6. Summary and Conclusion

The present work was undertaken mainly to screen medicinal plants based on its efficacy in controlling *Helicobacter pylori* in-vitro. In addition to the antimicrobial effect, the successful control of this pathogen also requires antioxidant activities. Thus, efforts were made to evaluate the potential target plants based on combined antibacterial and antioxidant properties.

To assay the plants, dry samples were extracted in a series of solvents, concentrated and placed on agar-cup or on a disc over bacterial culture plate having inoculums for confluent growth of the pathogen. The presence of antibacterial component in the extract would inhibit the growth of the pathogen leading to a formation of zone of inhibition.

*Azadirachta indica* (leaves), *Rosa indica* (leaves), *Emblica officinalis* (pulp and seeds), *Syzygium aromaticum* (buds) and *Datura metel* (fruit, buds, leaves and flower) inhibited the growth of *H. pylori* strains exhibiting 13-32 millimeter (including 6 mm disc diameter where sample was loaded) zone of inhibition. All the plant extracts tested were also effective against *Staphylococcus aureus* and *Bacillus cereus* strains with zone of inhibitions between 12-38 mm and 14-30 mm respectively.

Plants which did not show any activity against the *H. pylori* strains (reference and clinical isolates) were *Bacopa monnieri* (leaves), *Ocimum tenuiflorum* (leaves), *Fumaria parviflora* (leaves), *Camellia sinensis assamica* (leaves) and *Bryophyllum pinnatum* (leaves). *Ocimum tenuiflorum* (leaves) and *Fumaria parviflora* (leaves) were also found to be negative for *Pseudomonas aeruginosa* and *Bacillus subtilis*. Apart from these *Bacopa monnieri* (leaves), *Bryophyllum pinnatum* (leaves) and *Murraya koenigii* (leaves) were also found to be negative against *B. subtilis* strain.

Thus, according to the results obtained, it was seen that most of the plant extracts that were effective against *H. pylori* were also effective towards other human pathogens (*B. subtilis, B. cereus, S. aureus* and *P. aeruginosa*) indicating the antibiotic components present in these extracts are broad spectrum in nature. The plants which did not show any zone of inhibition either did not have any antibacterial activity or the concentration used in the extract was below the detection level. Since, our aim
was to select the plants with high antibacterial activity the plants with low antibacterial activity were not included later in the study.

The minimum inhibitory concentration (MIC) for *A. indica* (leaves) was 6.25-50 µg/µl, for *R. indica* (petals and leaves) were 16.93-33.87 µg/µl and 41.62 µg/µl respectively. For *E. officinalis* (pulp) extract, MIC was 18.25-37.5 µg/µl and for *S. aromaticum* extract, MIC was 62.5 µg/µl for all the *H. pylori* strains tested. The MIC value for *D. metel* fruit was 1.25-20 µg/µl, for *D. metel* bud it was 1.17-4.78 µg/µl, for *D. metel* leaves, it was 0.58-4.66 µg/µl and for *D. metel* flowers, it was 0.68-2.75 µg/µl for all the *H. pylori* strains. The phytochemical content and antioxidant activity was evaluated in crude extract of those medicinal plants which showed higher antimicrobial activity against the *H. pylori* strains. The reason for higher antimicrobial activity could be correlated to the presence of various phytochemical compounds. The phenolics and flavonoids constitute a large group of phytochemicals with antimicrobial potential. Total phenolic and flavonoid content were determined and both were found to be high in *E. officinalis* (pulp), *S. aromaticum* (bud) and *D. metel* (leaves and buds). Antioxidant activities was estimated by total reducing power content, FRAP assay, DPPH assay and ABTS assay and was found to be high in *E. officinalis*, *S. aromaticum*, *R. indica* and *D. metel* extracts.

Based on the result of antimicrobial and antioxidant activity, *D. metel* plant was selected for detail study. Moreover when different parts of *D. metel* – bud, flower, fruit and leaves were compared for antimicrobial and antioxidant activity along with the cytotoxicity study, the leaves of the plant was chosen since it exhibited highest antimicrobial activity with highest flavonoid content, relevant antioxidant activity and no cytotoxicity making it the best choice in terms of availability and abundance as a raw material from nature.

Isolation and purification of anti-*H. pylori* compounds from the crude extract of *D. metel* leaves was done by column chromatography using different solvent systems and bioactive compound/s with anti-*H. pylori* property were detected by TLC and contact bioautography. The most active antimicrobial spot from *D. metel* leaves was selected for further study. Moreover, it was also confirmed that the hallucinating
compound “atropine” present in *D. metel* did not show any anti-*H. pylori* activity so the fraction under study was not atropine.

The TLC separated bioactive spots was chemically characterized by spraying with reagents specific for alkaloids, essential oils, tannins, phenolics etc. It showed the presence of alkaloids, essential oil, tannins and phenolics. The multiple compounds present in the extract were further separated and purified by column chromatography.

Result of column chromatography revealed fraction no. 18 with a single spot having anti-*H. pylori* activity. Further purification of this bioactive compound from *D. metel* leaves was done by HPLC (high performance liquid chromatography). The HPLC chromatogram showed two sharp peaks with retention time 15min and 17min. Only the 1st peak with retention time 15 min was found to be bioactive against the *H. pylori* strain.

It was demonstrated through scanning electron microscopy that *H. pylori* when treated with the isolated purified compound acquired a coccoid shape and its content oozed out. However, the control *H. pylori* cells treated with only solvent retained its original spiral/helical shape.

The HPLC purified anti-*H. pylori* fraction from *D. metel* leaves was rich in flavonoid (3.8 mg rutin equivalent/g of dry weight of fraction) and also showed high antioxidant activity (0.83 mg trolox equivalent/g of dry weight). In HPTLC, the bioactive compound showed identical mobility with quercetin, one of the flavonoid compounds that have been reported earlier to be present in *Datura* species.

The results altogether suggest that the bioactive fraction present in *D. metel* leaves is either quercetin or a fraction very rich in quercetin. However, other studies like NMR and mass-spectrometry are required to confirm the identity of the compound. Our studies suggest that fraction isolated from *D. metel* could be used as a lead molecule in the synthesis of novel drugs with anti-*H. pylori* as well as antioxidant properties. However, further research is required to study the efficacy of the isolated fraction in animal model before it can be suggested for human use. In addition, toxicological and in-vivo potency studies are required to evaluate the safety parameters in the isolated compound.