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SHORT COMMUNICATION

Bactericidal effect of selected spices, medicinal plants and tea on *Helicobacter pylori* strains from Sri Lanka

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Abstract: The bactericidal activity of 21 plant extracts on *Helicobacter pylori* was investigated. Plants were boiled in water to produce aqueous extracts. Bactericidal activity of the extracts was assessed by a standard kill-curve using five strains of *H. pylori* isolated from Sri Lanka and the NCTC 11637 strain. Among the plants that showed bactericidal activity for *H.pylori,* turmeric and ginger were the most efficient followed by chilli and black tea. Nutmeg, liquorice, cinnamon, colombo weed, yellow-berried nightshade, threadstem carpetweed, sage, parsley, long pepper, and cumin also showed bactericidal activity against *H. pylori.* These could serve as potent alternative therapies for *H. pylori* infection, avoiding the problem of resistance associated with current antibiotic treatment.

Keywords: Bactericidal effect, ginger, *Helicobacter pylori*, inhibition, medicinal, plants, Sri Lanka, turmeric

INTRODUCTION

Helicobacter pylori is a strict human pathogen which has colonized half the world population[1]. If untreated it can lead to chronic active disease and gastric malignancy. According to current recommendations**,** treatment of infection by *H. pylori* includes triple therapy inclusive of a proton pump inhibitor.

Although antibiotic resistance of *H. pylori* is not known in Sri Lanka, it is reported to be increasing world over. The need therefore arises to find an alternative with anti microbial properties. Studies conducted mainly in the developed countries have demonstrated inhibition of *H. pylori* by extracts of ginger, black tea, garlic, thyme, and mint[2-6]. The overall data for the prevalence of *H. pylori* in Sri Lanka differ from 3-70 % and seems to correspond more to that of an industrialized country[7,8]. The reason for the low prevalence of *H. pylori* in Sri Lanka is an enigma. One of the reasons for the low prevalence may be the Sri Lankan diet. Rice is the staple food in a Sri Lankan traditional diet with a significant contribution from bread, pulses and vegetables. Spices and chilli are essential and popular additives to food. Tea is consumed in plenty.

A study in the UK used Italian strains of *H. pylori* to test the bactericidal activity of spices, tea and medicinal plants. The results of the study demonstrated inhibitory action in some of the tested plants extracts[6].

The genetic composition of *H. pylori* strains differ in various geographic regions of the world[6] and this study was designed to determine the inhibitory effects of certain spices, medicinal plants and tea against *H. pylori* strains isolated from Sri Lanka.

METHODS AND MATERIALS

Isolation of H. pylori: H. pylori NCTC 11637 and five strains of *H. pylori* isolated from patients presenting with peptic ulcer disease were included in the study. All strains were stored on beads at –80^{0}C until tests were performed. Bacteria were grown on Colombia blood agar (oxoid) plates supplemented with 5% sheep blood at 37^{0}C under micro aerophilic conditions.

Preparation of extracts: 10 g of each powdered substance (refer to Table 1) was dissolved in 100 mL of distilled water (100 mg/mL) and was boiled for 20 min. The extracts were filtered through sterile gauze and pH neutralized. Finally the extracts were sterilized by autoclaving. All extracts were stored in the dark at –20 ^{0}C until use.

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Viable colony count: Bactericidal activities of the extracts were determined by viable colony counts. One hundred micro liters (μ L) of bacterial suspension ($5x10^8$) from the NCTC strain and the five isolates were added to 900 μ L of each of the test extracts and incubated for 60 min in gas jars under microaerophilic conditions. The control consisted of *H. pylori* incubated with sterile normal saline. At the end of 60 min, 100 μL of this 1/10 dilution was inoculated on to Colombia agar plates and incubated for 3 d under microaerophilic conditions. The colony count was determined for each of the extracts (colony forming units per milliliter, cfu/mL). Plant extracts that inhibited growth of *H. pylori* (i.e. no colonies grew) were further tested. As described above, 1 in 10 dilutions were prepared for all plant extracts and incubated for varying time intervals e.g. 0,15,30, and 60 min. 100 μ L of this 1/10 dilution was then inoculated on to Colombia blood agar (oxoid) plates and incubated for 3 d under microaerophilic conditions. All experiments were performed three times. The effectiveness of the extracts in killing *H. pylori* was expressed as inhibition of colony growth.

RESULTS

Of the 21 plant extracts tested for bactericidal activity against *H. pylori,* 7 (fenugreek, coriander, bengal quince, nightshade, garlic, black pepper, fennel) did not show inhibition after 60 minutes of incubation under microaerophilic condition (Table 1).

Turmeric and ginger were the most efficient, with an ability to kill all 6 strains within 15 minutes. Chilli & black tea were able to kill all 6 strains within 60 minutes. Incomplete bactericidal activity were observed for 10 extracts (Table 2)

DISCUSSION

In this study we have demonstrated the bactericidal properties of 21 plant extracts against *H. pylori.* The extracts were obtained simply by boiling selected parts of plants in water. The current treatment for *H. pylori* infection with combination of antibiotics, is expensive and associated with adverse side effects. Further, the development of resistance in *H. pylori* to antibiotics is also a problem.

Table 1: Plants used in the study

Common English name	Common Sinhala name	Scientific name	Part of plant used	source	Used (in food/ beverage/ medicine
Bengal quince	Belli	*Aegle marmelos*	Fruit	SL	in food and medicine
Black pepper	Gammiris	*Piper nigrum*	Seed	SL	in food and medicine
Black tea	Thē	*Camellia sinensis*	Leaves & shoot	SL	in beverage
Chilli	Miris	*Capsicum anunum*	Fruit	SL	in food and medicine
Cinnamon	Curundu	*Cinnamomum verum*	Bark	SL	in food and medicine
Calumba root	Veni'val	*Coscinium fenestratum*	Climbing stem	SL	in medicine
Coriander	Kotta'malli	*Coriandrum sativum*	Fruit	SL	in food and medicine
Cumin	Suduru	*Cuminum cyminum*	Seed	SL	in food and medicine
Fennel	Ma'duru	*Foeniculum officinalis*	seed	SL	in food and medicine
Fenugreek	Ulu' hal	*Trigonella foenum-graecum*	Seed	SL	in food and medicine
Garlic	Sudu'lunu	*Allium sativum*	Bulb	SL	in food and medicine
Ginger	E'guru	*Zingiber officinale*	Rhizome	SL	in food and medicine
Liquorice	Val'mee	*Glycyrrhiza glabra*	Stem	SL	in medicine
Long pepper	Thipili	*Piper Longum*	Seed	SL	in medicine
Nightshade	Ela'battu	*Solanum surattense*	Fruit & root	SL	in medicine
Nutmeg	Sadikka	*Myristica fragans*	Kernel	SL	in food and medicine
Parsley	Parsley	*Petroselinum crispum*	Leaves	SL	in food and medicine
Sage	Minchi	*Salvia officinalis*	Leaves	SL	in food and medicine
Threadstem carpetweed	Pathpardagam	*Mollugo cerviana*	Seed	SL	in medicine
Turmeric	Kaha	*Curcuma longa*	Rhizome	SL	in food and medicine
Yellow-berried nightshade	Katu'val'batu	*Solanum xanthocarpum*	Whole Plant	SL	in medicine

Table 2: The bactericidal activity of 21 extracts to *H. pylori*

Common English name	Common Sinhala name	100% inhibition Time duration 15	30	60	Incomplete inhibition (Small colonies or some growth)	No activity
Bengal quince	Belli					√
Black pepper	Gammiris					√
Black tea	Thē			√		
Chili	Miris			√		
Cinnamon	Curundu				√	
Columba root	Veni'val				√	
Coriander	Kotta'malli					√
Cumin	Suduru				√	
Fennel	Ma'duru					√
Fenugreek	Ulu' hal					√
Garlic	Sudu'lunu					√
Ginger	E'guru	√				
Liquorice	Val'mee				√	
Long pepper	Thipili				√	
Nightshade	Ela'battu					√
Nutmeg	Sadikka				√	
Parsley	Parsley				√	
Sage	Minchi				√	
Threadstem carpetweed	Pathpardagam				√	
Turmeric	Kaha	√				
Yellow-berried nightshade	Katu'val'batu				√	

Seven plant extracts did not kill *H. pylori* after 60 minutes of incubation (black pepper, garlic, bengal quince, night shade, coriander, fenugreek and fennel) Several studies have shown that garlic does kill *H. pylori in vitro* and *in vivo*[9,10]. The observation in the current study may be due to the method of extraction. Boiling has been shown to reduce the inhibitory activity against *H. pylori*[4,6,12]. Fenugreek sprouts have been shown to have high anti-microbial activity against *H. pylori*[11]. In this study, fenugreek appeared inactive where the seeds were tested rather than sprouts, which may account for the difference in results, as compared to the European study[6]. In this study, only one Sri Lankan strain showed complete inhibition with cumin extract within one hour. However, in a study done in U.K., cumin extract was able to kill all strains within 30 minutes[6]. This difference is interesting and should be studied further.

In this study, black tea and chilli extracts killed all the strains within one hour, but in the U.K. study[6] no bactericidal activity against *H. pylori* was seen with black tea. The difference in these results may be due to different genotypes available in different geographic regions of the world. Green tea catechins have previously been reported to have antibacterial effect against *H. pylori* which was confirmed in Mongolian gerbils[3].

The plant extracts of nutmeg, cinnamon, columbo weed, yellow-berried nightshade, liquorice, long pepper, threadstem carpetweed, sage, parsley & cumin showed bactericidal activity against *H. pylori* but did not achieve complete inhibition within 60 minute.

As in the U.K. study[6] we observed a reduced colony size with some spices such as sage, cinnamon, nutmeg, yellow-berried nightshade and threadstem carpetweed.

Extracts of turmeric and ginger were the most efficient and killed *H. pylori*. These results have been also shown by a previous study done in the UK[6]. However, it is the first time that anti *H. pylori* properties of spices have been demonstrated against strains isolated from Sri Lanka. These plant extracts can be used in combination with antibiotics, possibly increasing the success of eradication, as has been shown *in vitro* for cranberry juice[12]. Plants contain multiple organic components including phenols, quinines, terpenoids, flavones and tannins which are known to have bactericidal effects[6,13]. These substances

are also water soluble and are probably responsible for the killing effect of the plant extracts on *H. pylori.*

The importance of this study is that it has demonstrated that several plant extracts are effective against *H. pylori* and are obtained simply by boiling parts of the plants.

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References

1. Feldman R.A. (2001). *Helicobacter pylori*: Molecular and Cellular Biology, (Eds M. Achtman & S. Suerbaum) pp. 29-51. Horizon Scientific Press, Norfolk, England
2. Mahady G.B., Pendland S.L.,Yun G.S., Lu Z.Z. & Stoia A. Ginger (2003). (*Zingiber officinale* Roscoe) and the gingerols growth of Cag A+ strains of *Helicobacter pyloriAnticancer Research* **23**(5A):3699-3702.
3. Takabayashi F., Harada N., Yamada M., Murohisa B. & Oguni I. (2004). Inhibitory effect of green tea catechins in cc.with sucralfate on *Helicobacter pylori* infection in Mongolion gerbils. *Journal of Gastroenterology* **39**(1):61-63.
4. Canizares P., Gracia I., Gomez L.A., Martain De Argila C., Boixeda D. & Garcia A. de Rafael L. (2004). Allyl-thiosulfinate bacteriostatic compounds of garlic against *Helicobacter pylori*. *Journal of Biotechnology Progress* **20**(1):397-401.
5. Tabak M., Armon R., Potasman I. & Neeman I. (1996). *In vitro* inhibition of *Helicobacter pylori* by extracts of thyme. *Bacteriology* **80**(6):667-672.
6. O. Mahony R., Al-Khtheeri Huda, Weerasekara D., Fernando N., Vaira D., Holton J. & Basset C. (2005). Bactericidal and anti-adhesive properties of culinary and medicinal plants against *Helicobacter pylori. World Journal of Gastroenterology* **11**(47):7499-7507.
7. De Silva M. (1999). Prevalence of *H.pylori* infection in patients with functional dyspepsia. *Ceylon Medical Journal* **44**(3):118-119.
8. Fernando N., Holton J.,Vaira D., De Silva M. & Fernando D. (2002). Prevalence of *Helicobacter pylori* in Sri Lanka as determined by PCR. *Journal of Clinical Microbiology* **40**(7):2675-6 .
9. Jonkers D., van den Broek E., van Dooren I., Thijs C., Dorant E., Hageman G. & Stobberingh E. (1999). Antibacterial effect of garlic and omeprazole on *Helicobacter pylori.* Journal of *Antimicrobial Chemotherapy* **43**(6)**:**837–839.
10. Iimuro M., Shibata H., Kawamori T., Matsumoto T., Arakawa T., Sugimura T. & Wakabayashi K. (2002). Suppressive effect of garlic extract on *Helicobacter pylori*-induced gastritis in Mongolian gerbils. *Cancer Letter* **187**(1-2)**:** 6 – 68.
11. Randhir R., Lin Y.T. & Shetty K. (2004). Phenolics, their antioxidant and antimicrobial activity in dark germinated fenugreek sprouts in response to peptide and phytochemical elicitors. *Asia Pacific Journal of Clinical Nutrition* **13**(3): 295 –307.
12. Shmuely H., Burger O., Neeman I., Yahav J., Samra Z., Niv Y., Sharon N., Weiss E., Athamna A., Tabak M. & Ofek I. (2004).Susceptibility of *Helicobacter pylori* isolates to the anti-adhesion activity of a high-molecular –weight constituent of cranberry. *Diagnostic Microbiology of Infectious Diseases* **50**(4)**:**231-235.
13. Cowan M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews* **12**(4)**:**564-582.