ORIGINAL ARTICLE

Study of Antimicrobial Activity of Few Medicinal Plants Extracts on Growth of Helicobacter Pylori

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ABSTRACT

Background: H. pylori is a microaerophilic Gram-negative bacterium, spiral in shape. It infects approximately half of the population across the world.

Aims: to assess the antibiotic resistance of H. pylori as well as the potential of medical plant extracts to inhibit resistant strains. Materials and Methods: The study was conducted in Microbiology Institute, Shah Abdul Latif University, Khairpur Mir's from January 2019 to December 2019. Endoscopy was used to obtain samples from the gastrointestinal ward of teaching hospital of KMC, Khairpur. H. pylori was isolated, identified and inoculated. From the local market, ginger roots, garlic roots, kalonji seeds, and mint leaves were acquired and extracts were prepared.

RESULTS: Antimicrobial sensitivity of extracts (Garlic, ginger, kalonji and mint) against H. pylori was tested by agar welldiffusion method. All herbal extracts showed more sensitivity extracted in ethanol as compared to distilled water. In contrast mint did not give any results. Ethanol extracts are found to be very effective against H. pylori as compared to distilled water extracts. Conclusion: Garlic, ginger, and kalonji all demonstrated antibacterial property towards H. pylori.

Keywords: Helicobacter pylori, Antimicrobial Sensitivity, Plant Extracts, Inhibition Zone

INTRODUCTION

H. pylori is a micro-aerophilic Gram-negative bacterium, spiral in shape. It infect approximately half of the population across the world ¹. Though, rate of infection is slowly declining in developed countries because of smaller size of family, reduced congestion, and better sanitization. Occurrence in developing countries is approximately 90%, whereas in developed countries, infection drops under 40%². It is related to chronic gastritis, gastric and duodenal ulcers and mucosal atrophy of stomach. Further, it is well documented class I carcinogen as It is thought to play a role in the persistent inflammation that causes duodenal ulcers and gastric disorders. As a result, understanding the path from acute mucosal inflammation to stomach cancer is critical ³. Conventionally for eradication of these infections, multiple drugs like tetracycline, clarithromycin, furazolidone, amoxicillin and metronidazole along bismuth or PPI⁴. Though conventional treatment for H. pylori eradication give high cure rate, failure rate vary from 5 to 20 %. This can be explained by firstly by non-compliance to follow the treatment properly in some patients and secondly by the resistance of antibiotics. In several countries, dual prevalence of resistance and multidrug resistances is amplified significantly that has become a main hurdle in H. pylori eradication⁵. Consequently the exploration of safe and effective medicines for treatment of particular bacterial infections is persistent. The scientists are currently giving attention to natural yields like herbal extracts or extracts of medicinal plant to epitomize as microbial agents 6, 7. Certain medicinal herbs show marvelous multi-functional actions for disease causing microbes along with treatment for some noncommunicable illnesses8. In the future, efforts and paths toward collecting baseline data on medicinal herbs, phytochemical and pharmacological studies, and innovation are quite limited. Because of the indiscriminate use of antibiotics, which is increasing the emergence of resistant microbial strains, many scientists have concentrated on the critical need for discovering new, safe, and inexpensive antibiotics with diverse chemical structures, new chemical actions, and no adverse side effects. The existence of bioactive ingredients in medicinal plants makes these plants candidates for future studies, includina are promising pharmacological studies and drug discovery⁹. Several natural compounds have shown antibacterial action against Helicobacter pylori, and for centuries a broad range of plants and plant-derived chemicals have been utilized to treat gastrointestinal diseases¹⁰.

The purpose of this study was to assess the antibiotic resistance of H. pylori as well as the potential of medical plant extracts to inhibit resistant strains, as these medicinal plants may be used in the pharmaceutical business (as alternatives) in the future.

MATERIALS AND METHODS

Study Setting: The study was conducted in Microbiology Institute, Shah Abdul Latif University, Khairpur Mir's, in the post-graduate research laboratory (PGRL) from January 2019 to December 2019. Endoscopy was used to obtain samples from the gastrointestinal ward of teaching hospital of KMC, Khairpur. For isolation and identification of H pylori samples were transferred and processed for further analysis within 24 hours of biopsy collection. The samples were correctly labeled with the necessary patient information.

For investigation and isolation, the biopsy samples from gastro duodenal dysfunctional patients, were inoculated on Columbia, Nutrient, MH agar containing 5% blood at 6.7pH for microscopy.

Approximately 0.5 to 2 mm transparent colonies were studied from selective agar plates using Gram staining and confirmed using rapid urease test and catalase test. 1

From the local market, ginger roots, garlic roots, kalonji seeds, and mint leaves were acquired. Fresh ginger, garlic, kalonji, and mint samples were thoroughly rinsed with tap water and dried before being crushed into fine powder. All herbal samples were extracted in a 75 percent ethanol or distilled water solvent.

Isolated colonies of H. pylori were taken and inoculated in 7-8 ml of sterilized nutrient broth containing test tube. Aseptically incubate test tube at 37°C for an overnight. On next day compare and adjust the turbidity with 0.5 McFarland standard (i.e.1.58 CFU/ml), aseptically.

A 0.5 McFarland turbidity standard was made by mixing 0.05 ml of dehydrated BaCl₂ with 9.95ml of sulfuric acid to match the absorbance of 0.08 to 0.1 at 600 nm.

Overnight incubated cultures of desired stains used and inoculums prepared and standardized with 0.5 Mac Farland, a fine lawn of cells made on Muller Hinton agar (thickness 4mm), 4-5 wells were made 6mm diameter by means of cork borer and different extracts poured on each well independently the test performed in triplicate and plates were kept at 37 0C for 24 hours anaerobically. All tests were carried out in triplicate, and data was

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collected. Microsoft Excel 2013 was used to compute the mean and standard deviation.

RESULTS

104 biopsy samples were used in current research. 70 were male while thirty four were female. From total 104 biopsies just 27 samples showed growth while 77 showed no growth. Out of 27 positive samples, seventeen (17) were males while ten (10) were females with Helicobacter pylori grown as etiologic aged.

Antimicrobial sensitivity of extracts (Garlic, ginger, kalonji and mint) against H. pylori was tested by agar well-diffusion method. Garlic, ginger, kaloji and mint extracted in distilled water showed zone of inhibition as 13.041±1.20mm, 8.52±0.86mm, 5.9±0.77mm respectively. Whereas Mint did not show any sensitivity.



Figure 1: Zone of Inhibition of extracts in distilled water.

Antimicrobial Sensitivity of Extracts in ethanol Against H. Pylori.

Diameter of zone of inhibition of garlic extract was 16.05 ± 1.69 mm in ethanol. While the diameter of Ginger and Kalonji was 11.19 ± 0.96 mm and 7.44 ± 1.11 mm respectively. In contrast mint did not demonstrated any result.



Comparison of results of zone of inhibition in ethanol and distilled water

Diameter of zone of inhibition $(13.04\pm1.20$ mm) of Garlic was in distilled water extract was smaller as compared to ethanol $(16.05\pm1.69$ mm). Diameter of zone of inhibition of Ginger in distilled water was smaller (8.52±0.86mm) than in ethanol (11.19±0.96mm). Kalonji zone of inhibition was also smaller (5.94±0.77mm) in distilled water as compared to ethanol (7.44±1.11mm).In contrast mint did not showed any results in both solvents.



Figure 3: Inhibition Zone of extracts in ethanol and distilled water

Determination of Antimicrobial Sensitivity of all herbal extracts mixed as consortia Against H. Pylori in distilled water and ethanol

Consolidate of diameter of garlic & ginger extract in in distilled water was smaller (18.50 ± 0.77) as compared to in ethanol (20.36 ± 1.47 mm). Zone of inhibition of Garlic & kalonji extracted was smaller (16.30 ± 0.81 mm) in distilled water as compared to in ethanol (17.74 ± 0.57). Zone of inhibition of garlic and mint was smaller (16.24 ± 0.67 mm) in distilled water as compared to in ethanol (16.54 ± 1.13 mm). Zone of inhibition Ginger & kalonji was smaller (10.60 ± 0.70 mm) in distilled water as compared to in ethanol (11.95 ± 0.96 mm.) Inhibition zone of Ginger & mint was smaller (9.44 ± 0.32) in distilled water as compared to in ethanol (11.46 ± 1.03 mm). Inhibition zone of Kalonji & mint was smaller (6.15 ± 0.75 mm) in distilled water as compared to in ethanol (7.69 ± 1.69 mm). Inhibition zone of Garlic, ginger, kalonji & mint consortia was smaller (24.38 ± 0.70) in distilled water as compared to in ethanol (26.62 ± 1.13 mm).



Figure 4: Mean and standard deviation of extracts as consortia in ethanol and distilled water

DISCUSSION

H. pyloriis ubiquitous nature and infect both malesand females equally without any difference. Helicobacter pylori infection is not homogeneously dispersed. There exists significant difference in and between different countries. The overall style displays that the normally frequency is lesser in developing countries in contrast to developed countries.

In this study antibacterial effects of garlic, ginger, kalonji and mint were observed by the well diffusion method, MIC was observed and antibacterial activity was observed against H. pylori. From 104 endoscopic samples, just 27 were found positive for of H pylori. Significant differences were found among garlic, ginger, kalonji and mint extracts and their consortia extracted in distilled water and ethanol.

Shumely, etal.,2016 has workedon antibacterial activityof Ginger (Zingiber officinale) and usedit asanti-ulcer, antiinflammatory, anti-tumor. He also observed that aqueous extract can protect against stress-induced stomach ulcers and secretions, as well as inhibit or limit Helicobacter pylori growth ¹².

In this study inhibition zone of Garlic extract was 13.04 ± 1.20 mm in distilled water, whereas 16.05 ± 1.69 mm in ethanol. Ginger & mint showed zone of inhibition extracted in distilled water as 9.44 ± 0.32 mm, and in ethanol as 11.46 ± 1.03 mm. The results are in agreement with earlier research of Attari et al $(2019)^{13}$. But in combination with other herbs, ginger kalonji and mint, Consolidate of garlic & ginger showed less $(18.50\pm0.77$ mm) inhibition zone in distilled water as compared to in ethanol extract (20.36 ± 1.47 mm). Inhibition zone of Garlic & kalonji was also smaller (16.30 ± 0.81 mm) in distilled water as compared to in ethanol (17.74 ± 0.57). Inhibition zone of Garlic and mint zone in distilled water was 16.24 and in ethanol as 16.54 ± 1.13 mm. The results are agreement with the results of sivam et al (2001) that combined form of herbs is more effective¹⁴.

In another sudy (Lawal, et al., 2014) Nigella sativa, often known as black seed or Kalonji, is a rananculacea plant that is widely grown throughout the world and is also grown in India and Pakistan as an annual herb. Fixed oil, protein, alkaloids saponin, and essential oil are all present. Antioxidant, hepato-protective, anti-parasitic, anti-cancer, anti-diabetic, and antibacterial properties have been described for N sativa, as well as analgesic and anti-inflammatory, anti-nociceptive, anti-ulcer, and anti-histaminic properties. In our study, Kalonji showed zone of inhibition extracted in distilled water as 5.94±0.77mm however in ethanol more as 7.44±1.11mm clearly supported by lawal et.al.in 2014with results on kalonji¹⁵.

All herbal extracts showed more sensitivity extracted in ethanol as compared to distilled water extracts and the comparison of both solvents is observed as: Garlic displayed zone of inhibition extracted in distilled water as 13.04 ± 1.20 mm, and in ethanol extract as 16.05 ± 1.69 mm. Inhibition zone of Ginger was 8.520.86mm in distilled water extract and 11.19 ± 0.96 mm in ethanol extract. For Kalonji zone of inhibition in distilled water was 5.9 ± 0.77 mm however in ethanol extracts are found to be very effective against Helicobacter pylori as compared to distilled water extracts.

CONCLUSION

Ethanol extracts were determined to have a greater effect than water and to be the greatest solvent and preserver for herbal activity. Garlic, ginger, and kalonji all demonstrated antibacterial property towards H. pylori. While mint did not show any effect against Helicobacter pylori. Herbs taken in combination and collectively (consortia) have a greater effect. Herbs as a medication require further development and research.

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