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### Antimicrobial effect of *emblica officinalis* and *cayratiapedata* against *helicobacter pylori*

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#### ABSTRACT

*Helicobacter pylori* have been attracting worldwide attention as a major factor or cofactor responsible for gastritis and peptic ulcer. To eradicate this micro-organism, numerous regimes containing various antimicrobial agents have been suggested. However, *H.pylori* antimicrobial resistance is a leading factor to treatment failure and recurrence of the disease. Traditional medicine based on herbal remedies has always played key role in the health system of many countries. Clinical research has confirmed the efficacy of several medicinal plants for the treatment of gastro-duodenal disease, and basic scientific research has uncovered mechanisms to explain their therapeutic effects, hence we have compared two plant extracts such as *Emblica officinalis* and *Cayratiapedata* for the antimicrobial activity of *H.pylori*. Thirty patients attending the OPD of Rajah Muthiah medical college and hospital, for Acid peptic disease (ulcer and non ulcer type) were subjected to endoscopy at RMMCH, Annamalai University, Chidambaram. Antral biopsies were collected from the patients with gastritis, duodenitis, duodenal ulcer, non-ulcer dyspepsia and gastric carcinoma. All the biopsy specimens were processed for *H.pylori* by Gram's stain, urease test and culture. All the positive culture was processed for the Antimicrobial resistance by Disk Diffusion Method. The minimum inhibition zone diameter of *Emblica officinalis* and *Cayratiapedata* were detected. *Emblica officinalis* and *Cayratiapedata* exhibited potential of in-vitro antimicrobial activity against *H.pylori*, which suggests that it may be useful for the treatment of *H.pylori* infection.

**Keywords:** *Emblica officinalis* and *Cayratiapedata*, *H.pylori*.

#### INTRODUCTION

*Helicobacter pylori* have been attracting worldwide attention as a major factor or co factor responsible for gastritis and peptic ulcer. The discovery of *Helicobacter pylori* by warren and Marshall not only introduced a

whole new group of bacteria to science but also Warren has proved beyond doubt that *H.pylori* is the cause of type β gastritis and is an co factor in the associated in the gastro duodenal disease (Marshall and Warren,1998).

The infection is associated with atrophic gastritis, duodenal ulcer or gastric ulcer, and gastric adenocarcinoma at a later stage (Covacci et al., 1999). Eradication of the organism has been shown to result in ulcer healing, prevention of peptic ulcer recurrence and may also reduce the prevalence of gastric cancer in high-risk populations (Sepulveda and Coelh, 2002). Several short course drug regimens have been prescribed for the eradication of *Helicobacter pylori* with different combinations of therapeutic agents such as antibiotics, bismuth subsalicylate, proton pump inhibitors and H<sub>2</sub> blockers (Hentschel et al., 1993). However, emerging resistance to antibiotics, especially clarithromycin and metronidazole limits and more in developing countries (Sullivan et al., 1990; Lind et al., 1999; Sherif et al., 2004). Furthermore, undesirable side effects of the drugs and the significant cost of combination therapy, require the exclusive need to search alternative approaches of eradicating or preventing infections (O'Gara et al., 2000). Phytotherapy has proved to be an untapped treasure for the discovery of model compounds to cure different diseases including gastrointestinal (GI) disorders (Thompson and Ernst, 2002).

Several studies have been focused to evaluate the anti-*Helicobacter pylori* effects of traditional herbal medicines from various parts of the world including Japan, Korea, Turkey, Taiwan, Cameron, etc. with promising bactericidal activities against *Helicobacter pylori* (Bae et al., 1998; Yesilada et al., 1999; Shin et al., 2004; Wang and Huang, 2005; Ndip et al., 2007). To the best of our knowledge, there is no report on the screening of herbal medicines, used for gastrointestinal disorders against *Helicobacter pylori* from South-Asia countries like India, Pakistan, and Bangladesh where traditional medicine is extensively employed in daily life for curative, preventive and promotive health care design.

Thus, in the present study we examined anti-*Helicobacter pylori* activities of medicinal plants from India which are widely used for the treatment of GI diseases in South-Indian countries. There has been a worldwide move towards the use of Extraction and traditional medicines because of its efficacy and being free from serious toxic effects. Several studies reported, that many natural plants extracts have anti-*H. pylori* activity (Ndip, R.N., A.N. Ajongle et al., 2008). The fruit of *Emblema officinalis*

commonly known as amla is highly valued in traditional Indian medicine. The fruit of *E. officinalis* is hepatoprotective, anti tumor, antioxidant and antiulcerogenic (Sairam, K., C.V. Rao, B.M. Dora, et al 2002). *E. officinalis* showed antibiotic activity against a wide range of bacteria (Chopra, D. and D. Simon, et al., 2000).

*Cayratia pedata* is an indigenous herb belonging to the family Vitaceae. The leaves of *Cayratia pedata* are used as astringent and refrigerant. The phytoconstituents present in this medicinal plant have been studied to possess diuretic activities. Traditionally the leaves of this plant were used in the treatment of ulcers and diarrhoea. The decoction of the leaves was used to check uterine and other fluxes (Patil et al., 2000) The plant has also found to possess anti-inflammatory (Veeradas Rajendran et al., 2011) and antinoceptive activities (Veeradas Rajendran and Indumathy et al., 2011). Therefore the aim of the present study was to identify the antimicrobial effect of various medicinal plant extract against *Helicobacter pylori* by using the conventional Kirby-Bauer's disk diffusion and minimum inhibitory concentration methods.

## MATERIALS AND METHODS

### Plant selection and Preparation of Extract

The plant and fruits were collected from in and around Annamalai Nagar area, Chidambaram and the taxonomic identification was made by Dr.V.Vengatesalu, Professor, Department of Botany, Annamali University, Annamalai Nagar, Tamilnadu, India. The voucher specimen was numbered and kept in our research laboratory for further reference. Either parts or whole plants were used for this study.

### Extract Preparation

Material of both *Cayratia pedata* and *Emblema officinalis* were dried at room temperature ( $28\pm3^{\circ}\text{C}$ ) by exposure to atmosphere. The dried materials were pulverized into very fine powder in an electric grinder. Aqueous extract was prepared by mixing 20g of dried powder with 200ml of water. They were added to soxhlet apparatus and the boiling point of water was set up at 100°C. The water evaporates continuously and was recycled, thereby extracting the compounds present in the sample.

The suspension of dried powder in water left form 3h, filtered through whatman no 1 filter paper, and the filterate was dried and stored in amber colored air tight bottle at 10°C temperature till use.

### Determination of the antimicrobial effect of herbal medicines

30patients both male and female attending the surgical OPD suffering with acid peptic disease were taken up for the study. H. pylori were isolated from antral biopsies of patients undergoing routine gastro duodenal endoscopy for stomach complaints in hospital at Rajah Muthiah Medical College in Chidambaram. The biopsy specimens were crushed and inoculated in skirrows medium containing 10% horse blood .The plates were incubated at 37°C in candle jar with pad of cotton soaked in water placed at the bottom. The plates were examined on 3<sup>rd</sup>,5<sup>th</sup> day of incubation. Characteristics colonies were identified by Gram staining, urease, catalase and oxidase tests.

### The disk diffusion method

To determine the inhibitory effect of various plant extract on H. pylori growth, the disk diffusion was tested using disks with varying amounts of *Embllica officinalis* , and *Cayratia pedata* at 200, 400 ,600 and 800 micrograms. H.pylori suspensions equivalent to a 3 McFarland standard were spread in to skirrows medium, and herbal disks were placed on the surface of the medium. The plates were incubated for 3 days at 37°C under microaerophilic conditions. The inhibition zone was recorded based on the contents of *Embllica officinalis* and *Cayratia pedata* concentration. The disk size was 6 mm.

## RESULTS

### *Embllica officinalis*

The inhibition zone diameter of 10 to 12 mm was noted in 600 micrograms and 12 to 14 noted at 800 micrgrams

### *Cayratia pedata*

The inhibition zone diameter of 8-10mm was noted at 200 micrgrams and 12-14mm was noted at 400 micrograms cayratia disk

### The result of disk diffusion method

	<u>Inhibition zone diameter</u>			
	200	400	600	800
Disk diffusion	200	400	600	800
<i>Embllica officinalis</i>	0	0	10-12	12-14
<i>Cayratia pedata</i>	0	8-10	12-14	12 -14

## DISCUSSION

Peptic ulcer disease is one of the most common medical and surgical problems encountered worldwide. H.pylori is considered the major cause of chronic gastritis and peptic ulcer disease in both adults and children and it can increase the risk of gastric carcinoma. From epidemiological data, it has been shown that in developing countries there is a high incidence of H. pylori infection in people ranging from 13% to 70% in the under 20 age group and from 70% to 94% in the over 30 age group(pounder Re, Ng D et al.,1995).

Many kinds of herbal medicines are supposed to have anti- inflammatory or antimicrobial effect against H.pylori .*Embllica officinalis* and *Cayratia pedata* has been widely used for centuries as anindigenous medicine for the treatment of a variety of inflammatory conditions and other diseases (Ammon HP et al., 1991).

In this study, we have found out the inhibition zone with E.officinalis 12 to 14mm with the concentration of 800 micrgrams where as *Cayratia pedata* 12 to 14 mm was found in 600micrograms/ml which proves beyond doubt that both emblicaofficinalis and *Cayratia pedata* has the antimicrobial effect against helicobacterpylori.

The present study investigated the anti-H. pylori effect of aqueous extracts of *E. officinalis* and its phytochemical content. In this study, gastric biopsy samples were obtained from patients presenting with gastro duodenal complications. *H. pylori* was isolated from the specimens using standard microbiology procedures and isolates subjected to aqueous extract of *E. officinalis* for susceptibility. Phytochemical studies revealed that the presence of phenolic, flavonoids and carotenoids which have been reported to have antibacterial activities (Alzoerky, N.S. and K. Nakahara et al 2003) The mechanisms behind the anti *H. pylori* activity are complex to understand and could be attributed to either inhibiting the cell division or damaging the cell wall or ability to intercalate with DNA (Phillipson,J.D. and M.J.O Neill et al., 1987), which however require to be further investigated in detail. *In vitro* studies of various crude extracts of *E.officinalis* has demonstrated anti-*H. pylori* effect. *E.officinalis* may provide novel or lead compounds, which could become template for the synthesis of new anti-*H. pylori* drugs of inexpensive, safe and effective treatment.

Traditionally, the plants of *Cayratia pedata* were used in the treatment of ulcers and diarrhea. The decoction of the leaves was used to check uterine and other fluxes (Patil et al., 2000). The plant has also found to possess anti-inflammatory and antinoceptive activities (Veeradas et al., 2011). The *Cayratia pedata* reduces the gastric volume, ulcer score, free and total acidity of gastric acidsecretion of pylorus ligature induced gastric ulceration in rats (Karthick et al., 2011). Plant extracts of *Cayratia pedata* possessed significant diuretic activity which was analysed by phytochemical screening and GC-MS analysis. The plant extract reveals the presence of alkaloids, carbohydrates, steroids, tannin, phenolic compounds, flavonoids and terpenoids. The GC-MS analysis of the ethanolic extract of *Cayratia pedata* reveals the presence of phytoconstituents belonging to the type-acids, esters, alcohols, ethers, etc. Thus, the medicinal plant *Cayratia pedata* is found to possess significant phytoconstituents. The presence of such a variety of phytochemicals may be attributed to the medicinal

characteristics of this plant *Cayratia pedata* (A Leo et al ., 2011).

The protection by *Cayratia pedata* against gastric ulcer to be produced by the suppression of pepsin levels and strengthening of mucosal barrier. Further it is clear that the antiulcer activity against *Helicobacter pylori*. Phytochemical studies of the extract of *Cayratia pedata* revealed the presence flavonoids,sterols and terpenes. Numerous flavonoids have shown reducing gastric acid secretion and protective properties. Phytosterols which have been shown various properties that necessary for protection againstulcer. Since flavonoids and sterols shown to be present in the extract of *Cayratia pedata*,these constituents may responsible for the antiulcer activity of the *Cayratia pedata* extract.

## CONCLUSIONS

Herbal medicine has become popular for healthcare. Research in medicinal plants has gained a renewed focus recently. The prime reason is that other system of medicine although effective come with number of side effects that often lead to serious complication. The plant based system of medicine being natural does not pose this serious problem .Herbal medicine can be tested for efficacy using conventional trial methodology. Several specific herbal extracts have been demonstrated to be efficacious for specific conditions. This study was designed to assess the *in vitro* anti-*H. pylori* potential of two herbal plant extract, namely *Emblica officinalis* and *Cayratia pedata*. It was found that *Cayratia pedata* and *Emblica officinalis* has potential of *in vitro* antimicrobial activity against *H. pylori*, suggesting that it may be useful for the treatment of *H. pylori* infection. Our major focus in future studies is to isolate and characterise the bioactive compounds from these plants.

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