Int.J.Curr.Microbiol.App.Sci (2020) *9*(9): 3225-3231

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 9 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.909.399

In-vitro Antibacterial Activity of the Product 'Xembran®' against *Helicobacter pylori*

Pankaj P. Gaonkar[1*], A. M. Das[2], Maithili Rembhotkar[3] and Amit Purnendu Goel[3]

[1]Department of Veterinary Pathology, Nagpur Veterinary College, Nagpur, Maharashtra India
[2]Gayatri Laboratory, Mumbai, Maharashtra India
[3]Grocare, Pune, Maharashtra, India

**Corresponding author*

Keywords

Helicobacter pylori, *Myristica fragrans*, Antimicrobial resistance, Xembran

Article Info

Accepted:
20 August 2020
Available Online:
10 September 2020

A B S T R A C T

Helicobacter pylori is a global pathogen, ultimately affecting fifty percent of the population of the world. It has developed resistance to routinely used antibiotics. Subsequently, it is imperative to locate an alternative treatment for *Helicobacter pylori.* Xembran®, a combination of *Myristica fragrans* and pure Shankha Bhasma was tested for its anti-*Helicobacter pylori* activity. The activity of Xembran was determined by disc diffusion and the minimum bactericidal concentration method. The complete inhibition of *Helicobacter pylori* was achieved at a concentration of 70mcg by the disc diffusion method. Xembran was able to show greater activity against *Helicobacter pylori* with an increase in contact time. However, 0.5% concentration was adequate to execute the bactericidal effect on Helicobacter *pylori* with a contact time of 20 minutes. Xembran can serve as an alternative to routinely used antibiotics. Thus, it is a potential agent to combat *Helicobacter pylori.*

Introduction

Helicobacter pylori is a human gastric motile, spiral, flagellated, microaerobic, Gram-negative pathogen. The global prevalence of *H. pylori* infection is 44.3%. However, it is highly prevalent in developing countries than in developed countries. Better hygiene practices and socioeconomic status are keeping a check on this infection in developed countries (Zamani *et al.*, 2018). *H. pylori* have infected almost half of the world's population. Hence it carries a tag of "Global Pathogen" (Zamani *et al.*, 2018). *H. pylori* can cause gastric cancer, irritable bowel syndrome (IBS), urolithiasis, gastritis, and piles/haemorrhoid (Helvaci *et al.*, 2009; Wroblewski *et al.*, 2009). It is also responsible for chronic disease leading to gastric adenocarcinoma or peptic ulcer (Basso *et al.*, 2010). The primary interaction of *H. pylori* takes place with the gastric mucosa epithelial cells. Their interaction promotes the production of DC activator and DC attracting chemokines. However, *H. pylori* promote the Treg response (Kao *et al.*, 2010; Kido *et al.*,

2010). It confers its pathogenicity by evading the immune system. Oncogenic effects are due to an imbalance between proliferation and apoptosis. Antiapoptotic action is a result of increased and decrease in expression of BCL1 and BAX respectively (Yan *et al.,* 2009). *H. pylori* is difficult to eradicate (cure) from the stomach because it is capable of developing resistance to commonly used antibiotics (antibiotic-resistant *H. pylori*). Therefore, two or more antibiotics usually are given together with a PPI and/or bismuth-containing compounds to eradicate the bacterium Graham *et al.,* 2000). WHO has classified clarithromycin resistant *H. pylori* as a high priority pathogen; seeking new antibiotics for its treatment (Palmitessa *et al.,* 2020). Hence it is a need of an hour to find an alternative treatment for *H. pylori*. In the pursuit of finding novel antimicrobials; herbal and Ayurveda origin drugs have found their place as a safe, reliable, and efficient tool to counter infectious diseases. Xembran® contains potent bioactive like Myristica fragrans and pure Shankha Bhasma. Shankha Bhasma has anti-ulcer, antacid properties, and also acts as a gastric cryoprotective agent (Richa et al.,1997; Pandit et al.,2000; Sur et al.,2013). Myristica fragrans has a broad range of antibacterial, anti-inflammatory, analgesic, and antioxidant activities. Dihydroguaiaretic acid in Myristica fragrans might be responsible for anti-*H. pylori* activity (Asgarpanah and Kazemivash, 2012). The present study was undertaken to determine the anti-*H. pylori* activity of the Xembran®; to find an alternative to routinely used antibiotics.

Materials and Methods

Test organism

Helicobacter pylori (Local isolate) was cultured on 5% sheep blood agar (5% SBA), brain heart infusion broth (BHIB) containing 5% horse serum + antibiotic supplements (vancomycin – 6 µg/ml, nalidixic acid - 20 µg/ml and amphotericin-B - 2 µg/ml) and Skirrow's campylobacter selective medium (SCSM) held at 37°C under microaerophilic (5% O2 + 10% CO2) atmosphere in an anaerobic jar containing 'Anaerogen' gas packs without catalyst for seven days. Cultures were maintained on 5% SBA slants at 4°C and subcultivation every week. A loopful of growth of the test strain was picked up from 5% SBA and mixed in a test tube containing 10 ml of dist. water till such time the opacity of the tube matched with the Brown's tube No. 3 corresponding to 9 X 10^7 organisms per ml. This served as a working inoculum. Grams staining, catalase, oxidase, urease, nitrate reduction tests, and the ability to grow at 37°C and 42°C was determined for the identification of *H. pylori*. Cephalothin and nalidixic acid sensitivity testing were performed (Adinortey *et al.,* 2018).

Screening of possibility of indigenously present *Helicobacter pylori* in the product – Xembran®

Five milligrams of Xembran® (powder) was added to 25ml of BHIB containing 5% horse serum + antibiotic supplements (vancomycin – 6 µg/ml, nalidixic acid - 20 µg/ml and amphotericin-B - 2 µg/ml). The swab of the suspension was plated onto the SCSM and incubated at 37°C under microaerophilic (5% O2 + 10% CO2) atmosphere in an anaerobic jar containing 'Anaerogen' gas packs without a catalyst for seven days. Plates were examined for the presence or absence of small grey and translucent colonies suggestive of *H. pylori*.

Survivability of *Helicobacter pylori* in distilled water

The test organism *H. pylori* after three subcultures in 5% SBA, was inoculated with a

loop in 10 ml of sterile distilled water. An inoculum of 0.1 ml of this suspension was plated onto 5% SBA after an exposure time of 30 min, 1 hr, 2 hr, 4hr, 6 hr, 8 hr, 10 hr, and 12 hr and incubated as described earlier.

Determination of minimum bactericidal concentration (MBC) of Xembran® (powder) against *Helicobacter pylori*

Dilution of the product was done by mixing Xembran® (powder) in sterile dist. water at a concentration of 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 % in 10 ml quantity. Two milliliters of working suspension of the test strain was mixed with respective dilutions of Xembran® (powder). Twenty microlitres of each suspension (*H. pylori* + Xembran®) was poured onto the surface of SCSM and spread gently with the help of an L-shaped glass rod at various exposure times (0, 5, 10, 15, 20, 25, 30, 45 and 60 min.). All plates were incubated as described earlier. The MBC of Xembran® (powder) was recorded as the lowest concentration that killed *H. pylori* strain under test. The presence or absence of growth was ascertained by observing plates under a stereoscopic microscope. The experiment was repeated thrice to check reproducibility.

Determination of the antibacterial activity of Xembran® (powder) against *Helicobacter pylori* by disc diffusion method

The Xembran® discs were prepared. A range of 10 mcg to 100 mcg of Xembran® (powder) was made available to Whatman filter paper No. 41 (for up to 30 mcg) and to No. 1 (for 40 - 100 mcg) for absorption. Briefly, one row consisting of ten agglutination tubes were filled with 10 - 100 mcg of Xembran® (powder) and acetone in the ratio of 1: 50 (i.e. 10 mcg of Xembran® (powder) + 500 microlitres of acetone) along with specified filter paper discs. Tubes were shaken occasionally during the drying period in an incubator heated up to 37°C. About 2-10 days were required for drying depending upon the quantity of Xembran® (powder) in each tube. Such dried discs were considered "Xembran® absorbed discs", to be used in the in-vitro disc diffusion test.

A quantity of 0.1 ml of the test strain of *H. pylori* was plated onto SCSM with the help of a sterilized L-shaped glass rod and allowed to dry. Ten discs with different concentrations (10-100 µl.) of Xembran® (powder) were placed equidistantly on the plate and incubated as described earlier. The zone of inhibition if seen was measured horizontally as well as vertically and the average of both was considered the actual diameter. The experiment was performed twice to observe the consistency in results.

Results and Discussion

The test strain used in the present study was spiral-shaped with gram-negative character. Biochemical tests characterized it as positive for Catalase and Oxidase, positive for Urease activity, and negative for nitrate reduction. *H. pylori* were distinguished from *H. cinaedi* and *H. fenneliae* based on the ability of our test strain to grow at 37°C and not at 42°C. The test organism was sensitive to cephalothin and resistant to nalidixic acid. *H. pylori* grew only at 37°C and not 25°C or 42°C affirms that the organism is neither *Helicobacter cinaedi* nor *Helicobacter fenneliae*. It is in accordance with the previous report (Adinortey *et al.*, 2018).

The product Xembran® was free of the indigenous presence of *H. pylori*. The test strain of *H. pylori* failed to survive in distilled water after 8 hrs. The results of the antimicrobial efficacy of Xembran® against *H. pylori* are presented in Table 1 and Table 2. Disc concentration of 70 mcg onwards of

Xembran® (powder) exhibited a zone of inhibition against *H. pylori*. Xembran® (powder) in 0.1% concentration eliminated *H. pylori* after 30 minutes. Thus Xembran® powder has potent anti-*H. pylori* activity.

Table.1 Effect of Xembran (powder) on Helicobacter pylori

Dilution of Xembran	Contact time (Min)								
	0	5	10	15	20	25	30	45	60
0.05%	+	+	+	+	+	+	+	+	-
0.1%	+	+	+	+	+	+	+	±	-
0.2%	+	+	+	+	+	+	±	-	-
0.3%	+	+	+	+	+	+	-	-	-
0.4%	+	+	+	+	+	-	-	-	-
0.5%	+	+	+	+	-	-	-	-	-

+ = Growth; ± = Poor Growth; - = No Growth

Table.2 Anti-bacterial activity of Xembran (powder) on Helicobacter pylori by disc diffusion method

Disc concentration (mcg)	*Helicobacter pylori*
10,20,30,40,50 and 60	R
70	S (10.0 mm)[a]
80	S (11.5 mm)
90	S (13.2 mm)
100	S (15.8 mm)

(Disc diameter = 7 mm) [a]Zone diameter, R = Resistant, S = Sensitive

Helicobacter pylori is present in the majority of the population throughout the world. Its oncogenic capability makes it more deadly along with its ability to cause gastrointestinal diseases. Previously generated data throughout the world points out the increasing antibiotic resistance toward *H. pylori* (Ghotaslou, 2015). In our study, Xembran® was able to completely inhibit *H. pylori* at the concentration as low as 0.05% but the contact time was higher. However, the contact time of 20 minutes was sufficient for the higher concentration of 0.5% to completely inhibit the *H. pylori*. Furthermore, the concentration of more than 70 mcg had anti-*H. pylori* activity. The zone of inhibition increased with the increase in the concentration of Xembran® treated discs. Generally, the treatment regime for *H. pylori* includes the combination of antibiotics and antacids. Drugs used against Helicobacter pylori are bismuth salts, proton pump inhibitors, azithromycin, clarithromycin, metronidazole, amoxicillin, and tetracycline (Kusters and Kuipers, 2001). For anti-*H. pylori* drug to be effective it must penetrate the gastric mucus layer. The mucus layer has a lower pH. It acts as an obstacle for the efficient action of antibiotics by affecting its stability and activity (Van *et al.*, 1992; Malanoski *et al.*, 1993). Therefore, triple therapy (amoxicillin, metronidazole, or clarithromycin and proton pump inhibitor) and quadruple therapy (metronidazole, tetracycline, bismuth, and PPI,) are the most commonly employed treatment strategies. Overall the regime is a combination of antibiotics and proton pump inhibitors. However, resistance to clarithromycin,

ciprofloxacin, metronidazole, tetracycline, amoxicillin has been reported almost two decades ago (Kusters and Kuipers, 2001). Eradication of *H. pylori* faces the biggest challenge of drug-resistant strains and side effects of therapy (Palmitessa *et al.,* 2020; Alizadeh *et al.,* 2020; Hu *et al.,* 2020). Among Asian countries' highest *H. pylori* resistance to clarithromycin, amoxicillin is reported from India. However, Furazolidon resistance is at the lower side in India compared to other Asian countries. Worldwide Asian countries contributed the highest clarithromycin and levofloxacin resistance. To some extent resistance against *H. pylori* is evident throughout the world (Ghotaslou, 2015).

Xembran® two vital components are Shankha Bhasma and Myristica fragrans. Shankha Bhasma has anti-ulcer, antacid properties, and also acts as a gastric cryoprotective agent (Richa 1997; Pandit *et al.,* 2000; Sur *et al.,* 2013). Myristica fragrans has a broad range of antibacterial, anti-inflammatory, analgesic, and antioxidant activities. Dihydroguaiaretic acid in Myristica fragrans might be responsible for anti-*H. pylori* activity (Asgarpanah and Kazemivash, 2012). Organic acids, esters, unsaturated side chain on the aromatic ring in Myristica fragrans is responsible for antibacterial activity. Their target is the bacterial cell wall (Narasimhan and Dhake, 2006).

To conclude, we have proposed a novel product Xembran® to tackle *H. pylori*. WHO has already suggested the need to find newer approaches against *H. pylori*. As per our results, Xembran® can fulfill the void of the requirement of potential antimicrobial for treating *H. pylori* infections. Xembran® is a natural product; hence it can pave the way for safe treatment. Taking into account the anti pylori activity of Xembran®, we have assessed the in-vitro activity of Xembran® but further comparative studies with triple and quadruple therapy are needed. It will help to develop a novel anti-*H. pylori* treatment regime by using Xembran®.

Acknowledgments

The authors are thankful to the staff Gayatri Laboratory for providing necessary help and facilities for the successful completion of this work.

Funding

This work was supported by Grocare, Pune, India

Conflict of Interest

All authors declare no conflicts of interest related to this article.

References

Adinortey, M. B., Ansah, C., Adinortey, C. A., Bockarie, A. S., Morna, M. T., and Amewowor, D. H. 2018. Isolation of Helicobacter pylori from gastric biopsy of dyspeptic patients in ghana and in vitro preliminary assessment of the effect of dissotis rotundifolia extract on its growth. *Journal of tropical medicine*. 2018, 8071081.

Alizadeh- naini, M., Yousefnejad, H., and Hejazi, N. 2020. The beneficial health effects of Nigella sativa on Helicobacter pylori eradication, dyspepsia symptoms, and quality of life in infected patients: A pilot study. Phytotherapy Research. 34(6), 1367–1376.

Asgarpanah, J., and Kazemivash, N. 2012. Phytochemistry and pharmacologic properties of *Myristica fragrans* Hoyutt.: A review. African Journal of Biotechnology. 11(65), 12787-12793.

Basso, D., Plebani, M., and Kusters, J. G.

2010. Pathogenesis of Helicobacter pylori infection. Helicobacter. 15, 14-20.

Ghotaslou, R., Leylabadlo, H. E., and Asl, Y. M. 2015. Prevalence of antibiotic resistance in Helicobacter pylori: A recent literature review. World journal of methodology. 5(3), 164.

Helvaci, M. R., Algin, M. C., and Kaya, H. 2009. Irritable bowel syndrome and chronic gastritis, hemorrhoid, urolithiasis. *The Eurasian journal of medicine*. 41(3), 158.

Hu, Y., Zhu, Y., and Lu, N. H. 2020. Recent progress in Helicobacter pylori treatment. Chinese Medical Journal. 133(3), 335.

Kao, J.Y., Zhang, M., Miller, M.J., Mills, J.C., Wang, B., Liu, M., *et al.*, 2010. Helicobacter pylori immune escape is mediated by dendritic cell-induced Treg skewing and Th17 suppression in mice. Gastroenterology.138:1046–54.

Kido, M., Tanaka, J., Aoki, N., Iwamoto, S., Nishiura, H., Chiba, T., *et al.*, 2010. Helicobacter pylori promotes the production of thymic stromal lymphopoietin by gastric epithelial cells and induces dendritic cell-mediated inflammatory Th2 responses. Infect Immun. 78:108–14.

Kusters, J. G., and Kuipers, E. J. 2001. Antibiotic resistance of Helicobacter pylori. Journal of Applied Microbiology. 90(S6), 134S-144S.

Malanoski, G.J., Eliopoulos, G.M., Ferraro, M.J. and Moellering, R.C.J. 1993. Effect of pH variation on the susceptibility of Helicobacter pylori to three macrolide antimicrobial agents and temafloxacin. European Journal of Clinical Microbiological Infection and Disease. 12, 131-133.

Narasimhan, B., and Dhake, A. S. 2006. Antibacterial principles from Myristica fragrans seeds. *Journal of medicinal food*. 9(3), 395-399.

Palmitessa, V., Monno, R., Panarese, A., Cuppone, R., Burattini, O., Marangi, S., *et al.*, 2020. Evaluation of Antibiotic Resistance of *Helicobacter pylori* Strains Isolated in Bari, Southern Italy, in 2017-2018 by Phenotypic and Genotyping Methods. *Microbial drug resistance (Larchmont, N.Y.)*. 26(8). 909–917.

Pandit, S., Sur, T. K., Jana, U., Bhattacharyya, D., and Debnath, P. K. 2000. Anti-ulcer effect of Shankha bhasma in rats: a preliminary study. Indian Journal of Pharmacology. 32(6). 378-380.

Qureshi, W. A., and Graham, D. Y. 2000. Antibiotic-resistant *H. pylori* infection and its treatment. *Current pharmaceutical design*. 6(15), 1537-1544.

Richa, A., Kadam, H. M., Kadam, S. S., and Paradkar, A. R. 1997. Studies on shankha bhasma-I antacid activity evaluation of Shankha Bhasma. Indian journal of pharmaceutical sciences. 59(5). 254.

Sur, T. K., Pandit, S., Sen, S., Bhattacharyya, D., and Debnath, P. K. 2013. Anti-ulcer activity of shankha Bhasma (calcined conch-shell). Explor. Anim. Med. Res. 3(1), 48-56.

van Zanten, S.J., Goldie, J., Hollingsworth, J., Silletti, C., Richardson, H. and Hunt, R.H. 1992 Secretion of intravenously administered antibiotics in gastric juice: implications for management of Helicobacter pylori. Journal of Clinical Pathology. 45, 225-227.

Wroblewski, L. E., Peek, R. M., and Wilson, K. T. 2010. Helicobacter pylori and gastric cancer: factors that modulate disease risk. *Clinical microbiology reviews*. 23(4), 713-739.

Yan, F., Cao, H., Chaturvedi, R., Krishna, U., Hobbs, S.S., Dempsey, P.J., *et al.*, 2009. Epidermal growth factor receptor activation protects gastric epithelial cells

from Helicobacter pylori-induced apoptosis. Gastroenterology. 136:1297–307.

Zamani, M., Ebrahimtabar, F., Zamani, V., Miller, W. H., Alizadeh- Navaei, R., Shokri- Shirvani, J., and Derakhshan, M. H. 2018. Systematic review with meta- analysis: the worldwide prevalence of Helicobacter pylori infection. Alimentary pharmacology and therapeutics. 47(7), 868-876.

How to cite this article:

Pankaj P. Gaonkar, A. M. Das, Maithili Rembhotkar and Amit Purnendu Goel. 2020. In-vitro Antibacterial Activity of the Product 'Xembran®' against *Helicobacter pylori*. *Int.J.Curr.Microbiol.App.Sci.* 9(09): 3225-3231. doi: https://doi.org/10.20546/ijcmas.2020.909.399