WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 3, 1066-1073. Research Article ISSN 2277– 7105

THE EFFECT OF pH ON THE ANTIBIOTIC ACTIVITY OF 2, 3 DIHYDROXYBENZOIC ACID (2, 3 DHB); AN ANTIBIOTIC ISOLATED FROM THE FRUITS OF *FLACOURTIA INERMIS* AND ITS PROSPECTS IN THE TREATMENT OF *HELICOBACTER PYLORI* (H.PYLORI).

Shibu George[1*], Suja N[1] and Benny P J[2]

[1]Department of Zoology, St. Aloysius College, Edathua, Alappuzha; affiliated to Mahatma Gandhi University, Kottayam, Kerala- India.

[2]Department of Zoology, St. Thomas College, Pala, Kottayam; affiliated to Mahatma Gandhi University, Kottayam, Kerala- India.

Article Received on
16 Dec 2014,

Revised on 10 Jan 2015,
Accepted on 04 Feb 2015

***Correspondence for Author**
Shibu George
Department of Zoology, St. Aloysius College, Edathua, Alappuzha; affiliated to Mahatma Gandhi University, Kottayam, Kerala- India.

ABSTRACT

2, 3 Dihydroxybenzoic acid, isolated from the fruits of *Flacourtia inermis* is an effective antibiotic against various microorganisms. *Helicobacter pylori* is a pathogenic bacterium of the gastric mucosa. Presently, there is no single effective antibiotic against it; lots of scientific searches are going on to develop a potent antibiotic with fewer side effects. In this study, an attempt was made to evaluate the effect of pH; both acidic and alkaline on the antibacterial activity of 2, 3 DHB, so as to suggest this compound for further studies in *H.pylori* treatment. For the study, the pH of 2, 3 DHB was modified to 1 and 10 by treatment with concentrated HCl and NaOH respectively. The antibacterial activity of the modified compound was tested by disc diffusion method against standard strains. This is based on the fact that the antibiotics that are used against the common bacterial diseases are being used as a remedy against *H.pylori*. The results of the study revealed that the 2, 3 DHB in pH 1 was highly active against all the tested strains such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Escherichia coli* and *Klebsiella pneumoniae*. The activity was slightly higher than that obtained against unmodified, natural 2, 3 DHB. However, the activity of 2, 3 DHB in pH 10 was ineffective to inhibit the growth of tested bacteria. As the compound 2, 3 DHB was inactive in alkaline medium, the oral

administration of 2, 3 DHB may not affect the alkaline intestinal fauna. This is one of the major advantages of 2, 3 DHB than any other antibiotics. 2, 3 DHB is also an effective antioxidant and its toxicity has not yet been reported. The study concluded that the antibiotic 2, 3 DHB in HCl is highly active. Therefore, it can be recommended for further studies in *H.pylori* treatment both *in vitro* and *in vivo*.

KEYWORDS: 2, 3 Dihydroxybenzoic acid, 2,3 DHB, *Flacourtia inermis*, antibiotic, *Helicobacter pylori, H.pylori*.

INTRODUCTION

Helicobacter pylori(H.pylori) is a silent but highly dangerous gram negative spiral bacterium. Unlike other bacteria that prefer intestine for the growth and survival, *H.pylori* prefers the high acidic medium of stomach for its survival. Except human, no other reservoir is reported.[1, 2] Infection statistics showed that developing countries (about 90% of the population) and even developed countries (50% of the population) are carrying this organism.[3,4] However, this pathogen resides in most of the human carriers asymptomatically [5] while in others it develops serious health problems such as hypochlorhydria, gastric/peptic ulcers, gastric cancer etc.[6] Several studies reveal that this pathogen could be closely associated with secondary problems such as acute ischemic stroke, low grade gastric mucosa associated lymphoid tissue (MALT) lymphoma, ventilator-associated pneumonia, cerebrovascular disease, dermatological disorders such as urticaria, alopecia etc.[7,8,9,10] The treatment of *H. pylori* is a great challenge in the modern medical applications because there is no single effective antibiotic against it. However, its current therapeutic regimens include the use of any one of the proton inhibitors that reduce the acid secretion such as lansoprazole, omeprazole, pantoprazole, rabeprazole, esomeprazole etc. along with two or sometimes more than two antibiotics.[5] In some patients, first course treatment of about 7-14 days is not sufficient for the eradication of *H.pylori*. In such cases, a second cycle treatment with different antibiotics is required.

This is the major threat in *H.pylori* treatment. A single antibiotic has too much side effects, then the side effects of two antibiotics would be unpredictable. In this context, development of a single effective drug is inevitable. However, the pre-clinical studies using the *H.pylori* are very difficult as it requires highly complex medium for the growth and maintenance of the pathogen. Sometimes, endoscopy-biopsy of the stomach is required for the isolation of

pathogen. Moreover, the *in vivo* effect of *H.pylori* against a drug would be different from those that obtained from the *in vitro* studies.[3]

All these attributes form the obstacles for pre-clinical studies in ordinary microbiology laboratories. In order to overcome these difficulties, standard bacterial strains can be used as a test organism instead of *H.pylori*. This is based on the fact that the antibiotics used against the *H.pylori* are those antibiotics that are commonly used in other bacterial diseases. Hence, *in vitro* studies of a drug using standard strains followed by the *in vivo* studies of the selected drug against *H.pylori* are the practical remedies for solving the above problems. However, the effectiveness of an antibiotic in acid/alkaline medium is another challenge. To be an effective drug in *H.pylori* treatment, the drug should be very active in acid medium of the stomach which is essential for inhibiting the growth of pathogen and at the same time, the same drug should not be active in alkaline medium of the intestine. Otherwise, the drug can cause the death of commensal microorganisms of the intestinal fauna. Therefore, attention should be given for identifying a pH sensitive antibiotic for *H.pylori* treatment.

In this investigation, 2, 3 dihydroxybenzoic acid (2,3 DHB); an antibiotic isolated from the fruits of *Flacourtia inermis* Roxb[11] was tested for evaluating its effect in acid/alkaline pH using standard bacterial strains, in order to suggest its prospects in *H.pylori* treatment.

MATERIALS AND METHODS

F. inermis is a common fruiting tree of South India, especially in Kerala and it belongs to the family Flacourtiaceae. From its fruits, a simple phenolic compound, 2, 3 dihydroxybenzoic acid (2,3 DHB) has been isolated and characterized by standard protocols. Its antibacterial, antifungal and antiprotozoal activities were well studied.[11, 12] In this investigation, the purified 2, 3 DHB of *Flacourtia inermis* was taken for evaluating the antibacterial effect of 2, 3 DHB in acid and alkaline medium.

Sample preparation

Purified 2, 3 DHB was taken for the study. The pH range of the 2, 3 DHB is 2.5 to 3. In order to study the effect of acidic and alkaline pH on the antibacterial activity of 2, 3 DHB, concentrated hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used respectively. After dissolving 10 mg of 2, 3 DHB in 1 ml warm distilled water, the pH of the sample was adjusted to 1 with concentrated HCl. The acidic sample mixture was then concentrated by heat evaporation and loaded on the 6 mm Whatmann No.1 sterile filter paper disc. The

sample concentration on the disc was 10 mg/disc. This was taken as the sample A. For the preparation of alkaline sample, 10 mg of 2, 3DHB was dissolved in 1 ml warm distilled water and the pH was adjusted to 10 by NaOH. This mixture was concentrated by heat evaporation and loaded on the disc. The sample concentration on the disc was 10 mg/disc. This was taken as the sample B. For the control experiments, 10 mg of 2, 3 DHB was dissolved in 1 ml warm distilled water, concentrated by heat evaporation and loaded on the disc with a concentration of 10 mg/disc. This was taken as sample C. The other two controls, sample D and E were also prepared by taking 1 ml each of warm distilled water whose pH was adjusted to 1 by concentrated HCl and 10 by NaOH respectively. To each sample, sterile disc was added and the sample was concentrated by heat evaporation.

Bacterial strains

Standard bacterial strains obtained from Institute of Microbial Technology, Chandigarh (IMTECH), India were used for the study. The strains were,

Staphylococcus aureus (MTCC 96)

Escherichia coli (MTCC 443)

Pseudomonas aeruginosa (MTCC 741)

Serratia marcescens (MTCC 97)

Klebsiella pneumoniae, sub species *pneumoniae* (MTCC 109)

Culture media

The dehydrated Muller Hinton Agar (MHA) medium purchased from Himedia Laboratories Pvt.Ltd, Mumbai, India was used. The medium was rehydrated, sterilized in an autoclave and was poured into sterilized petri dishes and allowed to set. The plates were stored at 4 - 10° C in refrigerator. Before inoculation, the surface of the petri dishes was dried in an incubator.

Antibacterial test by Disc Diffusion Method

The effect of pH on 2, 3 DHB against the growth of selected strains was tested by Disc Diffusion Method. [13] The dried plates were inoculated by test strains uniformly over the surface using a sterile cotton swab. A sterile 6 mm Whatmann No.1 filter paper loaded with appropriate sample (test samples A & B and control samples C, D and E) was placed on the surface of the inoculum and gently pressed by a sterile forceps. The plates were incubated at 37° C for 16 to 20 hrs. The zone of inhibition of bacterial growth around the disc was measured in millimeters. The experiment was repeated for three times and the average values were recorded.

RESULTS AND DISCUSSION

The effect of pH on the antibacterial activity of 2, 3 DHB is given in the Table 1 and Fig 1. The controls made up of HCl and NaOH (samples D and E respectively) were ineffective against tested strains. This indicates that there is no agent on disc for killing the bacteria after evaporating and drying the HCl/NaOH in distilled water. However, previous experiments showed that concentrated HCl or NaOH taken on the disc without evaporation and drying were active to inhibit the growth of bacteria. The control sample C which was made up of 10 mg of 2, 3 DHB alone was active to inhibit the growth of tested bacteria. Out of the two test samples A and B, sample B i.e., 2, 3 DHB in NaOH (pH 10) was ineffective to inhibit the growth of the tested strains. This clearly indicates that the antibacterial activity of 2, 3 DHB is lost in presence of an alkaline medium. However, the effect of 2, 3 DHB in HCl (sample A) was interesting. Instead of losing the activity in acid medium, its activity was found to increase against all the tested strains than those of the 2, 3 DHB alone. The zones of inhibition obtained from 2, 3 DHB in acid were ranged from 14 mm to 20 mm in contrast to 8 to 12 mm of inhibition zone of 2, 3 DHB. Among the tested strains, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Serratia marcescens* were the most susceptible organisms in 2, 3 DHB- HCl combination. *Escherichia coli* and *Klebsiella pneumoniae* showed least inhibition with an inhibition zone of 14 or 15 mm. However, this range of inhibition is also significant in antibacterial treatment.

Table 1:- Effect of pH on the antibacterial activity of 2, 3 DHB against standard bacterial strains

Sl.No.	Bacterial Strains	Zone of inhibition (in mm) against the samples				
		A	B	C	D	E
1	*Staphylococcus aureus*	20	0	10	0	0
2	*Pseudomonas aeruginosa*	19	0	12	0	0
3	*Escherichia coli*	14	0	8	0	0
4	*Klebsiella pneumoniae*	15	0	9	0	0
5	*Serratia marcescens*	20	0	12	0	0

***A**:- sample in HCl, **B**:- sample in NaOH, **C**:- 2, 3 DHB alone, **D**:- HCl alone, **E**:- NaOH alone*

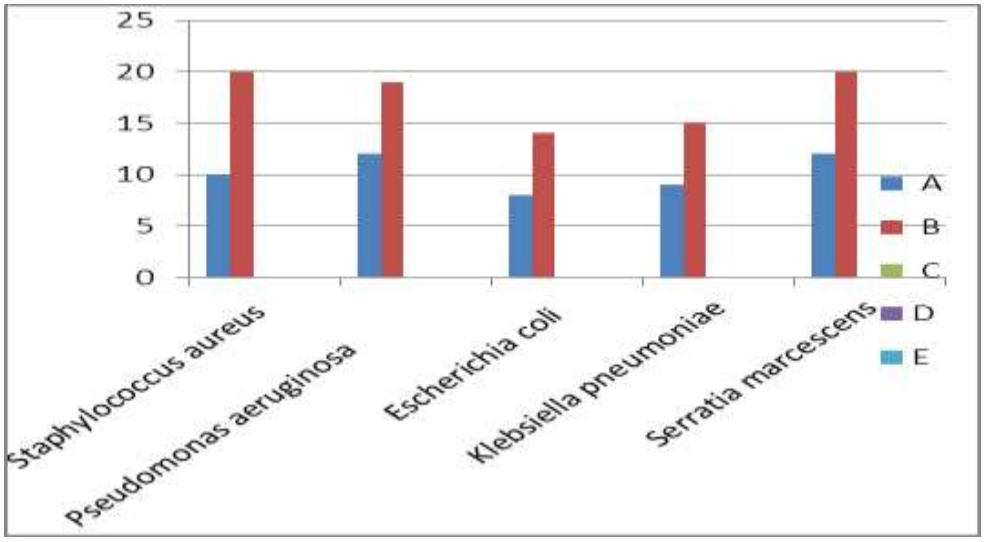

Fig 1:- Effect of pH on the antibacterial activity of 2,3 DHB against standard bacterial strains

2, 3 DHB is a simple phenolic acid [Fig 2], abundant in some plants or plant parts. The biological properties of this compound such as antibacterial, antifungal, antiprotozoal etc. have been well studied. It is also widely used as an iron- chelating agent and antioxidant in combination with antibiotics to prevent the oxidative organ damaging effect of the antibiotics. [14, 15] The hydroxyl group or the carboxylic acid of the 2, 3 DHB is responsible for the biological attributes of this compound.

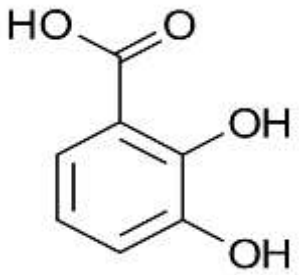

Figure 2:- Structure of 2, 3 Dihydroxybenzoic acid

Narrow spectrum antibiotics have not yet been discovered for the treatment of *H.pylori*. Therefore, in the current scenario of *H.pylori* treatment, the common antibiotics that are active against common bacterial diseases are used. Hence, developing a narrow spectrum drug with least side effects against *H.pylori* is worthy in this era of drug resistance. If the developing drug shall be effective for inhibiting the growth of *H.pylori* in acid medium, then the demand for concomitant use of Proton inhibitor such as omeprazole, pantoprazole etc. [5] can be avoided. On considering this aspect, the present drug 2, 3 DHB is effective only in acid medium. Hence, it could be suggested that this compound, 2, 3 DHB would be active in

stomach but not in intestine where the pH is alkaline. Therefore, this drug may not distort the normal intestinal fauna which is very essential for maintaining a healthy life. Moreover, it can also be expected that after its intestinal absorption to the blood, it may act as an antioxidant rather than an antibiotic. This is an added advantage of 2, 3 DHB, which is seldom present in other antibiotics. However, all these inferences are based on the preclinical studies with standard bacterial strains. Hence, preclinical studies with *H. pylori* followed by an *in vivo* study might be inevitable for reaching a final conclusion.

CONCLUSION

The pH of the natural compound 2, 3 Dihydroxybenzoic acid, isolated from the fruits of *Flacourtia inermis* was modified to 1 and 10 by treatment with concentrated HCl and NaOH respectively. It was tested against standard bacterial strains for determining its efficiency as an antibiotic in different pH so as to suggest this compound in *H.pylori* treatment. The results of the study revealed that the 2, 3 DHB in acidic medium (i.e. in pH 1) was highly active against the tested strains and the activity was slightly higher than that obtained against unmodified, natural 2, 3 DHB. However, the activity of 2, 3 DHB in alkaline medium (pH 10) was ineffective to inhibit the growth of tested bacteria. This experiment suggests that this compound can be considered as remedy against the gastric bacteria, *H.pylori*. This is based on the fact that the present day antibiotics for *H.pylori* treatment belong to the general class antibiotics, rather than specific for *H.pylori*. As the compound 2, 3 DHB is inactive in alkaline medium; it may not affect the intestinal fauna where the pH is alkaline. This is the major advantage of 2, 3 DHB than those antibiotics that are used in *H.pylori* treatment. As this findings were based on pre clinical studies with standard strains, we recommend for an elaborate experimentation on the activity of 2, 3 DHB against *H.pylori*, both *in vitro* and *in vivo*.

REFERENCES

1. Fatemeh A, Shamsi A, Parvin B, Mohammad R. *Helicobacter pylori* and Cag Aantibodies in Hyperemesis gravidarum (HG). Afr J Microbiol Res, 2011; 5(15): 2100-2102.
2. Nwodo EN, Yakubu SE, Jatau ED, Yabaya A. Seroprevalence of *Helicobacter pylori* Infection in Patients with Gastritis and Peptic Ulcer Disease in Kaduna, Kaduna State, Nigeria. Afr J Basic & Appl Sci, 2009; 1(5-6): 123-128.
3. Reza M, Mehdi M, Farideh S, Sadegh M. Treatment of *Helicobacter pylori* infection in Iran: low efficacy of recommended western regimens. Arch Iranian Med, 2004; 7(1): 1-8.

4. Sibel K, Burak S, Ahmet S, Mehmet A, Polat K, Ali A, Hasan D. Effect of subclinical *Helicobacter pylori* infection on gastric wall thickness: multislice CT evaluation. Diagn Interv Radiol, 2008; 14: 138-142.
5. Gisbert JP, Luna M, Gomez B, Herrerias JM, Mones J, Castro FM, Sanchez P, Cosme A, Olivares D, Pajares JM. Recurrence of *Helicobacter pylori* infection after several eradication therapies: long-term follow-up of 1000 patients. Aliment Pharmacol Ther, 2006; 23: 713–719.
6. Batool MM. The relationship between *Helicobacter pylori* infection and gastro-esophageal reflux disease. N Am J Med Sci, 2011; 3(3): 142-145.
7. Tsang KW, Lam SK. Extragastroduodenal conditions associated with *Helicobacter pylori* infection. H K Med J, 1999; 5(2): 169-174.
8. Chong VH, Lim KC, Rajendran N. Prevalence of active *Helicobacter pylori* infection among patients referred for endoscopy in Brunei Darussalam. Sing Med J, 2008; 49(1): 42-46.
9. Yasunori S, Iwao A, Maki H, Shigeru O, Takao M, Yuji T, Jun H. Association between chronic *Helicobacter pylori* infection and acute ischemic stroke: Fukuoka Harasanshin Atherosclerosis Trial (FHAT). Atherosclerosis, 2005; 178: 303–309.
10. Chandrashekar UK, Gnanadev NC, Vasudeva A, George KV, Ganesh B, Vidya M. Cronkhite-Canada syndrome with *Helicobacter pylori* infection. Calicut Med J, 2010; 8(4): e7.
11. Shibumon G, Benny PJ, Sunny K, Cincy G. Antibiotic activity of 2, 3-dihydroxybenzoic acid isolated from *Flacourtia inermis* fruit against multidrug resistant bacteria. Asian J Pharm & Clin Res, 2011; 4(1): 126- 130.
12. George S, Benny PJ, Kuriakose S, George C, Gopalakrishnan S. Antiprotozoal activity of 2, 3-dihydroxybenzoic acid isolated from the fruit extracts of *Flacourtia inermis* Roxb. Med Plants- Int J Phytomed & Rel Ind, 2011; 3(3): 237-241.
13. Kirby MDK, Bauer AW, Sherris JC, Turck M. Antibiotic susceptibility testing by standardized single disc diffusion method. Am J Clin Pathol, 1966; 45: 493-496.
14. Bijan N, Taghi G, Valiollah H, Ardeshir T, Diana T. The effect of 2, 3-dihydroxybenzoic acid and tempol in prevention of vancomycin-induced nephrotoxicity in rats. J Toxicology, 2003; 232(3): 192-199.
15. Stig AS, Mette K, Jens RN. 2, 3-Dihydroxybenzoic acid attenuates kanamycin-induced volume reduction in mouse utricular type I hair cells. J Hearing res, 2006; 212(1-2): 99-108.