THERAPEUTIC POTENTIAL OF SEAWEED BIOACTIVE COMPOUNDS AGAINST *Helicobacter pylori*

T. VinodhKumar¹*, G. Ramanathan², Henson Jebajose³, B. Ambika⁴

¹ Department of Microbiology AVS College of Arts and Science, Salem, Tamil Nadu, India.

², ⁴ Post Graduate Department of Microbiology, Sri Paramakalyani College, Alwarkurichi-627412.

³ Centre for Marine Science and Technology, Manonmaniam Sundaranar University Rajakkamangalam, Nagercoil, Tamil Nadu, India.

Corresponding Author - T. VinodhKumar¹

ABSTRACT

The prevalence of *H. pylori* is closely tied to socioeconomic conditions and accordingly, this infection is more common in developing countries than in developed countries such as the United States. Regardless, it has been estimated that 30–40% of the U.S. population is infected with *H. pylori*. Antimicrobial resistance is largely responsible for treatment failures. Resistance to metronidazole can frequently be overcome by increasing the dose and duration of treatment with acid suppression. Seaweeds or marine macroalgae are the renewable living resources which are also used as food, feed and fertilizer in many parts of the world. Seaweeds are of nutritional interest as they contain low calorie food, but rich in vitamins, minerals and dietary fibres. In these present investigation metabolites from seaweeds was evaluated for their antihelicobacterial activity. Two *Helicobacter* isolates were found in the samples and have been characterized based on selective biochemical and morphological characteristics. The isolates were screened for their antibiotics resistance / sensitivity pattern against twelve commercially availed antibiotics. Among the antibiotics ten of them exhibit sensitivity to the *Helicobacter pylori* isolates. But the isolates showed resistance to Nystatin, omeprazole, metronidazole, ranitidine, trimethoprim and methicillin. There are eleven marine seaweeds were collected from
Thondy coastal region and they were screened for antihelicobacterial activity with crude metabolites. Among the seaweeds screened against both *Helicobacter pylori* isolates the seaweed *Padina tetrastomatica* (13, 16mm), *Halmiola sp* (9, 12mm) and *Gracilaria edulis* (6, 20mm) exhibit better antihelicobacterial activity in *in vitro* assay. The mass spectral data of LC-MS report revealed that the peaks indicated, may be the presence of phenolic compounds and sulphated polysaccharides which were compared based on earlier reports on seaweeds.

**Keywords** – Seaweed, Antibiotics, Antimicrobial resistance, Gracilaria

### 1.0 INTRODUCTION

Peptic ulcer, lesion that occurs primarily in the mucous membrane of the stomach or duodenum (the upper segment of the small intestine); Between 10 and 15 percent of the world’s population suffers from peptic ulcer. Duodenal ulcers, which account for 80 percent of peptic ulcers, are more common in men than in women, but stomach ulcers affect women more frequently. The symptoms of gastric and duodenal ulcer are similar and include a gnawing, burning ache and hunger like pain in the mid-upper abdomen, usually experienced from one to three hours after meals and several hours after retiring. In the early 1980’s Australian researcher, Barry Marshall challenged previous theories of ulcer development with evidence that ulcers could be caused by *H. pylori*. *H. pylori* exists the world over and its prevalence in the population increases with age. In developed countries, prevalence increases about 1% per year of age where it is rare in children, and reaches 70% in the seventh decade. In developing countries, more than 50% children acquire the infection by the age of 10 years, and more than 80% of the population gets infected by the age of 20 years. In asymptomatic individuals prevalence of *H. pylori* infection varies from 31%-84%.

A recent study of 655 subjects from a teaching hospital in Rome found an overall prevalence of infection of 40%, with a higher prevalence among nurses and auxiliary employees than among physicians. Recently, *H. pylori* were defined as a class 1 carcinogen by the World Health Organization (Honda *et al.*, 1998). Despite of host’s vigorous immune response, the organisms evade humoral and cellular immune mechanisms and persist even for decades in the stomach environment (Baldari *et al.*, 2005). The currently most used eradicating treatment strategies
for *H. pylori* infection relies on a triple antibiotic therapy, but the cost of these drugs, poor patient compliance and emerging of antibiotic-resistant strains are some of the drawbacks of these therapies. In addition successful eradication therapy does not protect the host from re-infection (Unge 1999). Vaccination against *H. pylori* becomes an attractive approach for both therapeutic and prophylactic purpose. Various antigens which known to be involved in the pathogenesis of the infection have been proposed as suitable vaccine candidates, such as urease, the cytotoxin-associated antigen (Cag A), the vacuolating cytotoxin (Vac A), the *Helicobacter pylori* adhesion A (Hpa A), and others. The urease which is expressed by almost all *H. pylori* isolates is one of the most essential enzymes for virulence and colonization of *H. pylori* in the gastric mucosa by neutralizing the microenvironment of *H. pylori* in the stomach (Porta *et al.*, 1995). This enzyme consists of two major subunits, UreA (26.5 kD) and UreB (61 kD). The UreB is considered as a reliable vaccine candidate antigen and found to be protective in mice (Smythies *et al.*, 2005).

The development of safe anti- *H. pylori* compounds is therefore desirable. Studies have documented that some medicinal plant extracts have antibacterial activities, including *H. pylori* (Cowan, 1999; Isogai *et al.*, 2000; Funtogawa *et al.*, 2004; Ndip *et al.*, 2007). The course of treatment against helicobacteriosis is usually based on classic triple therapy including proton pump inhibitors and antibacterial therapy, clarithromycin and amoxicillin. For allergic patients, amoxicillin could be replaced by metrodinazole. The consumption of foods that inhibit the growth of bacteria may provide an alternative to current therapies or complement and expedite current treatments (Keenan *et al.*, 2010). Since seaweeds possess various classes of chemical constituents with pharmacological importance. The present study was initiated to screen antihelicobacterial efficacy of seaweeds.

**MATERIALS AND METHODS**

**Sample collection**

A total of 5 individuals (2 male; age 50-55 years, and 3 female; age 23-45 years). Those individuals who had previously received ulceration symptoms in stomach. Gastric juice as possible 5 to 10 ml was collected from each individuals during early morning episodes of intermittent reflux which may facilitate the passage viable organisms into the mouth.
**Bacterial isolates**

A total of two clinical isolates of *H. pylori* obtained from the gastric mucosa of patients. It was plated on Egg Yolk Emulsion agar medium. Suspicious growth was noted; bacteria were sub cultured and were incubated for 4 days in a micro aerobic gas environment which was handled in candle jar method. Bacterial growth was identified as *H. pylori* on the basis of colony morphology; positive biochemical reaction for catalase, urease, and oxidase and negative Gram stain.

**Collection of seaweeds**

Seaweeds (*Gracilaria edulis, Sargassum wightii, Bryopsis sp, Jama sp, Caloroba grapes, Euchoma sp, Chondrococus sp, Padina tetraestomatica, Caloroba sp, Gracilaria sp, Halmiola sp*) samples which were healthy and fully grown and submerged underwater from the tide pools were collected from Thondi Coastal region Ramnad District. The samples were washed with seawater and freshwater to remove salt, epiphytic microorganisms and other suspended materials. The clean algae were frozen. The dry material was stored.

**Extraction of bioactive metabolites from marine algae**

Organic solvent methanol was used for extraction. Each powdered sample (5g) was soaked in about 40 ml of the solvent for three days. The resultant crude extracts were filtered and then concentrated in a rotatory evaporator at a temperature of less than 400C. The residual water was removed with a vacuum pump. The crude extracts were weighed and deep frozen (-200C) until testing.

**Analysis of phytochemical constituents**

The crude extracts from chosen seaweeds were subjected for the qualitative identify of different classes of natural compounds, using the methodology of Sofowora (1982). The major phamaceutically valuable phytochemical compounds *viz.*, alkaloids, flavanoids, steroids, terpenoids, total sugars, total protein, tannin, phenolic compounds, anthroquinone, and saponins were investigated in the present study.
**Antibacterial activity of seaweed extract**

The helicobacterial activity was determined by well diffusion method. Muller Hinton Agar (MHA) was (3.1 g/100 ml) weighed and dissolved in 100 ml of distilled water in a sterile conical flask using test organism. The medium was sterilized by autoclaving and was allowed to cool at room temperature. The medium was poured into the sterile Petri plate. The inoculated plates were kept aside for few minutes, using well cutter. Two wells are made in those plates at required distance. In each step of well cutting, the well cutter was thoroughly wiped with alcohol, using sterilized micropipette, 100μl of methanol solvents with selected seaweed extract was added into one well and in another well the same volume of corresponding controls. The plate was incubated at 37°C for overnight. Inhibition of microbial growth was determined by measuring the diameters of zone of inhibition.

**FTIR spectroscopy analysis**

The maximum zone producing seaweeds were selected and it was analyzed by FTIR Spectroscopy method. The seaweed extract samples (10 mg) was mixed with 100 mg of dried Potassium bromide (KBr) and compressed to prepare as a salt disc. The disc was then read spectrometrically. The frequencies of different components present in active sample were analyzed.

**HPLC analysis**

Data was obtained at 280 nm by using UV-DAD (UV diode array detector). Data consisted of various peaks for crude extracts with their respective retention times. Analytical grade HPLC (high performance liquid chromatography) was performed on a Varian 9021 solvent delivery system equipped with Varian 9065 Polychrom UV-diode array detector (190-367 nm). Data was processed by Star Polychrom version 5.2. The system was maintained in a controlled room temperature at 21±10°C. A flow rate of 1ml/min-1 and injection volume of 10 μl were used. Sample analysis was performed by gradient elution on a 150 mm x 4.6 mm i.d., 5uM, Luna C-18(2) column (Phenomenex, Australia) with guard column (Phenomenex, Australia). The mobile phases were freshly prepared and degassed under vacuum using Phenomenex nylon 45 μm membranes and sonicated in a sanophon ultrasonic bath (Ultrasonic Industries Pty. Ltd, Sydney, Australia) for 15 minutes prior to HPLC analysis.
**LC-MS (Liquid chromatography mass spectrometry)**

Liquid chromatography-mass spectrometry (LC-MS) of the methanolic extracts of selected seaweed species was performed on a Micromass Quattro micro tandem quadrupole mass spectrometer (Waters, Manchester, UK). LC separation was attained by a Waters liquid chromatograph (Waters, Milford, USA), consisting of a 2695 Separation Module and 2487 dual wavelength UV detector operated at 240 and 280 nm. Columns and gradients were same as described previously for analytical scale HPLC. An injection volume of 10 μL and a constant flow of 1 ml/min were used for each analysis. The entire flow from the LC was directed into the mass spectrometer. Data was acquired by the Masslynx data system for both the MS and UV data.

**Molecular mass from LC-MS data**

LC-MS chromatograms obtained for two crude extracts were analysed by Masslynx to get spectra of various peaks. Spectra were recorded in negative and positive ion mode to estimate molecular masses of various components. Only major peaks in each chromatogram were analyzed for determination of molecular masses. Determination of molecular mass from LC-MS data can be a useful tool to identify components on the basis of molecular masses.

**RESULT & DISCUSSION**

In this present investigation there are two Helicobacter isolates retrieved from five patients with the Age group of 23-53 was analyzed. Among them 3 female and 2 male all are susceptible to ulcer symptoms of intermitted reflux. The isolates were subjected for to screen against commercially availed antibiotics to evaluate their sensitivity / resistant pattern against 12 antibiotics. Among the two strains adopted, the bacterial isolates 2, was more susceptible than isolate 1. *Helicobacter* isolate 1 was resistance to Nystatin, Omeprazole, Metronodazole and Ranitidine. Su et al., (2013) revealed that the resistance rates to clarithromycin, metronidazole, levofloxacin, amoxicillin, gentamicin and furazolidone were 21.5, 95.4, 20.6, 0.1, 0.1 and 0.1%, respectively. Double, triple and quadruple antibacterial resistant percentages were 25.5, 7.5 and 0.1%, respectively. A positive association between the resistance to levofloxacin and to clarithromycin was found, but there was a negative correlation in the resistances to levofloxacin and to metronidazole.
Antihelicobacterial efficacy was accessed by crude seaweed extracts, which was sourced from Thondy coastal region against Helicobacter isolates 1 and 2. It was found that, among the seaweed extract screened, the seaweeds *Gracilaria*, *Padina* and *Halmolia* exhibit better antihelicobacterial activity. Among the Helicobacter isolates, the isolate 2 was more susceptible with seaweed extract. Time kill assay was also performed; it was found that, higher concentration of seaweed extract of both *Padina tetrastomatica* and *Gracilaria edulis* control the multiplication of the pathogen with increased concentration at 12 hours.

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The potential seaweed extracts which shows significant helicobacterial efficacy was further evaluated to characterize the active metabolites by FTIR, HPLC and LC-MS analysis based on that it was indicated that the active principles may be phenolic, sulphated polysaccharides which is present as major phytochemical constituents in seaweeds. Time hill assay was performed against *H. pylori* isolated by using potential seaweed metabolites with 1x, 4x, and 16x concentrations for 12 hours. Efficiency of reduction in bacterial population was collected in log CFU for every 3 hours incubation. It was found that 16x concentrations of all the four seaweed extracts reduced the population considerably. FTIR analysis was followed to the potential seaweed extract. The peak value reveals the stretching point of the particular compound which is present in the seaweed extract. *Gracilaria edulis* have 29 peaks and *Padina tetrastomactica* have 31 peaks.

Retention times of all the compounds which are presented in the extracts were detected. The identification of the peaks is based on the analysis of their retention time. The Retention time of identified peaks for *Gracilaria edulis are* 1.400, 2.183, 2.303, and 2.753 and
for Padina tetrastomatica is 1.427, which indicates the presence of halogenated / sulfated polysaccharides based on earlier reports on seaweed bioactive metabolites. The peak value of Gracilaria edulis and Padina tetrastomatica were analyzed and showed in. The major peaks obtained at four different alternative scans. The mass spectral data revealed that the peaks indicated, may be the presence of phenolic compounds and sulphated polysaccharides which were compared based on earlier reports on seaweeds.
Fig: 3 FTIR Analysis of *Gracilaria edulis*

![FTIR Analysis of Gracilaria edulis](image)

Fig: 4 FTIR Analysis of *Padina tetrastomatica*

![FTIR Analysis of Padina tetrastomatica](image)
Conclusion

In humans, *Helicobacter pylori* colonizes the stomachs of about half of the world’s population and highly associated with the number of most important disease of the upper gastrointestinal tract particularly prevalence in developing countries. Eradication of the *H. pylori* primarily dependent on continued application of antibiotics. Although effective, repeated use of these chemical drugs have sometimes resulted in the development of resistance and had undesirable effects. These problems have highlighted the need for the development of new strategies for selective *H. pylori* eradication. Marine plants may be an alternative source of materials for the *H. pylori* eradication because they constitute a rich source of bioactive chemicals. The present study also found that the seaweeds *Gracilaria edulis, Padina tetrastomatica* showed antihelicobacterial activity. The importance of seaweed for human consumption is well known in many countries, they offer a good source of recovery of various useful chemicals particularly polysaccharide present in most of the seaweeds may used for the treatment of intestinal and stomach disorders. In this present investigation *Padina tetrastomatica* has proven antihelicobacterial activity based on the outcome of this investigation the active metabolites derived from seaweeds may be an
alternative therapeutic agent for the treatment of Helicobacter infection but still further
evaluation and complete structural characterization of active metabolites is warranted

**Bibliography**

Anna Babarikina, Vizma Nikolajeva and Dmitry Babarykin. 2011. Anti-*Helicobacter* Activity of Certain Food Plant Extracts and Juices and their Composition *in Vitro*

*Food and Nutrition Sciences, 2*: 868-877.


Feng Ma, Ye Chen, Jing Li, He-Ping Qing, Ji-De Wang, Ya-Li Zhang, Bei-Guo Long and Yang Bai. 2010. Screening test for anti-Helicobacter pylori activity of traditional Chinese herbal medicines. *World J Gastroenterol*. 16(44): 5629-5634


Santhanam Shanmugapriya, Aseer Manilal, Sugathan Sujith, Joseph Selvin, George


