Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 2338-2342
Received: 28-07-2018
Accepted: 30-08-2018

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Antibacterial activity of endophytic bacteria isolated from few medicinal plants of BR hills, Karnataka

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Abstract
Endophytes are the microorganisms that live inside the plant host without causing any apparent diseases to the host plant and have known to produce various novel bioactive compounds. Medicinal plants have been collected from Biligirirangana Hill (BR Hill), Chamarajanagar district, Karnataka. In the present study the leaves were isolated in triplicates to evaluate the antibacterial activity of endophytic bacteria. A total of 90 bacterial isolates were obtained from 30 medicinal plants. These isolates were subjected to assess their antibacterial activity against *S. aureus, E. coli, B. subtilis, P. aeruginosa, Shigella sp, K. pneumoniae, P. mirabilis and L. monocytogenes.* Few isolates showed the antibacterial activity by showing the clear zone of inhibition against few human pathogens, out of which 5 isolates exhibited a contrasting antibacterial activity with the presence of highest zone of inhibition against all the test pathogens.

Keywords: BR hill, medicinal plants, human pathogens, antibacterial activity

Introduction
Microorganisms are abundant in nature. They found in all the ecological systems. Plants harbour diverse communities of microorganisms. These microorganisms which reside inside the plants without causing any apparent disease are called as Endophytes. These plants associated with bacteria colonize the rhizophere, phyllosphere (epiphytes) and inside the plants tissue (Endophytes). In Ayurveda, plants were used as a source of different medicines having broad applications in treating various diseases such as diabetes, jaundice, malaria, diarrhoea, dysentery, cholera, pneumonia, cardiovascular problems, dermal infirmities, cancer etc (Shukla *et al.,* 2014) [9]. A significant variation was detected in the colonization frequency of endophytic species at different seasons of the year, indicating the environmental factors such as rainfall and atmospheric humidity and their effect on host plant (Vinu and Jayashankar, 2017) Endophytes produce novel bioactive metabolites such as antibacterial, antifungal, antiviral, antitumor, antioxidant, anti-inflammatory, immunosuppressive drugs and many related compounds. These microbial bioactive metabolite compounds could be mainly classified as an alkaloids, terpenoids, steroids, lactones, phenolic compounds, quinones, lignans, etc (Sturz *et al*., 1997) [11]. Some of these endophytes may be producing bioactive substances that may be involved in a host-endophyte relationship many of them are capable of synthesizing bioactive compounds that can be used by plants for defense mechanism against human pathogens and some of these compounds have been proven useful for novel drug discovery metabolites of endophytes have been reported to inhibit a number of microorganisms (Fisher *et al.*, 1994) [4]. The metabolites of endophytes can increase the chance of finding novel antibacterial natural products. There are numerous new endophytic species may exist in plants, it follows that endophytic microorganisms are important components of microbial biodiversity and its antibacterial activity (Zhang *et al.,* 2009) [15]. The endophytic bacteria have similar properties with its host, the possibility of secondary metabolites produced by endophytic bacteria of the medicinal plant also has antibacterial activity. (Desriani *et al.*, 2013) [3]. Due to the increasing development of bacterial resistance and replacing plant antibacterial medicines, it is very important to focus on new antibacterial sources. The development of drug compounds from nature is one step that can be taken to minimize the disease and resistance problems. Application of endophytic bacteria is one of the techniques of biotechnology methods in producing the bioactive compound has several advantage, including rapidly to produce with a constant quality and can be produced on a huge scale. The aim of this study was to isolate and evaluate antibacterial activity of endophytic bacteria in few medicinal plants.

Materials and methods

1. Collection of Plant Sample

Medicinal plants were collected from BR. Hill, Karnataka. The healthy medicinal plants were selected to avoid interfering by plant pathogens. Collected medicinal plants are brought to the laboratory within 24 hrs in a cleaned sterile polythene bag.

2. Isolation of Endophytic bacteria from few selected medicinal plants

The selected medicinal plant leaves were used for the isolation of the endophytic bacteria. The collected leaves were washed thoroughly in running tap water and surface sterilized with Sodium hypochlorite (2%) for 3 mins. The disinfectant was removed by rinsing five times each in 2 washes of sterile distilled water and finally the leaves were dried on sterile paper**.** The surface disinfected leaves were cut with a sterilized razor blade into pieces 1-3 mm long, which were placed on Nutrient Agar media and the plates were incubated at 37^0C for 24 hrs to allow the growth of endophytic bacteria (Hallmann *et al*., 1997; Zinniel *et al*., 2002) [6, 16]. A total of 90 bacterial isolates were obtained from 30 medicinal plant leaves and were subjected to assess their antibacterial activity against few human pathogens

3. Antibacterial activity using Agar well diffusion assay

a. Pathogenic cultures and growth condition

The pathogenic cultures *Staphylococcus aureus, E. coli, Bacillus subtilis, Pseudomonas aeruginosa, Shigella sp., Klebsiella pneumonia, Proteus mirabilis* and *Listeria monocytogenes* were grown in Brain heart Infusion (BHI) media for 24 h at 37°C under constant shaking (150 rpm).

b. Test culture and growth condition

The bacterial cultures were grown fresh in Luria Bertani media (LB) at 37 °C for 24 h. The culture was centrifuged at 10,000 rpm for 10 min to collect the cell free supernatant (CFS) and filter sterilized which was then used for antibacterial assay.

c. Assay of Antibacterial activity

BHI agar plates were prepared by inoculating 1% of freshly grown pathogenic culture. Wells of 4 mm in diameter was made in the plate by using sterile cork borer. Then, 70 µl of given CFS was added in each well. The sample was allowed to diffuse for 20 min at 4°C. Later, plates were incubated at 37°C for 24-48 h. After incubation, the zone of inhibition was measured in mm and recorded. Antibiotic chloramphenicol was used as positive control (Xie *et al*., 2009) [14].

Table 1: Results showing the Antibacterial activity of bacterial isolates

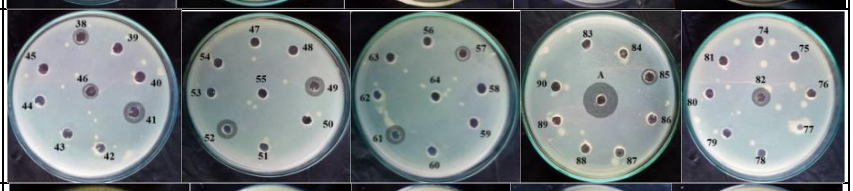
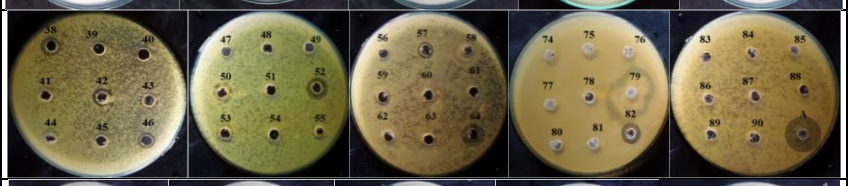
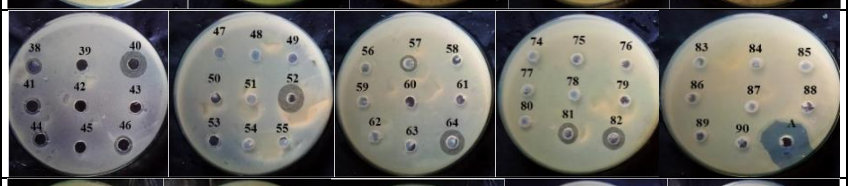
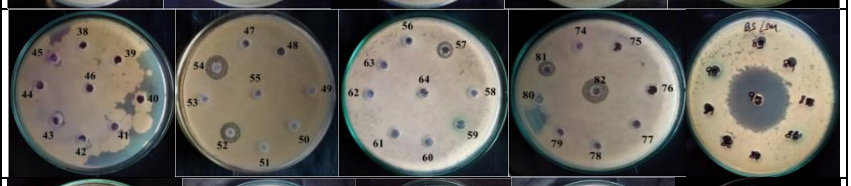
Culture code	Zone of inhibition (mm)							
	S. aureus	***E. coli***	***B. subtilus***	***P. aerogenosa***	***Shigella***	***K. pneumoniae***	***P. mirabilis***	***L. monocytogens***
C-1	-	-	-	-	-	-	-	-
C-2	-	-	-	-	-	-	-	-
C-3	-	-	-	-	-	-	-	-
C-4	-	-	-	-	-	-	-	-
C-5	-	-	-	-	-	-	-	-
C-6	++	+	-	-	+	-	+	+
C-7	-	-	-	-	-	-	-	-
C-8	-	-	-	-	-	-	-	-
C-9	+	-	-	+	-	+	-	-
C-10	-	-	-	-	-	-	-	-
C-11	-	-	+	-	+	-	-	-
C-12	+	-	-	-	+	-	++	-
C-13	-	-	-	-	-	-	-	-
C-14	-	-	-	++	-	-	-	-
C-15	-	-	-	-	-	-	-	-
C-16	-	-	+++	-	-	-	-	-
C-17	++	++	-	-	-	-	+	-
C-18	-	-	-	-	-	-	-	-
C-19	-	-	-	+++	-	-	-	-
C-20	-	-	-	-	-	-	-	-
C-21	-	-	-	-	-	-	-	-
C-22	+++	+	-	++	-	-	-	-
C-23	-	-	-	-	-	-	-	-
C-24	-	-	-	-	-	-	-	-
C-25	-	-	-	-	-	-	-	-
C-26	-	-	-	-	-	-	-	-
C-27	-	-	-	-	-	-	-	-
C-28	-	-	-	-	-	-	-	-
C-29	-	-	-	-	-	-	-	-
C-30	-	-	-	+	-	-	+++	-
C-31	-	-	-	-	-	-	-	-
C-32	-	-	-	-	-	-	-	-
C-33	++	+++	+	-	-	+	-	-
C-34	-	-	-	-	-	-	-	-
C-35	-	-	-	-	++	-	-	-

C-36	-	-	-	-	-	-	-	-
C-37	-	-	-	-	-	-	-	-
C-38	++	+	+	+	+	+	+	+
C-39	-	-	-	-	-	-	-	-
C-40	-	-	-	-	-	-	-	-
C-41	-	-	-	+++	-	-	++	-
C-42	-	+	-	-	-	-	-	-
C-43	-	-	-	-	-	-	-	-
C-44	-	-	-	-	-	-	-	-
C-45	-	-	-	-	-	-	-	-
C-46	+	+	+	+	+	+	+	+
C-47	-	-	-	-	-	-	-	-
C-48	-	-	-	-	-	-	-	-
C-49	++	-	-	-	+	-	++	-
C-50	-	-	-	-	-	-	-	-
C-51	-	-	-	-	-	-	-	-
C-52	+	++	++	+++	++	++	++	++
C-53	-	-	-	-	-	-	-	-
C-54	-	-	+++	-	-	-	-	-
C-55	-	-	-	-	-	-	-	-
C-56	-	-	-	-	-	-	-	-
C-57	++	+	+	+	+	+	+	+
C-58	-	-	-	-	-	-	-	-
C-59	-	-	-	-	-	-	-	-
C-60	-	-	-	-	-	-	-	-
C-61	-	-	-	-	-	-	++	-
C-62	+++	-	-	-	-	-	-	-
C-63	-	-	-	-	-	-	-	-
C-64	+++	+	-	++	++	+++	-	-
C-65	-	-	-	-	-	-	-	-
C-66	-	-	-	-	-	-	-	-
C-67	-	-	-	+	-	-	-	-
C-68	-	-	-	-	-	-	+	-
C-69	++	-	-	-	-	-	-	-
C-70	-	-	-	-	-	-	-	-
C-71	-	-	-	-	-	-	-	-
C-72	-	-	-	-	-	-	-	-
C-73	-	-	-	-	-	-	-	-
C-74	-	-	-	-	-	-	-	-
C-75	-	-	-	-	-	-	-	-
C-76	-	-	-	-	-	-	-	-
C-77	-	-	-	-	-	-	-	-
C-78	-	-	-	-	-	-	-	-
C-79	-	-	-	-	-	-	-	-
C-80	-	-	-	-	-	-	-	-
C-81	++	+	+	+	+	-	-	-
C-82	++	++	++	++	++	++	++	++
C-83	-	-	-	-	-	-	-	-
C-84	-	-	-	-	-	-	-	-
C-85	-	-	-	-	-	-	+	-
C-86	-	-	-	-	-	-	-	-
C-87	-	+	-	-	-	-	-	-
C-88	-	-	-	-	+++	-	-	-
C-89	-	-	-	-	-	-	-	-
C-90	-	-	-	-	-	-	-	-
Antibiotic	21	20	22	23	20	20	20	20

Note a) C1 to C90 are the bacterial cultures isolated from few selected medicinal plants.

b) Inhibition Zone Diameter index: + (9mm) Weak activity, ++ (10mm) Moderate activity, +++ (11mm) Strong activity, - No activity

c) A-Antibiotic chloramphenicol was used as positive control.

Test Pathogens	Plates showing the Antibacterial activity against test pathogens
E. coli	
S. aureus	
P. mirabilis	
K. pneumoniae	
P. aeruginosa	
Shigella sp	
B. subtilus	
L. monocytogenes	

Result and discussion

It is not worthy that of the nearly 3,00,000 plant species that exists on earth each individual plant is host to one or more endophytes (Strobel, 2003) [10]. The present study was attempted for the isolation of endophytic bacteria from few selected medicinal plants of BR Hill, Karnataka, followed by screening and evaluation of antibacterial activity of endophytic bacteria was tested by Agar well diffusion assay against few human pathogens. The pretreatment of the leaves from the medicinal plants to isolate the Endophytic bacteria method was followed by (Hallman *et al*., 1997) [6]. Based on the morphological charateristics a total of 90 bacterial isolates were obtained from 30 different plants and were subjected to assess their antibacterial activity. Few isolates showed the antibacterial activity by showing the clear zone of inhibition. Out of which 5 isolates showed broad spectrum of contrasting antibacterial activity by forming the highest zone of inhibition observed against all the test organisms and standard antibiotic are summarized in the table followed by the plates showing the highest zone of inhibition.

Many researchers found new drugs from the endophytes for the control of human diseases due to its antibacterial properties. In the present study the susceptibility pattern of endophytic bacteria were tested using 8 different human pathogens. Most of the endophytic bacteria are sensitive against the test organisms but some are resistant. Antibacterial activity of plant endophytes have been already reported by few researchers. Gram negative bacteria with potent antibacterial activity against few plant pathogens from roots of *Solanum* sp was reported by (Long *et al* 2003) [7].

Endophytic Bacteria from the Medicinal plant of *Andrographi s paniculata* showed promising antimicrobial activity against few human pathogens *S. aureus*, *E. coli*, *S. typhi*, *Proteus* sp, *Pseudomonas* sp and *Klebsiella* sp (Arunachalam and Gayathri, 2010) [1]. Antibacterial activity of endophytic Mycoflora of bark, leaf and stem tissues of *Azadirachta indica* A. Juss (Neem) against *E. coli* (Verma *et al* 2007) [12].

Endophytic bacterial isolates from the plants of *Azadirachta indica*, *Terminalia arjuna* and *Catharanthus roseus* exhibited a highest antimicrobial activity against *S. aureus* (Arun Kumar *et al* 2015) [2]. Bacterial endophytes from the plant *Passiflora foetida* produced potential antimicrobial activity against *B. cereus, K. pneumoniae, E. coli* (Pal *et al* 2012) [8].
The endophytic bacterial cultures C38, C46, C52, C57 and C82 showed the highest antibacterial activity against all the test pathogens with the highest zone of inhibition. Overall these result suggests that it has very good antibacterial activity against *Staphylococcus aureus, E. coli, Bacillus subtilis, Pseudomonas aeruginosa, Shigella sp., Klebsiella pneumonia, Proteus mirabilis* and *Listeria monocytogenes.*

Conclusion
The present study is concluded that this work is reported on endophytic bacteria from few selected medicinal plants of BR Hill, Karnataka. This study showed the promising antibacterial activity against 8 different human pathogens. Endophytes are chemical synthesizers within the plants which are the excellent sources of bioactive natural products that can be use to satisfy the demand of Pharmaceutical and Medical industries. Many of them are capable of synthesizing bioactive compounds that can be used by plants for defense against human pathogens and some of the bacteria are resistance among the pathogenic microorganisms. Detailed investigation on endophytic bacteria were needed to prove its potential to produce novel bioactive compounds with numerous high value metabolites further and it will leads for the discovery of new drugs.

Acknowledgement
The authors are thankful to Mangalore University for providing fellowship and facilities to carry out the research work.

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