5. Discussion:

Plants and plant derived agents have traditionally been used by herbalists and indigenous healers for the prevention and treatment of peptic ulcers (Borrelli and Izzo, 2000). Currently the most preferred treatment against the \textit{H. pylori} infection is the triple therapy which employs the use of a proton pump inhibitor and two antibiotics (Bytzer and O’Morain, 2005) however, multiple therapy treatment and constant usage of drugs has resulted in the emergence of the antibiotic resistant strains associated with adverse side effects (Bytzer and O’Morain, 2005; Cameron \textit{et al}., 2004). Increasing complications in the usage of the conventional triple therapy has led a bid to identify potential sources for new drugs that are safe, highly effective and have specific cellular targets (Colombo and Bosisio, 1996; Falcao \textit{et al}., 2008).

Thirteen Indian medicinal plants and their parts enlisted herein have been checked for antimicrobial property against \textit{Helicobacter pylori} and some other human pathogens (\textit{Bacillus cereus}, \textit{Bacillus subtilis}, \textit{Pseudomonas aeruginosa} and methicillin resistant \textit{Staphylococcus aureus}). Medicinal plants are a mixture of both polar and non-polar compounds. On the basis of a report by Cowan, (1999) which says almost all the identified components from plants which are bioactive against the pathogens are aromatic or saturated organic compounds and are usually obtained through either ethanol or methanol extraction giving a greater antimicrobial activity. Therefore, in this study though the initial extraction included solvent of different polarity however, later the study was continued with ethanol as the only extraction solvent.

Studies have shown that antioxidants have provided support in the treatment against \textit{H. pylori} infection by the potential to neutralize the DNA-damaging free radicals and also lower the risk of gastric cancer (Akyon, 2002). When an antioxidant astaxanthin was given to \textit{H. pylori} infected mice, there was an increase in the interleukin-4 release level which made a shift in the T helper cell (Th 2) T-cell response. There was a downward regulation of the Th 1 cells and an upward regulation of the Th 2 cells with the neutralization of the reactive free oxygen metabolites in the mucosa (Bennedsen \textit{et al}., 1999). Thus, in this particular study, plants and plant derived agents were isolated and their effect was targeted against \textit{H. pylori} strains taking in
account the antioxidant activity to create the combinatorial effect on the target pathogen.

The study was also targeted specifically against the Indian \textit{H. pylori} strains as they are known to be geographically distinct from the East Asian and the Western strains. The drug resistance pattern in \textit{H. pylori} strains from Kolkata, India shows 85\% to be resistant to metronidazole (8 µg/µl), 7.5\% of the strains resistant to tetracycline and all the strains were sensitive to clarithromycin, furazolidone and amoxicillin (Datta \textit{et al.}, 2005). However, the drug sensitivity pattern in the \textit{H. pylori} strains was different among strains of \textit{H. pylori} from other regions in India.

Resistance to the antibiotic metronidazole is very common in the \textit{H. pylori} strains (Gerrits \textit{et al.}, 2004; 2006). It is usually between 29-52\% in the developed countries (Jones \textit{et al.}, 2008) and in the developing countries, it is upto 100\% (Njume \textit{et al.}, 2009; Tanih \textit{et al.}, 2010). The other commonly used antibiotic in the treatment of \textit{H. pylori} is clarithromycin (Megraud and Lehours, 2007). It is the most efficient drug \textit{in-vitro} and is less affected by the pH. The prevalence of clarithromycin resistant strains varies immensely among countries. The clarithromycin resistance in \textit{H. pylori} has declined the eradication success rate by 40-50\% (Njume \textit{et al.}, 2009).

Since reports show that many strains are currently becoming resistant to clarithromycin, our work gave importance by including two clarithromycin resistant strains (\textit{H. pylori} 34A and 39A) from Delhi, NCR area in the study.

5.1 Antibacterial activity of the plant extracts against \textit{H. pylori} strains (reference and clinical) and pathogenic strains (\textit{B. cereus}, \textit{B. subtilis}, \textit{S. aureus} and \textit{P. aeruginosa})

Thirteen plants and their parts were checked against reference strains (26695, SS1 and J99); clinical isolates from Kolkata, India [154(1A), 225(1A) and 216(1A)]; clinical isolates from Delhi, NCR, India (3B, 58A, 34A, 39A, 75A, 76A and 70A); and human pathogenic strains (\textit{S. aureus}, \textit{B. subtilis}, \textit{B. cereus} and \textit{P. aeruginosa}). Plants extracts tested were \textit{B. monnieri} leaves, \textit{O. tenuiflorum} leaves, \textit{A. indica} leaves, \textit{A. vera} leaves, \textit{F. parviflora} leaves, \textit{M. arvensis} leaves, \textit{R. indica} (petals and leaves), \textit{E. officinalis} (seed and pulp), \textit{B. pinnatum} leaves, \textit{C. sinensis} leaves, \textit{M. koenigii} leaves, \textit{S. aromaticum} buds and \textit{D. metel} (fruit, bud, leave, flower).
The ethanolic extract of *B. monnieri* leaves did not show any activity against *H. pylori* strains tested by us. Although not many studies on anti-*H. pylori* activity of *B. monnieri* extract were reported, yet a study by Goel *et al.*, (2003) reported anti-*H. pylori* activity of methanolic extract of *B. monnieri* against a single strain of *H. pylori* NCTC at a higher concentration of 1000 µg/ml. In our study 65 µg/µl was used. Since our concentration was quite low as compared to the studies done against *H. pylori*, there was no zone of inhibition seen in our study against all the *H. pylori* strains tested for this plant.

We also checked the ethanolic leaves extract of *B. monnieri* against different pathogenic bacteria and found that the extract gave 13 mm, 20 mm and 19 mm of zone of inhibitions against *S. aureus*, *B. cereus* and *P. aeruginosa* respectively. No zone of inhibition was seen against *B. subtilis* strain. Sampathkumar *et al.*, (2008) checked the antimicrobial efficacy of *B. monnieri* plant extracts made in aqueous, ethanolic, diethyl ether and ethyl acetate against pathogenic strains *Staphylococcus aureus*, *Proteus vulgaris*, *Candida albicans* and *Aspergillus niger*. Results showed that the ethyl acetate and ethanolic extract had moderate effect against *S. aureus* strain with zone of inhibitions between 7-14 mm which supports our result of 13 mm against the *S. aureus* strain.

The ethanolic extract of *O. tenuiflorum* leaves did not show any activity against *H. pylori* strains (both reference and clinical isolates), *B. subtilis* and *P. aeruginosa* strains. The extract exhibited 15 mm and 21 mm of zone of inhibitions against *S. aureus* and *B. cereus* respectively. No zone of inhibition was seen against *B. subtilis* strain. According to Mishra and Mishra, (2011) very few data are reported regarding the antibacterial activity of the leaves of *Ocimum* sp. Research work till now has been done on the antibacterial activity of the oil of *Ocimum* sp.

In our study, against the reference strains of *H. pylori*, *A. indica* extract exhibited zone of inhibition between 16-19 mm; 13-19 mm against the clinical isolates of *H. pylori*; 15-26 mm against *S. aureus*, *B. subtilis*, *B. cereus* and *P. aeruginosa*. Clinical isolates of *H. pylori* from Delhi, NCR, exhibited 7 mm to 9 mm of zone of inhibition. Not many observations have been reported on the antimicrobial activity of *A. indica* against the *H. pylori* strains. Bandhopadhyay *et al.*, (2004) in his work compared *A. indica* extract with the known antiulcer drugs, ranitidine and
omeprazole in the pyloric ligation and the stress induced ulcer models, the extract was found to be almost equipotent to the standard drugs.

According to the study by Hoque et al., (2007) A. indica extracts were checked against various food borne pathogens and spoilage bacteria. The ethanolic extracts of A. indica showed higher inhibition against the pathogenic strains tested which included Escherichia coli O157:H7, Listeria monocytogenes, Staphylococcus aureus, Salmonella enteritidis, Bacillus cereus, Vibrio parahaemolyticus, Pseudomonas putida, Pseudomonas aeruginosa, Aeromonas hydrophila and Alcaligenes faecalis.

The antimicrobial activity of ethanolic extract of A. vera leaves against the reference strains of H. pylori exhibited 10-11 mm of zone of inhibitions; 7-9 mm against the clinical isolates of H. pylori. Against the pathogenic strains, 17 mm and 13 mm of zone of inhibitions were seen for S. aureus and B. subtilis and 21 mm and 14 mm were seen for B. cereus and P. aeruginosa. Although according to Kumari et al., (2010) there are only few studies till now which have demonstrated the antimicrobial activity of A. vera against H. pylori strains. Thong-Ngam and Chatsuwan, (2007) checked the antibacterial activity of A. vera gel spray dried powder in water against a standard strain of H. pylori (NCTC 11637) and against 9 other clinical isolate strains of H. pylori. They found no zone even with a high dose of A. vera (MIC > 512 µg/ml) reporting no antibacterial effect of A. vera against H. pylori strains. The reason can be attributed to the difference in the solvent and the material type used for the extract. The same researchers in their previous study had reported the anti-inflammatory effects of A. vera on leukocyte endothelium interaction in the gastric microcirculation of H. pylori infected rats. Thenmozhi and Shanthi, (2012) reported the antibacterial efficacy of A. vera against some clinical isolates. The ethanolic fraction of A. vera in their result gave a 17 mm of zone of inhibition against S. aureus strain which was in accordance to the result obtained in our study. A study by Lawrence, (2009) reported the antibacterial property of A. vera gel ethanolic extract against B. cereus to be 23 mm which was close to the result obtained by us in our study as 21 mm. Rodriguez et al., (2005) reported the activity of the yellow liquid fraction of A. vera against the fungal and bacterial pathogens affecting humans and plants. In another study, Reynolds and Dweck, (1999) observed control activities against 18 human pathogenic microorganisms.
No zone of inhibition was seen for *F. parviflora* leaves extract against *B. subtilis*, *P. aeruginosa* and all the *H. pylori* strains tested. A zone of inhibition of 14 mm and 16 mm was seen against *S. aureus* and *B. cereus* respectively. High antibacterial effect of *F. parviflora* has not been reported in literature also.

*F. parviflora* has been known to be an important ingredient of mouthwash as it is useful in reducing gums inflammation (Abdeirahman *et al.*, 2002). According to Vahabhi *et al.*, (2011) no report on antimicrobial activity of *F. parviflora* has been found. However, Parekh and Chanda, (2007) have evaluated antibacterial activity of *Fumaria indica* (aqueous and ethanol) extracts against some selected members of Enterobacteriaceae.

The ethanolic extract of *M. arvensis* leaves against the reference strains of *H. pylori* gave 11.33-12 mm of zone of inhibition; 9-13 mm against the clinical isolates of *H. pylori*; 30 mm and 17 mm of zone of inhibitions against *S. aureus* and *B. cereus* and 13 mm and 10 mm against *B. subtilis* and *P. aeruginosa* and 7-10 mm against the clinical isolates of *H. pylori* from Delhi, NCR area. Londonkar and Poddar, (2009) studied the activity of various extracts of *M. arvensis* Linn against drug induced gastric ulcer in mammals and in their study they reported, that 375 mg/kg body weight of the extract showed protective effect against acid secretion and gastric ulcers in ibuprofen plus pyloric ligation ulcer models. According to a study by Nair and Chanda, (2007) the aqueous extract of *M. arvensis* did not show any activity against the *S. aureus* and *P. aeruginosa* strains and the ethanolic extract of the same plant showed 7 mm against *B. cereus* and only 2 mm against *S. aureus* strain.

In our study, against the reference strains of *H. pylori*, the ethanolic extract of *R. indica* petals exhibited 13.33-14.16 mm; 12-15 mm against the clinical isolates of *H. pylori*; 26 mm and 19 mm of zone of inhibitions against *S. aureus* and *B. cereus* respectively. *B. subtilis* and *P. aeruginosa*, exhibited 14 mm and 16 mm of zone of inhibitions. The antimicrobial activity of ethanolic extract of *R. indica* leaves against the reference strains of *H. pylori* exhibited zone of inhibitions between 17-18.5 mm; against the clinical isolates of *H. pylori* 14-20 mm; the extract exhibits 28 mm and 27 mm of zone of inhibitions against *S. aureus* and *B. cereus*. 16 mm and 13 mm of zone of inhibitions were seen against *B. subtilis* and *P. aeruginosa*. 
There were no reports against the activity of *R. indica* petals and leaves against *H. pylori* strains. However, Koday *et al.*, (2010) have mentioned about the potent antibacterial activity of *R. indica* petals. Extracts of *Rosa* plants have been reported to show antibacterial activity against plant pathogen like *Xanthomonas axonopodis* (Basim *et al.*, 2003) and human pathogens like *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* and *Bacillus cereus* (Kamijo *et al.*, 2008). In a study by Pandey *et al.*, (2012) the antibacterial activity of the ethanolic extract of *R. indica* petals and leaves was checked against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and the zone of inhibition was seen between 18-27 mm which was according to the result obtained by us.

In our study, the ethanolic extract of *E. officinalis* pulp against the reference strains of *H. pylori* exhibited 19-22 mm of zone of inhibition; 16-32 mm against the clinical isolates of *H. pylori*; 31 mm and 32 mm against *S. aureus* and *B. cereus* respectively. 12 mm and 23 mm of zone of inhibitions were seen against *B. subtilis* and *P. aeruginosa*,

The ethanolic extract of *E. officinalis* seed against the reference strains of *H. pylori* exhibited 19-24.5 mm of zone of inhibition; 14-18 mm against the clinical isolates; 16 mm and 15 mm against *S. aureus* and *B. cereus*, 12 mm against *B. subtilis* and *P. aeruginosa*. The extract of *E. officinalis* (pulp and seed) against the clinical isolates of *H. pylori* from Delhi, NCR area showed zone of inhibitions between 20-30 mm. The results were of particular importance as big zone of inhibition was observed against clinical isolates of *H. pylori* which included the clarithromycin resistant strains.

Our study is the first to report on the antimicrobial activity of *E. officinalis* against *H. pylori*, eventhough, the fruit pulp of the plant is known to contain potent antimicrobial activity. The ethanolic extract of *E. officinalis* was found to be highly potent against the *H. pylori* strains mainly because most of the antibacterial compounds already reported in the extract are either aromatic or saturated hydrocarbon making ethanol an ideal solvent (Ndip *et al.*, 2007). The clinical isolates of *H. pylori* 225(1A) and 216(1A) used in the study were resistant to furazolidone and metronidazole and the clinical isolate 154(1A) was sensitive to furazolidone but resistant to metronidazole and amoxicillin (Datta *et al.*, 2005). The
results indicate anti-\textit{H. pylori} activity of the extract against all the tested clinical and laboratory strains that were differentially resistant to furazolidone, metronidazole and clarithromycin. Though variation in sensitivity to the synthetic drugs was observed yet, \textit{E. officinalis} extract could inhibit the growth of all the \textit{H. pylori} strains which showed variation towards drug sensitivity. The antimicrobial activity of aqueous infusion and decoction extracts of \textit{E. officinalis} has been evaluated against pathogenic strains like, \textit{Staphylococcus aureus}, \textit{Staphylococcus haemolyticus}, \textit{Staphylococcus saprophyticus}, \textit{Micrococcus varians}, \textit{Micrococcus lylae}, \textit{Micrococcus roseus}, \textit{Micrococcus sedenterius}, \textit{Bacillus subtilis}, \textit{Bacillus magaterium} and \textit{Candida albicans}. The zone of inhibition was 18-22 mm against \textit{S. aureus} and 20 mm against \textit{B. subtilis} strain at a concentration of 200 µg/µl (Saeed and Tariq, 2007) which was quite high against the concentration of 65 µg/µl of extract used by us. The results suggested that the constituents of \textit{E. officinalis} have been found to be active against a range of bacteria.

No zone of inhibition was seen for \textit{C. sinensis assamica} leaves extract against all the \textit{H. pylori} strains tested in our study and there were no reports as such against \textit{H. pylori} in the available literature (Yee and Koo, 2000). Yee and Koo, (2000) used Chinese tea, Lung Chen and checked against 20 clinical isolates of \textit{H. pylori} and found at a concentration of 0.5 %, the tea suppressed the growth of \textit{H. pylori}. They reported the presence of epigallocatechin gallate as an active ingredient responsible for the anti-\textit{H. pylori} effect. However, the extract exhibited 11 mm, 12 mm and 13 mm of zone of inhibitions against \textit{B. subtilis}, \textit{S. aureus}, \textit{P. aeruginosa} and 18 mm against \textit{B. cereus}. According to Yam \textit{et al.}, (1997) aqueous extracts of \textit{C. sinensis} from various sources exhibited a wide range of antibacterial activity against pathogenic bacteria including methicillin-resistant \textit{Staphylococcus aureus}, \textit{Escherichia coli} and \textit{Klebsiella pneumonia}. Many studies have revealed the anticarcinogenic, antimutagenic, antioxidative and hypocholesterolemic effects of \textit{C. sinensis}. (Yee and Koo, 2000).

In our result, the \textit{B. pinnatum} extract (65 µg/µl) did not show any antibacterial activity against all the \textit{H. pylori} strains tested. Not many observations were seen reporting the anti-\textit{H. pylori} activity of \textit{Bryophyllum}. However, Ghasil \textit{et al.}, (2011) in their study have reported the inhibition of development of acute ulcers induced in the stomach and duodenum of rats and guinea pigs by methanol soluble fraction. In
our study, the extract exhibits 13-14 mm of zone of inhibitions against *S. aureus*, *B. cereus* and *P. aeruginosa*, no zone of inhibition was seen against *B. subtilis* strain. Okwu *et al.*, (2011) have reported the isolation of two novel flavonoids from *B. pinnatum* at a very high concentration of 0.5 gm/ml which have showed potential antimicrobial activities against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Aspergillus niger*.

The antimicrobial activity of ethanolic extract of *M. koenigii* leaves against the reference strains of *H. pylori* is 9.66-11 mm of zone of inhibition; 7-9.5 mm against the clinical isolates of *H. pylori* from Kolkata, India; 7-8 mm against the clinical isolate strains of *H. pylori* from Delhi, NCR. Patidar, (2011) conducted a study with the aqueous leaf extract of *M. koenigii* and checked its activity against pylorus ligation induced gastric ulcer model at a dose of 200 and 400 mg/kg of the plant extract. Results showed a significant reduction in the ulcer index, antiulcer activity, antisecretory and cytoprotective effect. In our study, the extract also exhibited 19 mm and 20 mm of zone of inhibition against *S. aureus* and *B. cereus* respectively. However, against *B. subtilis* and *P. aeruginosa*, no zone of inhibitions was seen. Handral *et al.*, (2012) have reported a moderate inhibitory effect of aqueous extracts of *M. koenigii* (concentration 50 mg/ml) against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* strains. The result was due to the ethanolic extract used by them which was responsible for a slightly higher activitymainly because of the higher solubility of phytoconstituents in the ethanolic extracts.

The *S. aromaticum* bud extract against the reference strains of *H. pylori* exhibited zone of inhibition between 15.33–18.33 mm; 13-15 mm against the clinical isolates of *H. pylori* from Kolkata; 20 mm and 24 mm against *S. aureus* and *B. cereus* and 7-9 mm against *B. subtilis* and *P. aeruginosa* strains. Results against the *H. pylori* clinical isolates from Delhi, NCR showed 13 mm, 14 mm, 19 mm, 26 mm, 28 mm of zone of inhibition was seen against *H. pylori* 70A, 76A, 75A, 3B and 58A respectively. Against the two clarithromycin resistant strains (34A and 39A), 17 mm and 25 mm of zone of inhibitions were seen. Not many reports in the literature have been found on the antimicrobial activity of *S. aromaticum* bud against *H. pylori* strains. Li *et al.*, (2005) studied the effect of *S. aromaticum* and 29 other Chinese plants against a standard strain of *H. pylori* (ATCC 43504) and 6 other clinical
isolates. The ethanolic and the aqueous extract of the plant gave strongest zone of inhibition against all the *H. pylori* strains tested. Also they reported that the main reason for anti-*H. pylori* activity of ethanol extract of *S. aromaticum* plant might be due to the presence of an active ingredient, eugenol. Ali *et al.*, (2005) have reported the antimicrobial activity of eugenol of *S. aromaticum* against 30 *H. pylori* strains including the standard reference strain *H. pylori* 26695. Their study also showed that at a concentration of 2 µg/ml, eugenol produced significant decrease in the viability of *H. pylori*. In a study by Saeed and Tariq, (2008) aqueous extract and decoction of *S. aromaticum* were checked against gram-negative bacteria of 10 different species. Maximum zone of inhibition (10mm) of the extracts was seen against the *P. aeruginosa* bacteria, which was almost the same result obtained by us. The antibacterial activity of the *S. aromaticum* extract against *S. aureus* strain was also reported by Betoni *et al.*, (2006).

The antimicrobial activity of *D. metel* fruit extract against the reference strains of *H. pylori* exhibited zone of inhibition between 11-12 mm; against the clinical isolates of *H. pylori* from Kolkata, India gave 11-16 mm; 30 mm and 18 mm against *S. aureus* and *B. cereus*. 12 mm and 11 mm of zone of inhibition was seen against *B. subtilis* and *P. aeruginosa*.

*D. metel* buds against the reference strains of *H. pylori* exhibited 19-21 mm of zone of inhibition; 17-25 mm against the clinical isolates of *H. pylori*; 38 mm and 25 mm against *S. aureus* and *B. cereus*. 22 mm and 12 mm of zone of inhibitions were seen against *B. subtilis* and *P. aeruginosa*.

The antimicrobial activity of *D. metel* leaves against the reference strains of *H. pylori* exhibited zone of inhibition between 18-21.5 mm; 17-25.66 mm against the clinical isolates of *H. pylori*; 29 mm against *S. aureus* and *B. subtilis*. 30 mm and 25 mm of zone of inhibitions were seen against *B. cereus* and *P. aeruginosa*.

The antimicrobial activity of *D. metel* flower against the reference strains of *H. pylori* gave zone of inhibition between 16-19 mm; 13-24 mm against the clinical isolates of *H. pylori*; 31mm, 22 mm, 20 mm and 15 mm of zone of inhibitions against *S. aureus*, *B. cereus*, *B. subtilis* and *P. aeruginosa* were seen.
The extract of *D. metel* (fruit, bud, leaves and flower) against the clinical isolates of *H. pylori* from Delhi, NCR area gave 18 mm and 25 mm of zone of inhibition against *H. pylori* 76A and 3B. 20 mm of zone of inhibition was seen for *H. pylori* 58A, 70A and 75A. Against the two clarithromycin resistant strain 34A and 39A, 22 mm and 24 mm of zone of inhibitions were observed.

Extracts of *Datura* plant have been reported to show antibacterial activity against few gram-positive bacteria (Eftekhar *et al.*, 2005) but no report were obtained on the antibacterial efficacy of the plant against *H. pylori* strains. Sakthi *et al.*, (2011a) checked the antimicrobial activity of the leaves extract prepared separately in ethanol and ethyl acetate against nine pathogenic strains like, *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumonia, Vibrio cholerae* and *Pseudomonas aeruginosa*. The ethanolic extracts of *Datura* leaves at a concentration of 100 mg/ml were effective against *Pseudomonas aeruginosa, Escherichia coli* and *Bacillus subtilis* strains which was quite high compared to the concentration of 65 µg/µl used by us.

In another study by Akharaiyi, (2011) the crude aqueous and ethanolic extracts of leaves, stem bark and roots of *D. metel* at a concentration of 20 mg/ml was checked against eight bacterial pathogenic strains like, *Streptococcus β hemolytic, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Bacillus cereus* and *Streptococcus dysenteriae*. Results showed that the ethanolic extract of the leaves and bark gave highest inhibition against the *S. aureus* strain and leaves extract showed 30 mm of zone of inhibition against *B. cereus* and *P. aeruginosa* strain.

A report by Britto and Gracelin, (2011) showed the potential antibacterial qualities of methanolic extract of *D. metel* (leaves, stem, flower and fruit) against the plant pathogen *Xanthomonas campestris*. Sakthi *et al.*, (2011b) also in another study checked the leaves extract made in ethanol and ethyl acetate for antifungal activity against six different fungal isolates like, *Candida albicans, Candida glabrata, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* and *Penicillium chrysogenum*. The ethanolic extract of *D. metel* at a high concentration of 300 mg/ml gave maximum zone of inhibition against all the six tested fungal isolates.
High zone of inhibition obtained by *D. metel* extracts used by us against all the tested pathogenic strains gives a valid reason to choose *D. metel* as a potent fraction for the development of drug against pathogens which are resistant to the available antibiotics.

5.2 Minimum inhibitory concentration (MIC) of the plant extracts against the *H. pylori* strains:

Plants showing high antimicrobial activity with zone of inhibitions ranging between 12-22 mm (65 µg/µl concentration) against the *H. pylori* strains were selected to measure the MIC which is defined as the lowest concentration of plant extract that completely inhibits the growth (Hammer et al., 1999; Delaquis et al., 2002) and was determined by visual observation. Plant extracts tested were *A. indica* leaves, *R. indica* (petals and leaves), *E. officinalis* pulp, *S. aromaticum* buds and *D. metel* (fruit, bud, leave and flower) extracts.

The MIC is accepted and well used criterion for measuring the susceptibility of microorganisms to the inhibitors / plant extracts. Many factors affect the MIC value including temperature, inoculum density and the type of the organism tested (Lambert, 2000).

In our study, the MIC results of *A. indica* leaves extract against the two reference strains of *H. pylori*, 26695 and SS1 was 50 µg/µl and against the clinical isolates 225(1A), 216(1A) and 154(1A) was 6.25 µg/µl, 12.5 µg/µl and 25 µg/µl respectively. The variation in the MIC results was significant in the case of the clinical isolates tested mainly because of the most distinctive features of *H. pylori*, its genetic diversity between the clinical isolates obtained from different patient populations (Blaser, 2005). Fabry et al., (1996) have reported >4000 µg/ml of MIC in *A. indica* (stem and leaves) extracts against 12 *H. pylori* strains.

Bandhopadhyay et al., (2004) has mentioned about the antiulcerogenic effects of *A. indica* in human clinical trials. According to them, the gastroprotective property of the *A. indica* acts mainly by inhibiting acid secretion and blocking oxidative damage of the gastric mucosa. Furthermore, when the extract was compared with the known antiulcer drugs, ranitidine and omeprazole in the pyloric ligation and the stress ulcer models, the extract was found to be almost equipotent to the standard drugs.
According to Garg et al., (1993) the leaves of A. indica were investigated in rats exposed to 2 hour cold resistant stress. The leave extracts were administered in doses of 10, 40, 160 mg leaf / kg body weight either as single or five-dose pretreatment regimens. The treatment resulted in the prevention of mucus depletion and mast cell degranulation.

Koday et al., (2010) have mentioned about the potent antibacterial activity of the R. indica petals. Considering very few reports on the antibacterial activity of this plant against various pathogens, we evaluated the MIC of the plant extract against the H. pylori strains.

The MIC of dried petal extract of R. indica against the reference and the clinical isolates of H. pylori is shown in Figure 4.52. The MIC was 16.93 µg/µl against the two reference strains of H. pylori 26695 and SS1 and against the three clinical isolates it was 33.87 µg/µl.

Extracts of Rosa plants have been reported to show antibacterial activity against plant pathogen like Xanthomonas axonopodis (Basim et al., 2003) and human pathogens like Staphylococcus aureus, Escherichia coli, Salmonella, Bacillus cereus (Kamijo et al., 2008) However, there is no report on its antibacterial activity against human pathogen like H. pylori.

The MIC of dried pulp extract of E. officinalis against the reference and the clinical isolates of H. pylori is shown in Figure 4.53. The MIC was reported to be 18.25 µg/µl against the clinical isolate H. pylori 225(1A) and 37.5 µg/µl against the two reference strains of H. pylori 26695 and SS1 and the two other clinical isolates of H. pylori 154(1A) and 216(1A).

Our study was the first to report the activity of E. officinalis against H. pylori, eventhough, the fruit pulp of the plant is known to contain potent antimicrobial activity (Mehrotra et al., 2011). The clinical isolates of H. pylori 225(1A) and 216(1A) used in the study were resistant to furazolidone and metronidazole and the clinical isolate 154(1A) was sensitive to furazolidone but resistant to both metronidazole and amoxicillin (Datta et al., 2005). Franklin et al., (2012) have reported MIC value of 100 µg/disc for the ethanolic extract and a MIC value of 750 µg/disk for the aqueous extract against the H. pylori strain. However, at the same
concentration there was no susceptibility seen by them for the petroleum ether extract.

The ethanolic extract of *E. officinalis* was found to be highly potent against the *H. pylori* strains mainly because most of the antibacterial compounds already reported in the extract are either aromatic / saturated making ethanol an ideal solvent (Ndip *et al.*, 2007).

Reports on antibacterial study on *R. indica* petals and leaves till now are very less (Sahoo *et al.*, 2011). Considering very few reports on the antibacterial activity of this plant against various pathogens, we evaluated the MIC of the plant extract against the *H. pylori* strains.

The MIC of dried leaves extract of *R. indica* against the reference and the clinical isolates of *H. pylori* is shown in Figure 4.54. The MIC showed similar pattern in all the *H. pylori* strains tested. It was reported to be 41.62 µg/µl against the two reference strains of *H. pylori* 26695, SS1 and the three clinical isolates of *H. pylori* 154(1A), 225(1A) and 216(1A).

According to Sahoo *et al.*, (2011) at a MIC concentration of 20 mg/ml, the ethanolic and the methanolic extracts of *R. indica* leaves were effective against the *S. aureus* strain. However, at this concentration the extract was totally resistant to *B. subtilis* and *E. coli*. Yet, in another report, Pandey *et al.*, (2012) reported a MIC value of 2.314 mg/ml against *Escherichia coli* and 0.01 mg/ml against *P. aeruginosa* strains. There were no reports as such against the *H. pylori* strains.

The MIC of dried bud ethanolic extract of *S. aromaticum* against the reference and the clinical isolates of *H. pylori* is shown in Figure 4.55. The MIC for *S. aromaticum* extract exhibited the same value for all the *H. pylori* strains tested. The MIC was 62.5 µg/µl against the two reference strains of *H. pylori* 26695, SS1 and the three clinical isolates of *H. pylori* 154(1A), 225(1A) and 216(1A).

According to Ma *et al.*, (2010) the MIC of an aqueous extract of *S. aromaticum* buds was >500 mg/ml against the *H. pylori* SS1 strain which was quite high as compared to the result obtained by us. Not many reports have been found on the antimicrobial activity of *S. aromaticum* bud extract against the *H. pylori* strains. Ali *et al.*, (2005) have in fact reported the antimicrobial activity of eugenol, a phenolic compound and
as a main component of *S. aromaticum* against 30 *H. pylori* strains including the standard reference strain *H. pylori* 26695. According to their study, the 26695 strain of *H. pylori* was sensitive at an MIC <0.016 µg/ml to all the three drugs (metronidazole, amoxicillin and clarithromycin) tested. Their study also showed that at a concentration of 2 µg/ml, eugenol produced significant decrease in the viability of *H. pylori*.

The MIC of dried fruit extract of *D. metel* against the reference and the clinical isolates of *H. pylori* is shown in Figure 4.56. The MIC value was 1.25 µg/µl against the clinical isolate 225(1A) and 20 µg/µl against the clinical isolate 216(1A). It was 2.5 µg/µl against the clinical isolate 154(1A) and against the two *H. pylori* reference strains 26695 and SS1 was 10 µg/µl.

The MIC of dried bud extract of *D. metel* against the reference and the clinical isolates of *H. pylori* is shown in Figure 4.57. The MIC was 1.17 µg/µl against clinical isolates 154(1A) and 225(1A). It was 2.39 µg/µl against the clinical isolate 216(1A) and against the *H. pylori* reference strains 26695 and SS1 was 4.78 µg/µl.

The MIC of dried leaves extract of *D. metel* against the reference and the clinical isolates of *H. pylori* is shown in Figure 4.58. The MIC was 0.58 µg/µl against the clinical isolate 225(1A). It was 1.16 µg/µl and 2.33 µg/µl against the clinical isolate 154(1A). However, MIC result for the *H. pylori* reference strains 26695 and SS1 was 4.66 µg/µl.

The MIC of dried flower extract of *D. metel* against the reference and the clinical isolates of *H. pylori* is shown in Figure 4.59. The MIC value for *D. metel* flowers extract against *H. pylori* strains was 0.68 µg/µl against the clinical isolate 225(1A). It was 1.37 µg/µl against the clinical isolates 154(1A) and 216(1A). The MIC value for the *H. pylori* reference strains 26695 and SS1 was 2.75 µg/µl.

Extracts of *Datura* plant have been reported to show antibacterial activity against a few gram-positive bacteria (Eftekhar et al., 2005) but till yet there are no reports on the antibacterial efficacy of the plant against the *H. pylori* strains. Sakthi et al., (2011a) checked the antimicrobial activity of the leaves extract in ethanol and ethyl acetate against nine pathogenic bacterial strains like, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Salmonella typhi*, *Shigella*
flexneri, Klebsiella pneumonia, Vibrio cholerae and Pseudomonas aeruginosa. The ethanolic extracts of Datura leaves at a concentration of 100 mg/ml were effective against the P. aeruginosa, E. coli and B. subtilis strains which is quite high as compared to the results obtained in our study.

In another study by Akharaiyi, (2011) the crude aqueous and ethanolic extracts of leaves, stem bark and roots of D. metel at a concentration of 20 mg/ml was checked against eight bacterial pathogenic strains like, Streptococcus \(\beta\) hemolytic, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Bacillus cereus and Streptococcus dysenteriae. The MIC of only the leave extract was determined and it was found to be 20 mg/ml against all the eight strains tested.

A report by Britto and Gracelin, (2011) shows the potential antibacterial qualities of methanolic extract of D. metel (leaves, stem, flower and fruit) effective against the plant pathogen Xanthomonas campestris. Sakthi et al., (2011b) also in another study checked the leaves extract made in ethanol and ethyl acetate for antifungal activity against six different fungal isolates like, Candida albicans, Candida glabrata, Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger and Penicillium chrysogenum. The ethanolic extract of D. metel at a concentration of 300 mg/ml gave maximum zone of inhibitions against all the six tested fungal isolates.

There are also some reports on the isolation and presence of alkaloids like hyoscyamine and scopolamine from D. metel (Chopra et al., 1986; Oliver-Bever, 1986).

### 5.3 Phytochemical estimation in the plant extracts:

The phytochemical estimation was done for the selected plants which showed higher antimicrobial activity against the H. pylori strains. The reason for a higher antimicrobial activity could be correlated to the presence of phytochemicals within that fraction like phenolics is the major group widely reported to be antibacterial in nature. Plant extracts tested were A. indica leaves, R. indica petals and leaves, E. officinalis pulp, S. aromaticum buds and D. metel (fruit, bud, leave and flower) extracts.
Phenolic compounds are major plant constituents because of their free radical scavenging property facilitated by their hydroxyl groups. The total phenolic concentration could be used for estimation of the antioxidant activity (Pelczar et al., 1998). In addition to the antioxidant activity in phenolic compounds it is also known to be anti-allergenic, anti-atherogenic, anti-inflammatory, anti-thrombotic, cardioprotective and have vasodilatory effects (Benavente et al., 1997; Manach et al., 2005; Middleton et al., 2000; Puupponen-Pimia et al., 2001; Samman and Cook, 1998).

Phenols are present in the essential oils of many plants and are proved to be active against many pathogenic strains including bacteria (Suresh et al., 1992; Shashidhar, 2002), fungi (Shashidhar, 2002; Bilgrami et al., 1992) and viruses (Pacheco et al., 1993). Phenolics have also been effective against the H. pylori strains (Ali et al., 2005). Therefore, the study involved the estimation of phenolic content along with the antimicrobial activity of the plant extracts and the active compounds present in them against the H. pylori strains.

In our study, gallic acid was used as a standard compound for the quantification of the total phenolic content. Results showed S. aromaticum bud with the highest value of total phenolic content at 52 gallic acid equivalent mg / g of dry weight of extract.

Li et al., (2005) studied the effect of S. aromaticum and 29 other Chinese plants against a standard strain of H. pylori (ATCC 43504) and 6 other clinical isolates. The ethanolic and the aqueous extract of the plant gave strongest zone of inhibition against all the H. pylori strains tested. Also they reported that the main reason for the anti-H. pylori action of the ethanol extract of S. aromaticum plant might be due to the presence of an active ingredient, eugenol.

The essential oil of S. aromaticum contains 44.2 % of eugenol (Ozturk and Ozbek, 2005) which is a phenolic compound and induces glutathione-s-transferase enzyme which plays a key role in detoxification in liver and intestines. The induction of glutathione-s-transferase inhibits chemical carcinogens, proving to be anticarcinogenic (Ali et al., 2005). They also showed that at a concentration of 2 µg/ml, eugenol produced significant decrease in the viability of H. pylori.
Considering, *S. aromaticum* as the positive control in our study, phenolics were estimated in *A. indica, R. indica, D. metel* and *E. officinalis* also. Out of the extract prepared from flower, bud, fruit and leaves of *D. metel*, the bud showed the highest content of phenolic compounds at a value of 5.54 gallic acid equivalent mg/gm of dry wt. of extract. Sakthi et al., (2011b) and Akharaiyi, (2011) in their study have reported the presence of phenolic compounds in the extracts (fruit, bud, leaves and flower) of *D. metel*.

The exact mechanism by which the phenolic compounds inhibit the growth of *H. pylori* is unknown but it can be because of the inhibition of the urease activity (Lin et al., 2005), adhesion to the human gastric mucus (Burger et al., 2000), disintegration of the outer membrane (Nohynek et al., 2006) and inhibition of VacA cytotoxin activity which causes the development of inflammation and ulceration in patients (Ruggiero et al., 2006; Yahiro et al., 2005).

Flavonoids are a group of about 4000 naturally occurring polyphenolic compounds that are present in plants. They are known to be an important constituent of daily human diet. Study reveals that a daily diet should contain approximately 1 gram of flavonoids per day (Di Carlo et al., 1999). They exhibit high biological effects including anti-ulcer activity (Deineka et al., 2004), cardiovascular disease, cancer, inflammation and against various allergies (Yao et al., 2004; Ferguson et al., 2004; Havsteen, 1983; Lyons-Wall and Samman, 1997; Ma et al., 2004; Moon et al., 2006).

Flavonoids have also been effective antimicrobial substances against a wide range of microorganisms. Their mode of action may be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. They are also capable of disrupting the microbial membranes (Tsuchiya et al., 1996).

Fukai et al., (2002) have reported in their study that flavonoid constituents from three plants *Glycyrrhiza glabra, Glycyrrhiza uralensis* and *Glycyrrhiza inflate* were effective against the *H. pylori* strains (including clarithromycin and amoxicillin resistant strains). Researchers have also reported that flavonoids like kaempferol isolated from plants have shown to exhibit antiulcer effect due to the inhibition of platelet-activating factor (PAF) formation by gastric mucosa (Izzo et al., 1994). Several extracts with flavonoids have known to exert gastro-protective activity. The
most studied anti-ulcer flavonoids are naringin, quercetin, silymarin, anthocyanosides and sophoradin derivatives (Di Carlo et al., 1999).

In our study, rutin was used as standard compound for the quantification of total flavonoid content. The result was expressed as milligram of rutin equivalents per gram of dry weight of extract. Chemically, rutin is 5,7,3’,4’, tetrahydroxy flavonol-3-rhamnoglucoside and reported to possess antioxidant antidiabetic activities and reduce low density lipoproteins (LDL) oxidation (De-whalley et al., 1990).

Results of the total flavonoid content showed S. aromaticum bud with the highest value at 34.26 rutin equivalent mg / g of dry weight of extract and it was included as a positive control. The total flavonoid content was determined using a reagent containing aluminium chloride and sodium nitrite, giving rise to a pink coloured flavonoid-aluminium complex in the alkaline medium (Yanping et al., 2004).

It has been reported that variety of Syzygium species are source of many important phytoconstituents like polyphenols, gallic and ellagic acid derivatives, tannins and flavonol glycosides. S. aromaticum contains about 15-20 % essential oil, 13 % tannins, 10 % fixed oil. The main components of the essential oil are identified to be eugenol, eugenyl acetate and β-caryophyllene. Eugenol (4-allyl-2-methoxyphenol) constitutes almost 70–90 % by weight, eugenol acetate > 17 %, cariofilen > 12 %, β-caryophyllene 9 %, 1,8-cineole 0.1 %, linalool 0.2 %, α-copaene 1.2 %, α-humulene 3.5 %, β-cadinene 0.5 %, epizonarene 0.1 %, α-muurolene 0.1 %, eugenyl acetate 4.2 %, δ-cadinene 3.6 %, α-copaen 1.0 %, methoxy benzaldehyde, benzyl alcohol, benzaldehyde, carvacrol, 2-heptanone,methyl salicylate, isoeugenol, methyl eugenol, phenyl propanoides, dehydrodieuugenol, transconfreryl aldehyde, biflorin, kaempferol, rhamnocitrin, myricetin, gallic acid, ellagic acid, oleanolic acid, thymol, cinnamaldehyde, acetyl salicylate, vanillin, and crategolic acid. Apart from these phytoconstituents, S. aromaticum also comprises of tannins (gallotannic acid), flavonoids (eugenin, rhamnetin, and eugenitin), triterpenoids (oleanolic acid, stigmasterol and campesterol) (Singh et al., 2012). In this study, out of the four D. metel extracts, leaves showed the highest content at 8.24 rutin equivalent mg /g of dry weight of extract. Presence of flavonoids were also reported in D. metel by Akharaiyi, (2011) along with other phytochemical constituents like, saponin, tannins, alkaloids, glycocides, steroids and terpenoids.
Many of the dietary flavonoids are also potential anticancer agents and have effects on cancer, chemoprevention and chemotherapy. The mode of action of the flavonoids against cancer may be due to the carcinogen inactivation, anti-proliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, antioxidation and reversal of multidrug resistance or a combination of these mechanisms (Ren et al., 2003).

Flavonoids exhibit gastro-protective effect and several mechanisms have been proposed to testify them. They cause an increase in the mucosal prostaglandin content, decrease of histamine secretion from mast cells by inhibition of histidine decarboxylase and also an inhibition of \( H. \text{pylori} \) growth. Flavonoids are also free radical scavengers which play a key role in the ulcerative and erosive lesions of the gastrointestinal tract.

5.4 Antioxidant activity estimation in the plant extracts:

The combination of antibacterial and antioxidant activity is an ideal way of treating the various infections caused by the pathogenic bacterial strains and antioxidants are known to play an important role in the gastro duodenal mucosal inflammation, peptic ulcer disease and against gastric cancer (Nair et al., 2000). However, synthetic antioxidants like, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) according to few reports might cause cancer and hormonal perturbation. Therefore, researchers are working on the potential antioxidant activity of natural substances (Mogana et al., 2011). Studies have shown that antioxidants have provided support in the treatment against \( H. \text{pylori} \) infection (Akyon, 2002). Since, the combination of antibacterial and antioxidant activity is an ideal way of treating various infections caused by pathogenic bacterial strains, measurement of antioxidant activity became very crucial for this study.

Antioxidant estimation was done by evaluating the total reducing power content, antioxidant activity by FRAP assay, DPPH assay and ABTS assay. Due to the extreme complex nature of phytochemicals to evaluate the antioxidant activity in the plant extracts, it was important to employ all the three commonly accepted methods. Several antioxidant methods have been developed to evaluate the antioxidant activity because of the difficulty in measuring each antioxidant component separately and interaction amongst antioxidants. Of these, total antioxidant activity, reducing
power, DPPH assay are the most commonly accepted assays (Frankel and Meyer, 2000; Sanchez, 2002).

Plants which showed higher antimicrobial activity against *H. pylori* were selected for estimation of antioxidant activity. Plants extracts tested were *A. indica* leaves, *R. indica* (petals and leaves), *E. officinalis* pulp, *S. aromaticum* buds, *D. metel* (fruit, bud, leave and flower) extracts.

In our study, vitamin-C was used as standard compound for the quantification of total reducing power. The result was expressed as milligram of vitamin-C equivalents per gram of dry weight of extract.

Vitamin-C is an acidic molecule by nature and exhibits high reducing activity. It is an essential component and resides in almost all living issues. Vitamin-C (ascorbic acid) exists in two inter-convertible forms as ascorbic acid and as de-hydroascorbic acid (DHA) (Bates, 1997). It has been reported that both ascorbic acid and DHA have capability of influencing the cell growth either by affecting the cell proliferation or by inducing cell death (Brigelius-Flohe and Flohe, 1996; Sakagami and Satoh, 1997).

According to Akyon, (2002) many antioxidant studies involving the use of vitamin-C have shown that the *H. pylori* infected patients have lower gastric ascorbic acid levels. Ekstrom *et al.*, (2000) have revealed the potential properties of antioxidants. According to them, antioxidants have the power to neutralize the DNA damaging free radicals (DNA damage as a result of the free radicals could lead to harmful consequences like gene modifications that are potentially carcinogenic) which are a result of many factors, like a chronic *H. pylori* infection and may thus, lower the risk of gastric cancer. It has also been reported that *H. pylori* is urease positive and vitamin-C inhibits urease activity (Nilius *et al.*, 1991).

The mode of action of ascorbic acid against the gastric cancer might be: as it is a potential nitrite scavenger, it may reduce the formation of carcinogenic compounds like N-nitroso in the stomach, since, ascorbic acid itself resides in the gastric juice. Also if there is an increase in the mucosal concentration of ascorbic acid in the epithelium, it may inhibit the free radical damage by the *H. pylori* associated gastritis (Waring *et al.*, 1996).
Out of all the plant extracts tested, *E. officinalis* pulp showed the highest value of total reducing power content at 75.8 vitamin-C equivalent mg / g of dry weight of extract. However, out of the four *D. metel* extracts, the flowers showed the highest content at 3.44 vitamin-C equivalent mg / g of dry wt. of extract. In this assay, the yellow colour of the sample changes to green and blue depending on the reducing power of the extracts or compounds. The presence of the reductants in the solution induces the reduction of the Fe$^{3+}$/ferricyanide complex to the ferrous form. Therefore, the Fe$^{2+}$ can be monitored by the measurement of the formation of Perl’s prussian blue at 700 nm (Yanping et al., 2004).

Many researchers have indicated *E. officinalis* as the super rich source of natural ascorbic acid. The reason for high ascorbic acid content in the *E. officinalis* fruit may be that ascorbic acid content increases as the fruit ripens (Lim et al., 2006). The increase in the ascorbic acid content in the mature fruit is due to the breakdown of starch to glucose which is used in the biosynthesis of ascorbic acid (Singh et al., 2005).

The fruits of the plant also contain other phytochemicals like, hydrolyzable tannins, emblicanin A, emblicanin B, pedunculagin and punigluconin. The presence of all these phytochemicals have implicated in its role as antioxidant (Ghosal et al., 1996).

Not much study till now has been done on the reducing power content of *D. metel*. Though, *D. metel* is a potential source of a large number of plant secondary metabolites like, a number of alkaloids including hyoscine, hyoscyamine, meteloidine, scopolamine, tigloidine, tropine, withametelline and daturametelins (Rastogi and Mehrotra, 1998).

FRAP is a simple procedure which is used to measure the ferric reducing ability of plasma (FRAP) to measure the antioxidant activity. In this method, the yellow Fe$^{3+}$-TPTZ formed complex is reduced to the blue Fe$^{2+}$-TPTZ complex by electron donating substances under the influence of acidic conditions. If the electron donating substances have a half reaction of lower redox potential than Fe$^{3+}$/Fe$^{2+}$ TPTZ, the formation of the blue complex would occur. FRAP assay is inexpensive, reagents used can be prepared easily, results are highly reproducible, dependable and assay procedure is swift (Benzie and Strain, 1996). Although according to Szollosi and Varga, (2002) FRAP method was usually used for human plasma, but along with the
mentioned researchers, we also wanted to check its reproducibility in plant extracts and thus, evaluated the antioxidant content through it.

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is a water soluble analogue of α-tocopherol. Trolox is an antioxidant like vitamin-E and is used in biological or biochemical applications to reduce oxidative stress or damage. In our study, trolox was used as standard compound for the quantification of the antioxidant power by FRAP assay. Result was expressed in mg of trolox equivalents per gram of dry weight of extract.

The mode of action of trolox is that it protects the membrane from lipid peroxidation by their free radical scavenging activity, have effects on the cell signaling pathways and gene expression (Herrera and Barbas, 2001). However, Sanderson et al., (1997) have shown in their study that lipid soluble antioxidants like, vitamin E have no exact role in preventing the mucosal cells from free radical damage and they easily and rapidly convert themselves back to their original form by redox processes.

In our study on antioxidant estimation by FRAP assay, *E. officinalis* pulp showed the highest antioxidant activity at 124.6 trolox equivalent mg / g of dry weight of extract. However, out of the four *Datura metel* extracts, *Datura metel* bud showed the highest content at 3.16 trolox equivalent mg / g of dry wt. of extract.

According to Ghosal et al., (1996) *E. officinalis* fruit contains two new hydrolysable tannins emblicanin A (2,3-di-O-galloyl-4,6-(S)-hexahydroxydiphenoyl-2-keto-glucono-d-lactone) and emblicanin B (2,3,4,6-bis-(S)-hexahydroxydiphenoyl-2-keto-glucono-d-lactone) and other tannins like punigluconino (2,3-di-O-galloyl-4,6-(S)-hexahydroxydiphenoylgluconicacid) and pedunclagin (2,3,4,6-bis-(S)-hexahydroxydiphenoyl-D-glucose) which have been reported earlier. They have also been found to preserve erythrocytes against oxidative stress induced by asbestos, generator of superoxide radical. According to them, emblicanin A when comes in contact with asbestos forms emblicanin B and together they have a higher protective and antioxidant action for erythrocytes than vitamin C. They also have been seen to improve the potential activity of vitamin C in reducing dihydroascorbic acid to ascorbic acid.
In our results, the ascorbic acid equivalent antioxidant activity was highest in *E. officinalis* pulp. High antioxidant activity in the fruit of *E. officinalis* proves the presence of maximum phytochemicals after the ripening of the fruit. Nevertheless, FRAP method is appropriate to measure the total antioxidant capacity and state of medicinal herbs if it is used in phytotherapy.

Not many reports till now have been seen on the antioxidant content of *D. metel*. Though, *D. metel* is a potential source of a large number of plant secondary metabolites like, a number of alkaloids including hyoscine, hyoscyamine, meteloidine, scopolamine, tigloidine, tropine, withametelline and daturametelins (Rastogi and Mehrotra, 1998). In our study we found the antioxidant activity of *D. metel* bud to be 3.16 trolox equivalent mg / g of dry weight of extract. According to Akharaiyi, (2011) the aqueous extract of *D. metel* stem and leaves exhibited 3.41 % and 25.51 % of antioxidant activity. The difference in the results is because of the chemical methods used for evaluating the antioxidant content. In our method, we spectrophotometrically analysed the result, however, Akharaiyi, (2011) analysed the antioxidant activity on the basis of the coloured spots obtained by TLC and the use of chemicals. Ramadan *et al.*, (2007) had characterized only the *D. metel* seeds extract and found a high amount of phytosterols along with stigmasterol, β-sitosterol, lanosterol, Δ5-avenasterol and sitostanol. They also revealed that the major component present was γ-tocopherol 80 % in this extract. The presence of numerous phytosterols in *D. metel* enhances its role as a good antioxidant.

DPPH (1,1-diphenyl-2-picrylhydrazyl) is found to be a stable free radical because of the presence of the spare electron delocalization. DPPH assay is one of the best antioxidant activity estimation methods, which is frequently used and accurate. The delocalization results in a deep violet colour with $\lambda_{\text{max}}$ as 515 nm. DPPH, a violet colored, stable free radical is reduced to the yellow-colored diphenyl-picrylhydrazine when antioxidants are added. More the decolorization more is the reducing ability. DPPH assay has been the most accepted assay for evaluating the free radical scavenging activity of any new drug (Patel and Patel, 2011).

The same standard compound, trolox as used in FRAP assay was used for the quantification of the antioxidant power by DPPH assay. Result was expressed in mg of trolox equivalent per gram of dry weight of extract.
E. officinalis pulp showed the highest antioxidant activity by DPPH assay at 58.06 trolox equivalent mg / g of dry weight of extract. However, out of the four D. metel extracts, flower and bud showed the highest content at 1.23 and 1.16 trolox equivalent mg / g of dry weight of extract. High antioxidant activity in the E. officinalis fruit may also be because of the presence of phenolic compounds, as hydrophilic antioxidants are found be present mostly in fruits (Macheix et al., 1990).

The results of DPPH assay were in correlation with the results obtained through FRAP assay. According to a study by Thaipong et al., (2006) the DPPH and FRAP assays does not shows much difference among determinations and this is because the working solutions in both DPPH and FRAP assays are used immediately after preparation while that of ABTS assay is required to be kept in dark for 12 hours and then is used within 4 hours.

ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) is a radical cation decolorisation technique. A blue/green ABTS•+ chromophore is generated due to the reaction between ABTS and potassium persulfate. The absorption maxima of the ABTS assay was at λ=734 nm. The addition of the antioxidants to the pre-formed radical cation reduces ABTS on a time scale dependent antioxidant activity. The extent of decolorization ABTS•+ radical cation is based on the concentration and time and is calculated relative to the reactivity of the standard trolox. ABTS method is not only applicable to the water soluble antioxidants but also to the lipid soluble antioxidants, the pure compounds and food extracts (Re et al., 1999).

Trolox was used as a standard compound for the quantification of the antioxidant power by ABTS assay. Result was expressed in mg of trolox equivalents (TE) per gram of dry weight of extract.

The working solution in the ABTS assay was treated in dark for 12 hours to generate free radicals from the ABTS salt and then only consumed within the next 4 hours. Since, there was a difference in the age of the ABTS working solution, the reaction between the solution and the tested plant extracts might be different among the determination times (Thaipong et al., 2006).

E. officinalis pulp showed the highest antioxidant activity by ABTS assay at 175.76 trolox equivalent mg / g of dry weight of extract. However, out of the four Datura
metel extracts, bud showed the highest content at 2.12 trolox equivalent mg/g of dry weight of extract.

The result shows almost 3 times more antioxidant activity in E. officinalis fruit by ABTS assay than by DPPH assay and the antioxidant activity in Datura metel bud is consistent in all the 3 assays evaluated. This is explained by Ozgen et al., (2006) since, the fruit extracts contain a number of different and majority of phenolic compounds, comparison of the antioxidant activity is not conclusive; difference in the averages due to the capacity for polymerization. Therefore, in our results there is a difference in the antioxidant activity by the three assays which gives different values for the fruit in absolute terms but shows the same relative ranking.

5.5 Stability of the plant extracts with antimicrobial activity at different temperature and pH:

The plant extracts were checked at different temperature range and also treated with buffers (acidic, neutral and alkaline pH) to check their stability. Stability of the plant at temperature (both high and low) were checked for shelve life, long storage and transportation. If the plant extract had the capability to be stable at extreme temperatures, it would make its handling and transport easier, less expensive and in addition it can be made easily available to the rural areas.

Stability of the plant extract at different pH levels was an important criterion for safe drug administration. The plant extract and the compound isolated from it at a particular pH has to remain active at different pH levels since it has to survive in the various pH environment of the body and particularly the digestive system while following its way for an effective drug delivery. Both pH and temperature stability were extremely important for formulation and packaging of the drug before it is into use.

Plants extracts tested for temperature stability were A. indica leaves, A. vera leaves, R. indica (petals and leaves), E. officinalis pulp, C. sinensis assamica leaves, S. aromaticum buds and D. metel (fruit, bud, leave and flower) extracts. They were treated at different temperatures like, 4 °C, 25 °C, 60 °C and 100 °C.

Results showed that A. indica leaves, R. indica (petals and leaves), E. officinalis pulp, S. aromaticum buds and D. metel (fruit, bud, leave and flower) extracts were
stable to the heat treatment and showed same zone of inhibitions at all temperatures (4 °C, 25 °C, 60 °C and 100 °C) against the *H. pylori* and the pathogenic strains. Study reveals that the compounds present in the extracts were highly stable at high temperatures and their activities against the tested pathogens were retained. However, *A. vera* and *C. sinensis assamica* leaves extract were not stable at high temperatures. Doughari, (2006) checked the antimicrobial activity of *Tamarindus indica* Linn at different temperatures at 4 °C, 30 °C, 60 °C and 100 °C for 30 min against *Salmonella paratyphi*, *Bacillus subtilis*, *Salmonella typhi* and *Staphylococcus aureus*. Their results showed that the bioactivity of the extracts remained unchanged even after the heat treatment. Also, it has been seen traditionally that the plant extracts have maintained their activity even when boiled at high temperatures for a longer period of time for the extract preparation. However, Ismail et al., (2004) in their study evaluated the total phenolic content in the vegetable extracts after 1 min heat treatment and reported a significant decline in the phenolic content in all the extracts. The reason being, since, plants contain a large number of polyphenolic compounds which differ structurally, the results are rationalized in terms of quinone oxidation intermediates and relative resonance stabilization of phenoxide ions.

Arabshahi et al., (2007) had evaluated the pH (4 and 9) and temperature (100 °C, 15 min) stability of 3 ethanolic extracts (*Moringa oleifera*, *Mentha spicata* and *Daucus carota*) and noted their effect on the antioxidant activity. Results showed that the antioxidant activity in *Mentha* and *Daucus* was higher at alkaline pH than acidic, while that of the *Moringa* extract remained unchanged. Also, the *Daucus* extract was more heat-stable than other extracts with same antioxidant activity after heat treatment. Similarly in our results, we have also observed in our results that the two bioactive components in the *A. indica* and *E. officinalis* were stable under different pH range of 2, 7 and 8. However, the 2\(^{nd}\) bioactive component (faster moving) in *S. aromaticum* bud extract was reduced at alkaline pH 8 and in *A. vera* leaves and *C. sinensis assamica* leaves extract, only the 2\(^{nd}\) bioactive component lost its activity at pH 2, 7 and 8.

Doughari, (2006) reported high antibacterial activity at acidic pH. Increased phytoconstituents activity at acidic pH was also supported by a study by Molan, (1992). Reduced bioactivity of the plant extracts at alkaline pH may be explained
that since, traditionally the local application of the plants involves the addition of high amount of potash which is a strong basic salt in nature, the activity of the extracts is drastically reduced at alkaline pH.

**5.6 Chemical characterization of the plant extracts:**

Plants extracts which showed high antimicrobial activity against *H. pylori* strains were tested for TLC separation and bioautography, they were *A. indica* leaves, *R. indica* (petals and leaves), *E. officinalis* pulp, *S. aromaticum* buds and *D. metel* (fruit, bud, leave and flower) extracts.

TLC which is a rapid technique and used for identification and segregation of plant constituents into groups was used along with semi-polar solvent to separate the plant fraction (Pascual *et al.*, 2002; Upadhyay *et al.*, 2010; Jasim, 2012).

Nine spots of Rf values 0.8, 0.72, 0.63, 0.51, 0.72, 0.7, 0.75, 0.86, 0.55 were separated with the toluene: chloroform: acetone = 40:25:35 from the nine ethanolic plant extracts tested for bioautography. This shows that ethanol was capable enough to extract a larger number of compounds. Many studies have also revealed that since, many active compounds are semi-polar in nature, it makes ethanol as an ideal solvent system for extraction (Ndip *et al.*, 2007). Solvent system toluene: chloroform: acetone (40:25:35) led to a better separation of the phytoconstituents in the ethanolic plant extracts considered in our study. However, it was observed that certain solvent system like, butanol: acetic acid: water (1:1:1), ethyl acetate: hexane (6:1 and 8:2), chloroform: methanol: water (4:3:2), methanol: acetic acid: water (1:1:1) etc. caused degradation of antimicrobial compounds and there was loss of bioactivity in contact bioautography.

Due to the very fastidious nature of *H. pylori*, initially it was difficult to perform contact bioautography as it resulted in frequent contamination on the assay plates and thus, the TLC plates from thereon were handled carefully. The dried TLC chromatogram was placed with face down onto the inoculated agar plate to enable diffusion for the observation of the bioactive spots against *H. pylori* strains.

For better visibility of the zones of inhibition by the plant extract’s active component, charcoal was added to the media. Nine Rf values of 0.8, 0.72, 0.63, 0.51, 0.72, 0.7, 0.75, 0.86, 0.55 from the TLC spots revealed antimicrobial activity by
contact bioautography against \textit{H. pylori}. The most active bioactive spots from \textit{D. metel} leave (Rf 0.86) extract was considered for further characterization.

Chemical characterization of the bioactive spots was done to determine the functional group present in them as recommended by Fiedler, (1993) and Pascual et al., (2002). After running and drying of the TLC sheets containing plant extract, they were sprayed with different chemical sprays (dragendorff for alkaloids, phosphomolybdic acid for essential oils and FeCl$_3$ for tannins and phenolics and TLC sheets were observed in the visible wavelength for the colour change.

Our results showed that the bioactive spot of \textit{A. indica} was positive for alkaloids, essential oil, tannins and phenolics. This result was in correlation with the findings from Joshi et al., (2011) reporting the presence of essential oil, tannins and phenolics. Similarly, the bioactive spot of \textit{R. indica} (petal and leaves) was found to be positive for essential oil, tannins and phenolics and in accordance with the result, the presence of flavonoids, saponins, tannins, steroids and phenols was indicated through the phytochemical screening by Sahoo et al., (2011). The bioactive spot of \textit{E. officinalis} was positive for essential oil, tannins and phenolics and it is in accordance with result provided by Ghosal et al., (1996) which have reported the presence of hydrolyzable tannins, emblicanin A, B, punigluconin and pedunculagin in \textit{E. officinalis} fruit extract. The bioactive spot of \textit{S. aromaticum} bud contained alkaloids, essential oil and phenolics. Singh et al., (2012) have also reported the presence of polyphenols, gallic and ellagic acid derivatives, tannins and flavonol glycosides in \textit{S. aromaticum} buds. Although, the bioactive spot reported by us did not contain tannins. The bioactive spots of \textit{D. metel} fruit, bud, leaves and flower were positive for alkaloids, essential oil, tannins and phenolics. According to Rastogi and Mehrotra, (1998) \textit{D. metel} is a potential source of a large number of plant secondary metabolites like, a number of alkaloids including hyoscine, hyoscyamine, meteloidine, scopolamine, tigloidine, tropine, withametelline and daturametelins are present.

Even atropine which is a hallucinating compound is reported to be present in all the species of \textit{Datura} (Jakabova et al., 2012). The activity of atropine was checked against the \textit{H. pylori} strain in our study and it did not give any zone of inhibition. The result suggests that the isolated fraction of \textit{D. metel} leaves was not atropine.
The bioactive spots after TLC run and contact bioautography were scrapped and eluted in ethanol. Plant extracts tested were *R. indica* (petals and leaves) and *D. metel* (fruit, bud, leaves and flower). Results showed that both the tested extracts (*R. indica* and *D. metel*) retained its activity against the tested *H. pylori* strains (26695, J99 and SS1).

On the basis of antioxidant and antimicrobial activity of plant extracts against *H. pylori* (both reference and clinical isolates), *D. metel* leave was selected for further study. Moreover when different parts of *D. metel* were compared for antimicrobial, antioxidant activity along with cytotoxicity study, the leaves of the plant were selected since it showed no cytotoxic effect on the human cell lines, highest antimicrobial activity with highest flavonoid content and relevant antioxidant activity making it the best choice in terms of availability and abundance as a raw material from nature.

5.7 Isolation of bioactive molecule with anti-*H. pylori* activity from *Datura metel* leaves extract by column chromatography:

The *D. metel* leaves extract was purified by column chromatography and all the fractions were checked for antimicrobial activity against the *H. pylori* strain SS1 by disc diffusion assay and they were also run in TLC for separation. Results showed column fraction no. 18 (16 µg/µl) with a single spot of Rf value 0.75 in solvent system 5 % methanol in chloroform with inhibition of 25 mm against tested *H. pylori* strain.

5.8 Estimation of phytochemicals and antioxidant activity in the isolated fraction of the *Datura metel* leaves:

Phytochemical constitutes of the crude extracts were checked and then the isolated bioactive column fraction no. 18 was also checked for the presence of phytochemicals. The initial flavonoid content in the crude extract was 8.24 mg of rutin equivalents per g of dry weight of extract and the purified fraction showed flavonoid content to be 3.873 mg of rutin equivalents per g of dry weight of fraction with 0.83 mg trolox equivalent per g of dry weight of fraction as antioxidant activity.

5.9 Identification and purification of the isolated bioactive fraction of *Datura metel* leaves by HPLC (high performance liquid chromatography):
Final purification of the isolated bioactive compound from the extract of \textit{D. metel} leaves was done by HPLC. The HPLC chromatogram showed two sharp peaks at 15\textsuperscript{th} and 17\textsuperscript{th} min retention time. Fractions were collected and only the 1\textsuperscript{st} peak i.e. the fraction collected at 15\textsuperscript{th} min of loading showed anti-\textit{H. pylori} activity. Dabur \textit{et al.}, (2003) identified a new pyrrole derivative with anti-fungal activity from the \textit{D. metel} leaves by using acetonitrile: water (70:30) as solvent system and we used methanol: 5 \% acetic acid (27:73).

5.10 Scanning electron microscopic (SEM) study of the \textit{H. pylori} cells treated with the isolated bioactive fraction:

Effect of the isolated bioactive fraction on \textit{H. pylori} was studied by electron microscopy. The \textit{H. pylori} cells were treated with the isolated bioactive fraction and compared with control treated with only solvent (70 \% ethanol). In addition to the cell disruption, \textit{H. pylori} cells showed degradation of the cell walls, turned coccoid and into clusters whereas no such effect was observed in the control \textit{H. pylori} cells.

According to Willen \textit{et al.}, (2000) \textit{H. pylori} exist in two morphological forms: spiral and coccoid. The coccoid form is usually viable but not cultivable. The reason for the coccoid form is controversial till now. Reports suggest coccoid form as dead \textit{H. pylori} cells and there are also reports that the \textit{H. pylori} acquires coccoid shape when exposed to stress (Mizoguchi \textit{et al.}, 1998)

5.11 Characterization of the isolated bioactive compound of \textit{Datura metel} leaves by HPTLC (high performance thin layer chromatography):

The isolated bioactive compound was characterized using HPTLC. Standards like hisperidine (0.5 mg/ml) and quercetin (1 mg/ml) were run along with the purified compound. Results showed that bioactive fraction co-migrated (Rf 0.57) with quercetin by HPTLC and Thakker \textit{et al.}, (2011) have reported the Rf value of quercetin as 0.55 in their study.

As known, quercetin is the found to be most abundant flavonoid molecule. It has been reported to prevent the gastric mucosal lesions (Martin \textit{et al.}, 1988; 1993). Quercetin also increases the content of neutral glycoproteins which are the most abundant and important in the gastric mucosa (Di Carlo \textit{et al.}, 1999). Finally the
isolated bioactive fraction from *D. metel* leaves shows to be either quercetin or fraction very rich in quercetin with high anti-*H. pylori* activity.