Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 5, Issue 2, 2012 ISSN - 0974-2441

Research Article

IN VITRO POTENTIAL OF ANTIBACTERIAL ACTIVITY OF 2H-FURO[2,3-H]-1-BENZOPYRAN-2-ONE AGAINST GRAM NEGATIVE HUMAN BACTERIA

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Received: 20 December 2011, Revised and Accepted: 4 February 2012

ABSTRACT

Antibacterial activity of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one against five human bacterial species viz., *Klebsiella pneumonia*, *Vibrio cholera, Escherichia coli, Shigella dysenteriae* and *Salmonella typhi* were tested at 100-1000ppm concentration. Among the five bacterial species tested, *K. pneumonia* recorded 32.0mm inhibition at 700mm concentration (MIC 700ppm). *V. cholera* showed 25.0mm inhibition at 600ppm concentration(MIC 600ppm), *E. coli* recorded 30.0mm inhibition at 900ppm concentration(MIC 900ppm), S. *dysenteriae* recorded 30.0mm in 600ppm concentration(MIC 600ppm. *S. typhi* recorded 30.0mm inhibition at 800ppm concentration(MIC 800ppm). All the result was compared to standard synthetic antibiotics Gentamycin and Tetracycline at 25mg concentration.

Keywords: *Psoralea corylifolia*, 2H-Furo[2,3-H]-1-benzopyran-2-one, bacteria, MIC.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicine. [1] About 80% of the worlds inhabitants relying mainly on traditional medicines for their primary health care. [2] The use of plants by man to treat common ailments is time immemorial and many of the traditional medicines are still included as part of the habitual treatment of various maladies. [3] About 60 % of the total global population remains dependent on traditional medicines for their healthcare system. [4] Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity and also play an important role in the modern medicine. [5] The screening of plant extracts and their products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes. [6] Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents, there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, over use of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms. [7] To avoid the use of synthetic antibiotics, there is a urgent need to search an alternative medicine. In the present study, bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one, isolated from seeds of *Psoralea corylifolia* L. belongs to family Fabaceae were evaluated *in vitro* against human bacteria.

MATERIALS AND METHODS

Plant material

Fresh and healthy seeds of *P. corylifolia* L., were washed with tap water thrice and two to three times with distilled water .The seeds were air dried at room temperature. Completely air dried seeds were powered using waring blender (Waring international, new hart-ford, CT, USA).

Isolation of Bioactive compound

Bioactive compound was isolated from seeds of *P. corylifolia* following the procedure of Harborne.[8]

Test organisms

Five pathogenic bacteria namely *Klebsiella pneumonia* (Gram negative), *Vibrio cholera* (Gram negative), *Escherichia coli* (Gram negative), *Shigella dysenteriae* (Gram negative) and *Salmonella typhi* (Gram negative) were collected from research center, CMR Institute of Management Studies (Autonomous), Bangalore. The obtained cultures were subcultured on nutrient agar medium. After 24 hours of incubation at 37°C the cultures were preserved aseptically in lower temperature until further use.

Preparation of Inoculum

A loopful of all the test bacteria were taken and sub-cultured in test tube containing 10 ml of nutrient broth. The test tubes were incubated at 37°C for 24 hours. The broth was standardized using sterile normal saline to obtain a population of 10 cfu/ml.

Antibacterial assay

Agar Cup Diffusion Method

Agar cup diffusion method described by Joshi [9] was employed. An overnight culture of *K. pneumonia, V. cholera, E. coli, S. dysenteriae*. and *S. typhi* were inoculated into petri plates containing nutrient agar medium. The culture medium was allowed to set. Thereafter, a sterile cork borer of 5.0 mm diameter was used to punch wells in the seeded nutrient agar. Five wells were made in the petriplate containing media (One in centre and Four at the border), the agar plugs were removed with a flamed and cooled wire loop. For each well 50 µl of different concentrations (100, 200,300,400,500,600, 700,800, 900 and 1000ppm) of the bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one were added. The plates were incubated at 37°C for 24 hours and the zone of inhibition was measured in millimeter. The experiments were repeated for six times.

STATISTICAL ANALYSIS

The data were subjected to Tukey's HSD analysis. Data on percentages were transformed to arcsine and analysis of variance (Anova) was carried out with transformed values. The means were compared for significance using Tukey's HSD (P=0.05).

RESULT

Isolation of the Bioactive compound

The bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one was isolated. From the observation it was recorded 0.47 R_f value and 138^0 C melting point.(Figure 1).

Antibacterial assay

Agar cup diffusion method

Antibacterial activity of bioactive compound 2H-Furo[2,3-H]-1-benzopyran- 2-one isolated from the seeds of *P.corylifolia* when

tested at 100 to 1000ppm concentration against five pathogenic species of bacteria. Among the five pathogens tested, *K. pneumonia* recorded a maximum inhibition of 32.0mm. The minimum inhibitory concentration (MIC) was observed in 700ppm concentration. At 100ppm concentration, the zone of inhibition was 5.0mm. The zone of inhibition was increased in 200ppm(9.0mm), 300ppm(15.0mm), 400ppm(23.0mm), 500ppm(29.0mm) and 600ppm(30.0mm) . In *V.cholera*, the MIC was recorded in 600ppm concentration. The inhibition zone was 25.0mm in 600ppm concentration and in 100ppm concentration, it was recorded 2.0mm, and in 200ppm, the zone of inhibition was 6.0mm. With the increase in concentration, the zone of inhibition was increased. In *E.coli*, the MIC was recorded in 900ppm concentration. In 100ppm concentration the zone of inhibition was 10.0mm and in 800ppm concentration the zone of inhibition was 24.0mm. In *S. dysenteriae*, the MIC was observed in 600ppm concentration and recorded 30.0mm inhibition. In 500ppm concentration the zone of inhibition was 26.0mm inhibition. Significant activity was observed form 100, 200,300 and 400ppm concentration. In *S.typhi*, the MIC was observed in 800ppm concentration and recorded 30.0mm inhibition. In 700ppm concentration, the inhibition zone was 27.0mm, in 600ppm(25.0mm), 500ppm(20.0ppm), 400ppm(17.0mm), 300ppm(12.0mm), 200ppm(7.0mm) and in 100ppm concentration the zone of inhibition was 3.0mm. Compared to standard antibiotics Gentamycin and Tetracyclin at 25mg concentration. Gentamycin recorded 35.0mm inhibition in *K. pneumonia* , 30.0mm in *V.cholera*, 33.0mm in *E.coli,* 32.0mm in *S. dysenteriae* and 34.0mm inhibition in *S. typhi* respectively. In Tetracycline, *K. pneumonia* recorded 34.0mm inhibition, *V.cholera* recorded 30.0mm, *E. coli* recorded 30.0mm, *S. dysenteriae* recorded 30.0mm inhibition respectively (Table 1).

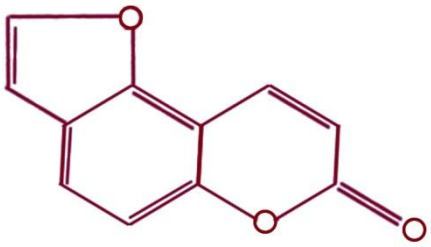

Figure 1: Molecular structure of the Bioactive compound 2H-Furo[2,3-H]-1-benzopyran-2-one isolated from seeds *of P.corylifolia* L.

Table 1: Antibacterial activity of bioactive compound 2H-Furo[2,3-H]-1-benzopyran- 2-one on bacterial species of human

Bacteria	Inhibition(mm)												
	Concentration of the Bioactive compound										MIC	Standard Antibiotics	
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm	700 ppm	800 ppm	900 ppm	1000 ppm		Gentamycin 25mg	Tetracycline 25mg
K. pneumonia	5.0^{a} ±0.0	9.0^{b} ±0.1	15.0^{c} ±0.0	23.0^{d} ±0.1	29.0^{e} ±0.1	30.0^{f} ±0.1	32.0^{g} ±0.1	32.0^{g} ±0.0	32.0^{g} ±0.1	32.0^{g} ±0.1	700^{b} ppm	35.0^{b} ±0.0	34.0^{a} ±0.0
V. cholera	2.0^{a} ±0.0	6.0^{b} ±0.0	10.0^{c} ±0.1	17.0^{d} ±0.1	22.0^{e} ±0.1	25.0^{f} ±0.2	25.0^{f} ±0.1	25.0^{f} ±0.0	25.0^{f} ±0.0	25.0^{f} ±0.1	600^{a} ppm	30.0^{a} ±0.0	30.0^{a} ±0.0
E. coli	10.0^{a} ±0.1	14.0^{b} ±0.0	15.0^{c} ±0.2	17.0^{d} ±0.1	19.0^{e} ±0.2	21.0^{f} ±0.1	22.0^{g} ±0.0	24.0^{h} ±0.1	30.0^{i} ±0.0	30.0^{i} ±0.0	900^{d} ppm	33.0^{b} ±0.0	30.0^{a} ±0.0
S .dysenteriae	6.0^{a} ±0.0	10.0^{b} ±0.1	16.0^{c} ±0.0	21.0^{d} ±0.1	26.0^{e} ±0.1	30.0^{f} ±0.1	30.0^{f} ±0.0	30.0^{f} ±0.0	30.0^{f} ±0.0	30.0^{f} ±0.0	600^{a} ppm	32.0^{a} ±0.0	33.0^{b} ±0.0
S. typhi	3.0^{a} ±0.1	7.0^{b} ±0.1	12.0^{c} ±0.0	17.0^{d} ±0.1	20.0^{e} ±0.1	25.0^{f} ±0.1	27.0^{g} ±0.0	30.0^{h} ±0.0	30.0^{h} ±0.0	30.0^{h} ±0.0	800^{c} ppm	34.0^{b} ±0.0	30.0^{a} ±0.0

- **Values are the mean of three replicates**
- **± Standard error.**
- **The means followed by the same letter (S) are not significantly different at P<0.05 when subjected to Tukey's HSD.**

DISCUSSION

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world. [7] Recently scientific interests in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. Therefore, the search for new drugs from plants continue to be a major source of commercial drugs. Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease. hence, further exploration of plant antimicrobials need to occur. [10,11] From the observation it can concluded that, the seeds of *P.corylifolia* is a potent source as antibacterial agent. Many bioactive compounds were observed in isolation prcedure other than 2H-Furo[2,3-H]-1-benzopyran- 2-one. Thus a further work is needed to isolate all the bioactive compounds and evaluating its antibacterial activity against different human and plant pathogens.

ACKNOWLEDGEMENT

The authors are thankful to the CMR Institute of Management Studies (Autonomous), PG Department of Biosciences, Kalyan Nagar, Bangalore, Department of Studies in Botany and Microbiology, Maharanis Science college for women, Palace road, Bangalore and Herbal Drug Technology laboratory, Department of Studies in Botany , University of Mysore, Mysore for providing facilities.

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