MANIPULATION OF PROBIOTICS FERMENTATION OF MILK BY CINNAMON ZEYLANICUM, GLYCYRRHIZA GLABRA OR ALLIUM SATIVUM AND THEIR EFFECTS ON INHIBITION OF HELICOBACTER PYLORI GROWTH IN VITRO

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2011
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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

FACULTY OF SCIENCE UNIVERSITY OF MALAYA KUALA LUMPUR 2011
AKNOWLEDGMENT

I would like to thank all people who have helped, inspired and supported me in any respect during the completion of this project.

I am heartily thankful to my supervisor, Associate Professor Dr. Ahmad Salihin Baba, whose encouragement, guidance and support from the initial to the final phases enabled me to grow and develop. I appreciate him for giving me a challenging and motivating research project which include the disciplines of Biochemistry, Medical Microbiology and Food Biotechnology.

I would like to express my sincere gratitude to Prof. Dato’ Dr. KL Goh, the head of Gastroenterology unit, Faculty of Medicine, for his valuable advice and friendly help.

I warmly thank Prof. Mohd Yasim Mohd Yusof from Microbiology Department, University of Malaya, who has guided me and allowed me to use the facilities in Microbiology Lab. Special thanks also goes to Ms. Chan Chui Lin for her assistance in the isolation of H. pylori isolates.

I take this opportunity to express my profound gratitude to my beloved families; especially my mother, grandfather and my aunt for their unflagging love and endless support through my life. Words alone can’t express my sense of gratitude; simply “God blessed me to have you around and thank you”.

Many thanks to my dearest husband, whose patience, accompany, love, and support enabled me to complete this project.

Thanks to all my friends and lab mates for helpful discussions and support.

Last but not least, thanks to God for guiding my life through all tests in the past three years. You have blessed me and made my life more bountiful. May your name be honored and glorified.

Thank you
Sara Behrad
November 2010
ABSTRACT

Dairy products containing probiotics (e.g. *Lactobacillus* ssp. and *Bifidobacterium*) and certain herbs have inhibitory effects on the growth of *Helicobacter pylori*. The objectives of the present study were to determine the effects of herbs traditionally used for the treatment of gastric ulcer on yogurt fermentation characteristics, probiotic bacteria and the growth of *H. pylori* *in vitro*. *Cinnamomum zeylanicum* (cinnamon), *Glycyrrhiza glabra* (licorice) or *Allium sativum* (garlic) was individually mixed with milk and the mixtures were fermented by probiotic bacteria to form herbal-yogurts. Changes in pH, titratable acidity, anti oxidant activity and the viable cell count of *Lactobacillus* ssp. and *Streptococcus thermophilus* were evaluated during refrigerated storage. The *in vitro* inhibition of *H. pylori* growth was determined using agar diffusion and minimum inhibitory concentration (MIC) methods. There were no significant differences in pH and TA between herbal-yogurts and plain-yogurt during fermentation and storage. Refrigeration up to 28 days increased (*p*>0.05) viable *Lactobacillus* ssp. counts to 15.8 x 10^6 cfu/ml in the plain-yogurt but the presence of cinnamon, licorice or garlic tend to inhibit the increase (*p*>0.05) in herbal yogurts. Garlic-yogurt showed the least favored (*p*>0.05) for all characteristics tested for organoleptic properties. Water extract of cinnamon-yogurt and licorice-yogurt on day 7 of refrigerated storage showed the highest inhibitory effect against *H. pylori* strains UM-1, UM-2 and UM-3. An MIC of 3ml was effective for all *H. pylori* isolates by cinnamon and licorice yogurt water extracts, but only for *H. pylori* isolate UM-3 by garlic yogurt extract. Licorice-yogurt water extract had MIC at 1ml for isolate UM-1 and UM-2, whereas cinnamon-yogurt water extract had MIC at 2ml. Garlic-yogurt water extract had weak inhibition on *H. pylori*. The present *in vitro* findings indicate that yogurt and herbs under study can decrease the growth of *H. pylori*. These herbs could be used as food additives for the production of novel dairy products because of their unique functional attributes and potential mitigation on *H. pylori* growth.

*Key words: Probiotics – Helicobacter pylori – Medicinal Herbs – Herbal-yogurt – Lactobacillus ssp.*
ABSTRAK

Produk-produk tenu su yang mengandungi probiotik (misalnya ssp. *Lactobacillus* dan *Bifidobacterium*) dan herba tertentu mempunyai kesan menghalang pada pertumbuhan *Helicobacter pylori*. Penelitian ini adalah untuk mengaji kesan dari herba yang digunakan secara tradisi untuk rawatan ulser perut ke atas ciri-ciri fermentasi yogurt, bakteria probiotik dan pertumbuhan *H. pylori in vitro*. *Cinnamomum zeylanicum* (kayu manis), *Glycyrrhiza glabra* (licorice) atau *Allium sativum* (bawang putih) dicampur berasingan dengan susu dan campuran difermentasikan oleh bakteria probiotik untuk membentuk yogurt herba. Perubahan pH, jumlah asid tertitrasi, aktiviti anti oksidan dan jumlah sel hidup *Lactobacillus* ssp dan *Streptococcus thermophilus* dinilai selama simpanan berpendingin. Perencatan pertumbuhan *in vitro* *H. pylori* ditentukan dengan menggunakan kaedah difusi agar dan kepekatan minimun perencatan (MIC). Tidak ada perbezaan yang signifikan pada pH dan TTA antara yogurt herba dan yogurt kawalan selama fermentasi dan simpanan berpendingin. Pendinginan sehingga 28 hari meningkat (*p > 0.05*) *Lactobacillus* ssp ke jumlah 15.8 x 10⁶ cfu/ml untuk yogurt kawalan namun kewujudan kayu manis, licorice atau bawang putih cenderung menghalang peningkatan (*p > 0.05*) pada yogurt herba. Yogurt bawang putih adalah paling tidak disukai (*p > 0.05*) untuk semua ciri-ciri diuji untuk sifat organoleptik. Ekstrak air yogurt kayu manis dan yogurt licorice pada 7 hari simpanan berpendingin menunjukkan kesan penghambatan yang tinggi terhadap strain *H. pylori* UM-1, UM-2 dan UM-3. Extrak yogurt kayu manis dan yogurt licorice pada MIC 3ml adalah berkesan merancat semua isolat *H. pylori* tetapi hanya pada MIC 3ml bagi ekstrak yogurt bawang putih. Ekstrak air yogurt licorice pada kepekatan 1ml berupaya merencat UM-1 dan UM-2, sedangkan air ekstrak yogurt kayu manis mempunyai MIC 2ml. Ekstrak air yogurt bawang putih menghalang pertumbuhan *H. pylori*. Yogurt dan herba-herba yang diteliti secara *in vitro* dapat merencat pertumbuhan *H. pylori*. Tumbuh-tumbuhan ini boleh digunakan sebagai makanan tambahan untuk pengeluaran produk tenu su baru kerana sifat unik berfungsi dan potensinya mengekang pertumbuhan *H. pylori*. 
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LIST OF ABBREVIATIONS

BF  Before Fermentation
μl  Microlitre
ml  Millilitre
μg  Microgram
mg  Milligram
nm  Nanometer
mm  Millimeter
rpm  Revolution Per Minute
LAB  Lactic Acid Bacteria
HCl  Hydrochloric Acid
NaOH  Sodium Hydroxide
DPPH  2,2-Diphenyl-1-Picrylhydrazyl
TA  Titratable Acidity
MIC  Minimum Inhibitory Concentration
DDM  Disk Diffusion Method
cfu  Colony Forming Unit
dH₂O  Distilled Water
BHIB  Brain Heart Infusion Broth
S. thermophilus  *Streptococcus thermophilus*
L. acidophilus  *Lactobacillus acidophilus*
*H. pylori*  *Helicobacter pylori*
GI  Gastrointestinal