–	24	15	24
Ginger	–	13.2	8.25	–
Myrrh	–	20	12.5	–
Licorice	–	14.44	9.025	–

market in Alexandria, Egypt) in percentages of 12.5, 25, 50 and 100 % where each concentration for each individual plant was calculated in mg/ml (Table 2). The prepared positive, negative and tested milk-mix combinations were all tested against *H. pylori* as mentioned above. After 72 h of incubation at 30 °C, the formed clear zones diameters were measured.

Cytotoxicity assay

Human blood lymphocytes separation

Peripheral blood mononuclear cells (PBMCs) were isolated, as reported by (Lohr et al. 1995).

Neutral red assay for toxicity

Every herbal extract must exert toxicity on cells after usage of certain concentration. So, firstly, in order to determine the nontoxic dose of the extract on normal cells, neutral red assay was done. To that end, peripheral blood mononuclear cells (PBMC) were chosen as a modeling for cytotoxicity assay. Briefly, pre seeded PBMC were treated with serial dilutions of the herbal mixture beginning with 20 mg/ml and ending with 0.156 mg/ml. After 3 days of incubation, neutral red was used as live/dead differentiated dye. Neutral red stain intensity was assayed using automated ELIZA microplate reader adjusted at 540 nm (reference filters 620 nm).

Cell viability was obtained using the following equation:

$$\text{Percent cell viability} = (\text{controlled cells} - \text{treated cells}) / \text{controlled cells} \times 100. \quad (1)$$

Antioxidant activity assay

The antioxidant activity of mix2 plants extract was detected according to (Braca et al. 2002) adopted with suitable modifications of (Kumarasamy et al. 2007) as following. Stock solution of 1 mg/ml of mix2 water extract was prepared and followed by different dilutions to get the concentrations of 100, 90, 80, 70, 60, 50, 40, 30, 20 and 10 µg/ml. Equal

Table 2 The concentration of each individual plant in mg/ml at the prepared mix2 with baby milk

Plants extract (mix2)	Concentration (mg/ml)			
	100%	50%	25%	12.5%
Cloves	10.12	5.06	2.53	1.265
Pomegranate peel	24	12	6	3
Ginger	13.2	6.6	3.3	1.65
Myrrh	20	10	5	2.5
Licorice	14.44	7.22	3.61	1.805

volumes of each dilution and 1 mg/ml methanolic solution of DPPH (1,1-Diphenyl-2-picryl-hydrazyl) were mixed together and incubated for 30 min at 25 °C. The absorbance was measured at 517 nm using UV–Visible spectrophotometer. The inhibition percentage was calculated using Eq. 2 whilst IC₅₀ values were estimated from the percentage of inhibition versus concentration plot, using a non-linear regression algorithm.

$$\text{Inhibition (\%)} = [(A \text{ of control} - A \text{ of sample}) / A \text{ of control}] \times 100. \quad (2)$$

Determination of total phenolic compounds

Total soluble phenolic in mix2 plants extract were determined using Gallic acid as standard phenolic compound. Briefly, 1 ml of Folin-Ciocalteu reagent was added to the diluted mix solution and the content of the flask was mixed thoroughly. After almost 3 min, 3 ml of 2 % Na₂CO₃ was added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm using UV–Visible spectrophotometer (LaboAmerica, USA). The concentration of total phenolic compounds in the mix sample was determined as microgram of Gallic acid equivalent using Eq. 3 obtained from the standard Gallic acid graph:

$$\text{Absorbance} = 0.0028 \times \text{Gallic acid (mg)}. \quad (3)$$

Qualitative phytochemical analysis of the medicinal herbs water extract

The phytochemical compounds of each plant extract including tannins, phenols, flavonoids, alkaloids, reducing sugars, volatile oils, glycosides, amino acids & proteins, saponins and terpenoids were all qualitatively estimated according to (Mbatchou and Kosoono 2012).

Determination of phenolic compounds and flavonoids of mix2 by high performance liquid chromatography (HPLC)

HPLC (Agilent 1000) analysis of ten phenolic compounds and (antioxidants) were performed on a reverse phase Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 5 µm), using a gradient program with two solvent systems; A: 0.5 % acetic acid in 50:50 acetonitrile: water (1:1); B: 2 % acetic acid in water at a constant solvent flow rate of 1.2 ml/min with injection volume of 20 µL. The signals were detected at 280 nm by UV–VIS detection.

Results and discussion

Antibacterial activities of individual herbal plants against *H. pylori*

In the current study, known concentrations of the selected plant water extracts were tested against *H. pylori* using the agar diffusion method. The concluded results showed that, as shown in Table 3, all of the tested extracts were able to effectively cease the growth of *H. pylori* except myrrh and licorice with inhibition zone ranged from 11 to 32 mm. The potentialities of plant extract antibacterial actions ranged from the highly effective as in pomegranate peel with inhibition zone diameter of 32 mm to less effective as in ginger and pepper with inhibition zone diameter of 11 mm and passed through the moderate action as in cumin, sage and clove that showed inhibition zone diameter of 14, 18 and 20 mm, respectively. Likewise, as shown in Table 4; clear zone diameters of the all plant prepared mixes were higher than those of the individual extracts. In addition, mix2 was the most efficient treatment against *H. pylori* with diameter zone 39 mm. Medicinal plants are considered as a rich natural sources of antimicrobial agents and safe alternatives for antibiotic agents especially broad spectrum antibiotics which kill the normal flora of the body and impairing normal body functions (Mahesh and Satish 2008; Ahmad et al. 2012). Each individual medicinal plant could have antimicrobial activity that might increase synergistically when combined with others. Our current study proved the ability of combined plant extracts to affect the growth of *H. pylori* in a better way than individual extracts. The obtained data clarifying the enhancement of *H. pylori* inhibition using Mix2 compared with that for the highest effective individual extract. The concept of

Table 3 Clear zones of the antibacterial activities of individual and mixed plant extracts against *H. pylori*

Plant extract	Diameter of inhibition zone (mm)
Cloves	20
Pepper	11
Cumin	14
Sage	18
Pomegranate peel	32
Ginger	11
Myrrh	ND
Licorice	ND
Plant mix 1	21
Plant mix 2	39
Plant mix 3	23
Plant mix 4	28

ND not determined

Table 4 Minimum inhibitory concentrations (MIC) of mix2 against *H. pylori*

Concentration (mg/ml)	Clear zone (mm)
3	12
6	15
9	19
12	22
15	24
18	26
21	27
24	27
27	27
30	27
45	28
60	29
75	29
90	29
105	30

combination between different medicinal plants or phyto-constituents and antibiotics have been reported (Aqil et al. 2005; Ahmad and Aqil 2007; Subramaniam et al. 2014). In addition, the aim of the current study was extend to involve dietary supplement concept, and that is why, both myrrh and licorice were chosen in mix 2 preparation in spite of their undetected antibacterial activity.

Antibacterial activity of baby milk-mix2 combination

H. pylori is well known by its ability to infect children in wide range of ages. This is strongly inviting the researchers to find a suitable and convenient solution to save children from this violent kind of bacteria. As previously mentioned in methods section; different mixed percentages between mix2 and baby milk had been prepared. The results demonstrated that all the prepared mix2-milk combinations have ability to cease in a positive way the growth of *H. pylori* strain in percentage dependent manner. Although mix2 inhibit *H. pylori* with clear zone of 39 mm in diameter, the tested mix2-milk combinations showed inhibition zones ranged from 18 to 30 mm in diameter compared with the negative control (powdered milk) that failed to show any antibacterial activity against *H. pylori*.

It worth to mention that the antibacterial potency of mix2 and milk combinations was in the following order; Mix2 alone > mix2-milk combinations (100% > 50% > 25% > 12.5%) > milk alone.

These results lead us to think about a probable supportive effect of milk toward the growth of bacteria of interest due to the presence of vitamins, fats and proteins as main ingredients

of baby milks. These ingredients might help the bacteria to slightly but not totally resist the antibacterial effect of the prepared mix. Here, we can clarify that, Breast feeding lactation can provide babies with almost all nutritional requirements in addition to beneficial components as IgG antibodies (Pang et al. 2012). Regarding this concept, mothers can act as a mediator to transfer the beneficial constituents of medicinal plants to babies through their milk. This kind of mother-baby convey relationship is not present in formula feeding babies. That is why our research idea depended on, helping non breast feeding babies to overcome the probability of bacterial infection incidences including *H. pylori* infections. This could be achieved by addition of natural medicinal plants with antibacterial activities to baby's milk.

Minimum inhibitory concentration (MIC) of mix2

Minimum inhibitory concentration (MIC) is an essential method to determine the lowest concentration of the tested substances that able to completely inhibit and stop the bacterial growth (Gobalakrishnana et al. 2013). As it was mentioned previously, the prepared mix2 was prepared through the combination between five different medicinal plants with different concentrations of mg/ml. These concentrations might be elevated enough to affect the *H. pylori* growth. To detect the lowest concentrations that able to affect the bacterial growth, different concentrations of mix2 were prepared and tested by the agar well diffusion method. The tested concentrations ranged from 105 to 3 mg/ml, all concentrations have sensible antibacterial activity with clear zones diameters ranged from 30 to 12 mm (Table 4; Fig. 1) while the recorded mix2 MIC value was about 3 mg/ml. It was observed that, the rate of decrease in the tested concentrations was matched with the rate of decrease in the recorded clear zones. Sen and Batra (2012) reported the importance of MIC to confirm the susceptibility limit of special microorganism to antimicrobial agent and also to monitor the activity of new antimicrobial agents.

Qualitative phytochemical analysis

The estimation of presence and absence of phytochemical compounds of each of the eight water extracts was qualitatively achieved (Table 5). The extent of presence or absence of each individual phytochemical compound was categorized as abundant, moderate or weekly present. With an overview, six compounds including alkaloids, tannins, glycosides, flavonoids, terpenoids and saponines were highly present in most of the tested plants and can be ranked as abundant.

Alkaloids were almost detected in all of the eight tested plants while tannins, glycosides, flavonoids, terpenoids and saponines were highly detected in seven plants and absent

Fig. 1 MIC determination using different concentrations of mix2 against *H. pylori*, the antibacterial activity of mix2 concentrations 3, 6 and 9 mg/ml (a); concentrations 12, 15, 18, 21, 24 and 27 mg/ml (b); concentrations 30, 45, 60, 75, 90 and 105 mg/ml (c)

from only one and so all of them considered as abundant. On the other hand, both of reducing sugars and phenolic compounds were ranked as moderately present where both of them was detected in five plants and absent from three. Finally, volatile oils plus amino acids and proteins were only recorded in three plants and absent from the rest and so they were ranked as weekly present. Finally we can say that, plants in the same category as abundant, moderate or week does not have exactly the same compounds or phytochemical contents. In addition to the antimicrobial properties, medicinal plants considered as a reservoir of phytochemical agents that improved its therapeutic usage (Nagesh and Shanthamma 2009). The current study results proved the presence of alkaloids, tannins, glycosides, flavonoids, terpenoids and

Table 5 Phytochemical analysis of water extracts of the eight medicinal plants; ‘(+)’ sign indicates ‘present’ and ‘(–)’ sign indicates ‘absent’

Plants	Tannins	Reducing sugars	Glycosides	Alkaloids	Flavonoids	Volatile oils	Amino acids and proteins	Terpoids	Saponines	Phenolic compound
Cloves	+	+	+	+	+	–	–	–	–	+
Pepper	+	+	++	+	+	–	–	++	+	+
Cumin	+	+	+	+	+	–	–	+	+	+
Sage	+	–	–	+	+	–	–	–	+	–
Pomegranate peel	+	+	+	+	+	–	+	+	+	+
Ginger	–	+	+	+	+	+	+	+	+	+
Myrrh	–	–	+	+	–	+	+	+	+	–
Licorice	+	+	+	+	+	+	–	–	+	–

saponines in the most of the tested extract. It had been shown that these phytochemical compounds have the ability to show an acceptable level of diseases treatment in addition to their antibacterial and antifungal properties (Wadood et al. 2013). Tannins have the ability to inactivate bacterial strains pathogenicity through formation of hydrogen bonds with bacterial proteins (Oyewole et al. 2010). In addition, flavonoids could interact with and inactivate bacterial proteins as adhesions (Mishra et al. 2009).

Cytotoxicity assay

The cytotoxicity results indicated that, generally, the herbal extract usage was completely safe even till the standard maximum herbal concentration usage on cells (20 mg/ml). The cytotoxicity results briefly revealed that, herbal maximum concentration did not reach inhibitory concentration (IC₅₀) of the experiment, it showed inhibition percentage 22.51. On the other hand, the recommended nontoxic herbal dose on PBMC (the dose that showed 10 % cellular inhibition) recorded 5 mg/ml. while, using mix2 with concentration up to 20 mg/ml will be acceptable and applicable as the inhibition percentage was in range below IC₅₀. To give a preliminary evaluation on the correlation between their beneficial and harmful effects, the antioxidative activity and cytotoxicity assays were investigated in vitro (Li et al. 2008). Pharmacologically, the obtained median lethal concentration LC₅₀, of 20 mg/ml is an indication that the mixture is slightly toxic to mammalian cell model (PBMC), according to the toxicity classification/scale (Hodge and Sterner 2005). Even if LC values would be exactly measured, this value could not be considered as precise numerical value, this due to extrapolation from the in-vitro level to volunteers through the in-vivo level is hardly possible (Lorke 1983). However, these methods are still considered as a first pointer to the safety or toxicity degree of a substance with unknown toxicity profile (Kagbo and Ejebe 2010).

Evaluation of phenolic compounds and flavonoids by high performance liquid chromatography (HPLC)

Analysis of phenolic compounds in the Mix2 has been carried out by high performance liquid chromatography. Most of the phenolic acids have maximum absorption in the UV absorption spectra at wavelength of 280 nm and they were identified by comparison of retention times (Peak normalization, PN) and UV spectra of samples with those of authentic standards. Ten phenolic acids and flavonoids including (Gallic acid, Itaconic acid, Protocatechuic acid, Catechin, Esculetin, Catechol, Tannic acid, Ferulic acid, Pyrogallol and Cinnimic acid) were analyzed and determined either high or low or not in our prepared Mix2 mixture. HPLC chromatograms of standard of phenolic compounds include six phenolic acids and four flavonoids are given in Fig. 2. We can say that, ten of sample peaks at specific retention time were identical to those peaks of the ten phenolic standards. Finally, data could be summarized as Mix2 contained Gallic acid (29.93 mg/g), Itaconic acid (124.74 mg/g), Protocatechuic acid (94.71 mg/g), Catechin (20.19 mg/g), Esculetin (60.25 mg/g), Catechol (40.02 mg/g), Tannic acid (80.32 mg/g), Ferulic acid (63.92 mg/g), Pyrogallol (72.79 mg/g) and Cinnimic acid (100.94 mg/g). These results showed that our prepared mix2 contains high contents of phenolic and flavonoid compounds indicating that these compounds contribute to the antioxidant activity. This concept could be supported by Arulselvan et al. (2012) who mentioned that most of the phyto-constituents like flavonoids, catechins, carotenoids and beta carotene can prevent cellular damage caused by free radicals via their antioxidant activity. Regarding these results, our prepared mix2 could be candidates as a promising natural source of antioxidants.

Determination and measurement of total phenolics

The concentration of total phenolics of the prepared mix2 was 472 mg Gallic acid/g extract. These results indicate the ability

Fig. 2 A high-performance liquid chromatography-UV-VIS analysis to mix2. All peaks were identified by comparison of retention time and UV spectra with commercial standards as follows. Zorbax Eclipse XDB-C18 column (4.6×150 mm, $5 \mu\text{m}$), gradient eluent acetic acid/acetonitrile/

water/, flow rate 1.2 mL/min. Peak identification from left to right: (1) Gallic acid, (2) Itaconic acid, (3) Protocatechuic acid, (4) Catechin, (5) Esculetin, (6) Catechol, (7) Tannic acid, (8) Ferulic acid, (9) Pyrogallol., (10) Cinnimic acid

of our prepared mixture to scavenge free radicals which considered a good benefit when added to baby milk powders.

DPPH free radical scavenging activity

The scavenging activity against DPPH free radical using different concentration of Mix2 was compared with ascorbic acid as standard. As shown in Fig. 3, mix2 concentrations range from 0.19 to 100 $\mu\text{g/ml}$ showed percentage of inhibition ranged from 6.45 to 93 %. While, lowest concentrations of 0.19, 0.39 and 1.96 $\mu\text{g/ml}$ were out of inhibition percentage range but the same concentrations of ascorbic acid showed sensible percentage of inhibition 0.22, 0.25 and 1.5 $\mu\text{g/ml}$, respectively. Nevertheless, the mix2 was able to record IC₅₀ of 3.75 $\mu\text{g/ml}$ compared with 12.5 $\mu\text{g/ml}$ for ascorbic acid. The antioxidant activity of most of the phyto-constituents like flavonoids, catechins, carotenoids, beta carotene and total

phenolics have been proved by preventing cellular damage caused by free radicals (Arulselvan et al. 2012; Atanassova et al. 2011). It has been previously recorded that the effect of antioxidants on DPPH is due to their hydrogen donating ability which able to react with and stop its free radical activity (Ghasemzadeh et al. 2010). Our obtained results conclude that, our prepared mix2 that riches in phyto-constituents could be candidates as a promising natural source of antioxidants. However, the fact that phyto-constituents act as pro-oxidants and mutagens makes their safety uncertain when at higher doses.

Conclusion

Combination of different medicinal plants to form mix2 showed high effect in controlling growth of *H. pylori*

Fig. 3 Antioxidant activity of mix2 and ascorbic acid as standard using DPPH scavenging activity method

in vitro with MIC of 3 mg/ml. The mix2 extract contained numerous active compounds including flavonoids, terpenoids, saponins, volatile oils, tannins, alkaloids, reducing sugars and phenolic compounds. These compounds could theoretically help to control gastric inflammation. It was detected that, mix2 is safe for administration as it has no cytotoxic effect against normal human peripheral blood cells (PBMCs). The obtained data could conclude that our prepared mix2 might be suitable for therapeutic use against *H. pylori* and gastric ulcer caused by it and might be added to baby milk and used as functional and therapeutical food for babies. Finally, although, this pilot study pointed to an advanced trend of investigation, more studies at animal level and on volunteers must be carried out to explain exactly its clinical effect.

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