The Antibacterial Activity of the Crude Extract of *Capsicum frutescens* Linn. (Hot Pepper) Against the Growth of *Helicobacter pylori* and Other Selected Enteric Bacteria

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by

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APPROVAL SHEET

This thesis entitled, "THE ANTIBACTERIAL ACTIVITY OF THE CRUDE EXTRACT OF *CAPSICUM FRUTESCENS* LINN (HOT PEPPER) AGAINST THE GROWTH OF *HELICOBACTER PYLORI* AND OTHER SELECTED ENTERIC BACTERIA", prepared and submitted by HAZEL S. GUDELOSAO-IQBAL, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN BIOLOGY, has been examined and is recommended for acceptance and approval for oral examination

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SUMMARY

As *Helicobacter pylori* is implicated in 95% of gastritis and gastric ulcer formations and *Escherichia coli* and *Staphylococcus aureus* in most gastrointestinal disorders, this study aimed to determine the antimicrobial activity of the crude extract of *Capsicum frutescens* (hot pepper) on the selected enteric bacteria mentioned above and on one probiotic, *Lactobacillus casei* The susceptibility of the four test organisms against varying concentrations of the crude extract of *Capsicum frutescens* was investigated using the disc diffusion method

The study commenced on the first week of December 2003 with preparation of the crude extract of the *Capsicum frutescens* and ended on the first week of March 2004 using *Staphylococcus aureus* as the last test organism Three batches of 500 g each of *Capsicum frutescens* were ground in a blender, soaked separately in three different solvents, namely, ethanol, ethyl acetate and dichloromethane (DCM) respectively. The resulting extracts were then filtered, concentrated by evaporating the solvents to form three different fractions These fractions were then dissolved again using ethyl alcohol as the final solvent for all. These are now the stock solutions Sterile filter paper discs were impregnated separately with the same volume of the three stock solutions for the initial screening of their inhibitory effect on the test organisms, which served as a qualitative test Final susceptibility testing was conducted after the fraction with the highest zone of inhibition was determined The stock solution of such fraction was subjected to serial dilution of 100,000, 10,000 and 1,000 ppm Positive and negative controls were included in the same plate to see clearly contrasting effects of a negative and positive reaction for inhibition Approximately 5µg Metronidazole for *Helicobacter pylori* and approximately

30 µg Tetracycline for *Escherichia coli, Lactobacillus casei* and *Staphylococcus aureus* were used as positive controls whereas 95% ethanol was used as negative control.

Test organisms were subcultured from the pure cultures obtained from the Philippine National Collection of Microorganisms (PNCM) and Velez College except for one that needed isolation from the gastric biopsy collected from a Cebu Doctors' Hospital (CDH) patient positive for gastritis and gastric ulcer Pure Mueller Hinton agar was used in the subculture of two of the four organisms and the same but supplemented medium was used for *Helicobacter pylori,* where 5-10% defibrinated horse blood was added Fresh horse blood was obtained from the City Abattoir with permission from the City Veterinary Office. Rogosa agar was used in the selective subculture of *Lactobacillus casei* All test organisms have grown optimally at 37°C Both *Helicobacter pylori* and *Lactobacillus casei* required a microaerophilic condition during incubation and this was met using the AnaeroPack-Microaero inside an anaerobic jar while *Escherichia coli* and *Staphylococcus aureus* were grown aerobically.

In the isolation and identification of the *Helicobacter pylori,* confirmatory tests were required which included· characteristic motility in hanging drop method, cell morphology, gram stain reaction, urease test reaction and colony morphology The other three test organisms did not need to go through biochemical tests since they were obtained as pure cultures although gram staining was necessary for all for photo documentation purposes All procedures in the subculture and susceptibility testing of the test organisms were performed aseptically and with extra care as to avoid contamination All cultures were incinerated after they have been observed and before being disposed of to prevent the spread of the pathogenic test organisms to the immediate environment.

The growth curve was determined for each of the four test organisms using the Spectronic 20 and the Techcomp Ultraviolet Visible Spectrophotometer. This was necessary to know whether the cells were at their lag phase or exponential growth phase which was very essential in determining the approximate time when the culture plates for susceptibility testing was to be appropriately observed Dilutions of succeeding bacterial solutions were adjusted to meet the standardized turbidity to have a uniform number of colony forming units (CFU) before inoculation

The growth curve showed that at a wavelength set at 600 nm and using nutrient broth as the inoculating solution, *Helicobacter pylori* has longer lag phase and takes more time (days) to reach its optimal growth, whereas the three selected enteric bacteria were able to reach their exponential growth phase in a matter of hours, The DCM as the initial fraction was observed to be more effective in inhibiting the test organisms during the initial screening, In the final susceptibility test however, it was observed that *Escherichia coli, Lactobacillus casei* and *Staphylococcus aureus* were all resistant to the effect of low or high concentrations of the crude extract while *H pylori* showed a considerable varying size of inhibition at increasing concentrations, from 1,000 to 100,000 ppm

It was concluded that the crude extract had an antimicrobial activity on *Helicobacter pylori*, and not on *Escherichia coli, Lactobacillus casei* and *Staphylococcus aureus*

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