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Evaluation of antibacterial activities of *Barleria Prionitis* Linn

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In the present study, 1 g/ml each of *Barleria prionitis* leaves (BPL) and *B. prionitis* stem (BPS) were extracted from different solvents like petroleum ether, chloroform ethyl acetate and methanol. Ethyl acetate extract of BPL showed maximum inhibition zone on Gram positive *Bacillus pumilus* (9.83 mm) and methanol extract of BPS showed minimum inhibition zone on Gram negative *Escherichia coli* (0.16 mm). Petroleum ether extract did not show inhibition except petroleum ether extract of BPS on Gram positive *B. pumilus* (0.46 mm). Minimum inhibition concentration (MIC) was shown by petroleum ether extract of BPL on Gram positive *B. pumilus* and Gram negative *Pseudomonas aeruginosa* (1.0 mg/ml). Leaves and stem extract of *Barleria prionitis* L. showed difference in antibacterial activity.

Key words: Plant extracts, antimicrobial activity, minimum inhibitory concentration.

INTRODUCTION

Medicinal plants have played an essential role in the development of human culture, for example religions and different ceremonies. Also, they are important sources of unknown chemical substances with potential therapeutic effects. Many drugs presently prescribed by physicians are either directly isolated from plants or are artificially modified versions of natural products (Wang et al., 2007).

In recent time, traditional medicines derived from herbs have huge therapeutic potential to heal many infectious diseases without associated side effects compared to synthetic drugs. In indigenous system of medicine in India, the aerial parts of *Barleria prionitis* are used for the treatment of anemia, toothache, and bacterial disorders. In the present work, an attempt has been made to study the efficacy of non-polar compounds fractionated from *B. prionitis; they were* evaluated for their anti- bacterial activity and compared with the efficacy of polar compounds fractionated from the same plant. Leaves of *B. prionitis* were extracted from petroleum ether containing non-polar compounds followed by ethanol extract containing the polar compounds of *B. prionitis* (Aiswarya and Ravikumar, 2014).

Over the past few years, medicinal plants have regained a wide recognition due to an escalating faith in herbal medicines, whose side effects are lesser compared to allopathic medicines. And they also meet

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Figure 1. Selected different parts of barleria prionitis L. plant.

the necessary requirements of medicine for an increasing human population. With the continuous erosion of traditional knowledge of plants used for medicine in the past and the renewed interest at the present time, there is a need to review this valuable knowledge of medicinal plants with the purpose of developing medicinal plants sectors across the different states in India.

In fact, it is well known that even in developed countries, the use of traditional medicines is quite prevalent. This culture has been passed down from one generation to another (Nurliani et al., 2004); hence, promoting the use of medicinal plants for health purpose. In India alone, less than 10% of the medicinal plants traded in the country are cultivated, about 90% are collected from the wild, very often in a destructive and unsustainable manner (Natesh, 2000).

Natural products have been applied to human healthcare for thousands of years. According to the World Health Organization (WHO) estimates, out of 4 billion people, 80 percent of the world populations presently use herbal medicine for some aspects of primary health care (Behera, 2006). WHO notes that of 119 plant-derived pharmaceutical medicines, about 74 percent are used in modern medicine directly proportional to the traditional use of plant medicines by native cultures (WHO, 2002).

MATERIALS AND METHODS

Plant sample

The plant selected in the present study was *B. prionitis* L. This plant was collected from Boriavi, Anand, Gujarat, India (Figure 1). Throughout the study, specific codes were allotted to various parts of the tested plant material: BPL for *B. prionitis* leaves and BPS for *B. prionitis* stem. Upper and lower ground parts of the tested plant serve as sources of young leaf and stem tissue used during the antibacterial activity, minimum inhibitory concentration, phytochemical test and antioxidant studies.

Preparation of plant extracts

For preparing the various parts of the plant extract, leaves and stems of *B. prionitis L.* were air dried at room temperature (27°C) for one week, after which they were ground into a uniform powder. The following four different solvent extracts were used for preparing the plant extracts: petroleum ether, chloroform, ethyl acetate and methanol. 10 g each of the dry powdered plant material was soaked in 200 ml of respective solvent at their respective boiling temperature for 48 h in Soxhlet assembly. The extracts were filtered after 48 h, first through a Whatman filter paper No. 42 (125 mm) and then through cotton wool. The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40°C. The extracts were kept in sterile bottles under refrigerated condition until use. Then 10 gm of dry extracts was resuspended in 10 ml of respective solvents. The final concentration of the plants extracts was 1 g/ml.

Microorganisms used

The test organisms used for screening the antibacterial activity of the plant extracts were both Gram positive (*Bacillus subtilis, Bacillus pumilus* MTCC *7615, Bacillus cereus and Streptococcus pyogenes* MTCC 1923) and Gram negative bacteria (*Escherichia coli* MTCC 448 , *Pseudomonas aeruginosa* MTCC 7436, *Comomonas acidovorans* MTCC 3362 and *Serratia marcescens* MTCC 3124).

Preparation of culture media

All bacterial strains were cultivated in nutrient agar medium (NA), and incubated at 37°C for 24 h. These were used for the microbial activity by disc diffusion assay

Testing for antibacterial activity

Crude plants extracted from different solvents (petroleum ether, chloroform, ethyl acetate and methanol) were used to determine the antibacterial activity by cup-plate agar diffusion method, which was slightly modified, according to Kirby-Bauer. It was used to assess the antibacterial activity of the prepared extracts. 20 ml of the inoculated nutrient agar and 0.5 ml of standardized bacterial stock suspensions (108-109) colony- forming units per ml were poured on sterile Petri plate. Negative controls were prepared using the same solvents which were employed to dissolve the plant extracts. TE30 (tetracycline 30 mcg/disc), GEN10 (gentamicin10 mcg/disc), E15 (erythromycin 15 mcg/disc), S10 (streptomycin 10 mcg/disc) were used as positive reference standards to determine the sensitivity of one strain in each bacterial species tested.

Four wells of 9 mm diameter were bored in the medium with the help of sterile cork-borer having 9 mm diameter. They were labeled properly. Fifty microliters of the working suspension/solution of the different extracts of the plant's parts and same volume of solvent extract for control were filled in the wells with the help of micropipette. They were allowed to diffuse at room temperature for two hours. The plates were then incubated in an upright position at 37°C for 18 h. Three replicates were carried out for each extract against each of the test organism. Simultaneous addition of the respective solvents instead of extracts was carried out as controls. After incubation, the diameters of the results and growth inhibition zones were measured averaged and the mean values were tabulated.

Determination of minimum inhibitory concentration

Although the results of the disc diffusion assay cannot always be compared to the MIC data (Njenga et al., 2005), plant extracts having positive antibacterial activity against most of the microorganisms tested in the disc diffusion bioassay were further tested for the determination of minimum inhibitory concentration (MIC) by two fold serial broth dilution method. Plant extracts with more than 4.5 mm inhibition zone were selected for MIC. Selective broth medium was used for dilutions as well as preparing inoculums. The bacterial cell density was maintained uniformly throughout the experimentation at 1 x 108 CFU/ ml by comparing with 0.5 Mc Far land turbidity standards. Plant extract of 40µl from stock solution (100 mg/ml) was put in the first dilution tube containing 960 µl of selective medium broth and mixed well. From this, 500 µl well was transferred to second tubes containing 500 µl broths. This step was repeated nine times and from last tube 500 µl solution was discarded. 100 µl of test organism was added in each tube. The final volume of the solution in each tube was made up to 0.6 ml. Tubes were incubated at optimal temperature and time in an incubator. Growth indicator 2-3-5 tri phenyl tetrazolium chloride solution (100 µl of 0.1 %) was incorporated in each tube to find out the bacterial growth inhibition. Tubes were further incubated for 30 min under dark condition. Bacterial growth was visualized when colorless 2-3-5 tri phenyl tetrazolium chloride was converted to red color from zone in the presence of bacteria.

RESULTS

Antibacterial activity against Gram positive bacteria

In the present finding, different solvent extracts from leaves and stem (BPL and BPS) parts of *Barleria prionitis* L showed antibacterial activity against all Gram positive bacteria studied (*B. pumilus, B. subtilis, Streptococcus pyogenes* and *Bacillus cereus*) (Figures 2, 3, 4 and 5).

The control plate representing petroleum ether, ethyl acetate, chloroform and methanolic extracts did not inhibit growth activity of the tested Gram positive microorganisms (Figures 2, 3, 4 and 5). For standard antibiotics, discs zone of growth inhibition was noted against selected strains of Gram positive bacteria (Figures 2, 3, 4 and 5). These results were compared with the response of the plant's extracts.

Antibacterial activity against Gram negative bacteria

In the present finding, the result revealed that the different solvent extracts of leaves and stem parts of *Barleria prionitis* L. also showed antibacterial activity against Gram negative bacteria (*E. coli, Serratia marcescens*, *Comomonas acidovorans* and *P. aeruginosa*) (Figures 6, 7, 8 and 9).

The control plate representing petroleum ether, ethyl acetate, chloroform and methanolic extracts did not inhibit the tested Gram negative microorganisms (Figures 6, 7, 8 and 9). For standard antibiotics, discs zone of inhibition was noted against selected strains of Gram negative bacteria (Figures 6, 7, 8 and 9). These results were compared with the plant's extracts zone of inhibition.

Minimum inhibitory concentration (MIC)

Ethyl acetate extract of leaves showed maximum MIC against *B. pumilus* and *P. aeruginosa*. It required minimum concentration of 1.0 mg/ml which was sufficient to inhibit the growth activity.

DISCUSSION

Numerous antibacterial screening has been performed with respect to location of microorganism such as skin infections, uterine infections etc. As oral bacterial infections are linked with various chronic diseases, screening of antibacterial activity of medicinally important

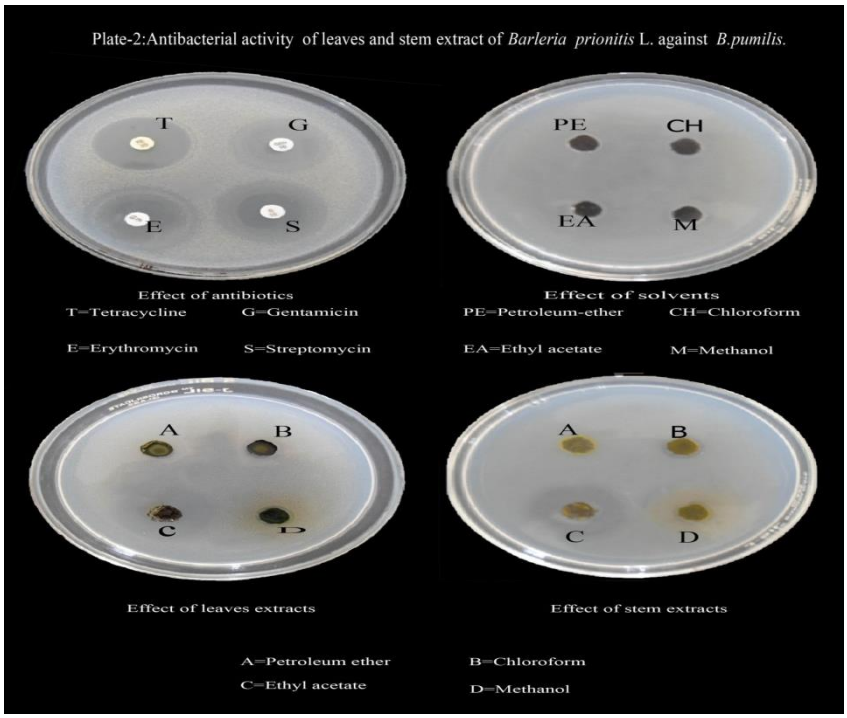

Figure 2. Antibacterial activity of leaves and stem extract of *Barleria prionitis L*. against *B. pumilus.*

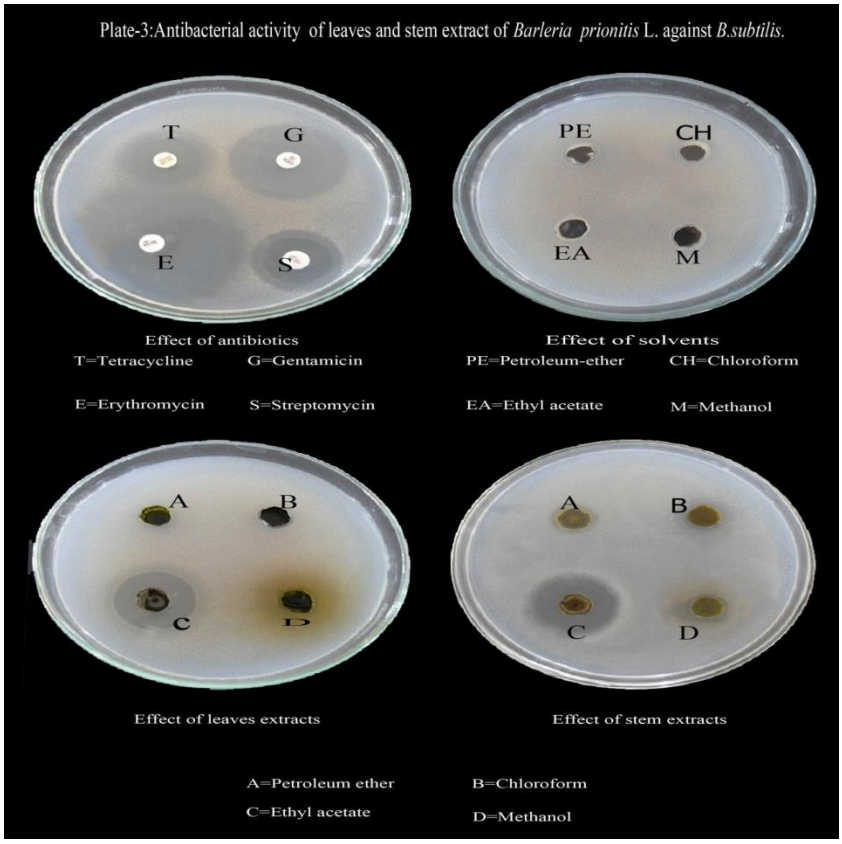

Figure 3. Antibacterial activity of leaves and stem extract of *Barleria prionitis L*. against *B. pumilus.*

Figure 4. Antibacterial activity of leaves and stem extract of *Barleria prionitis L*. against *S. pyogenes subtilis*.

Figure 5. Antibacterial activity of leaves and stem extract of *Barleria prionitis L*. against *B. cereus.*

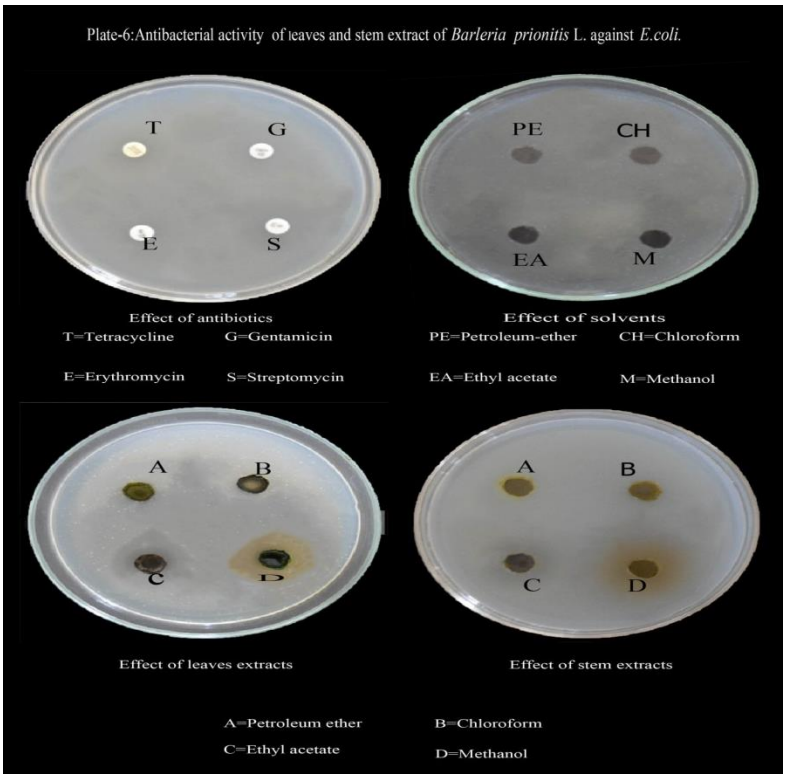

Figure 6. Antibacterial activity of leaves and stem extract of *Barleria prionitis L*. against *E. coli.*

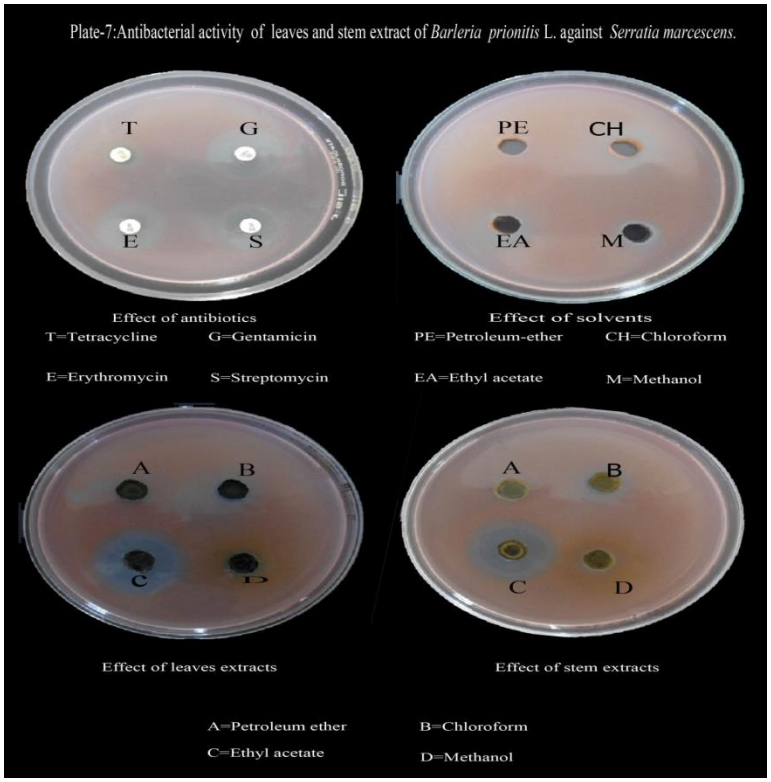

Figure 7. Antibacterial activity of leaves and stem extract of *Barleria prionitis L*. against *Serratia marcescens*

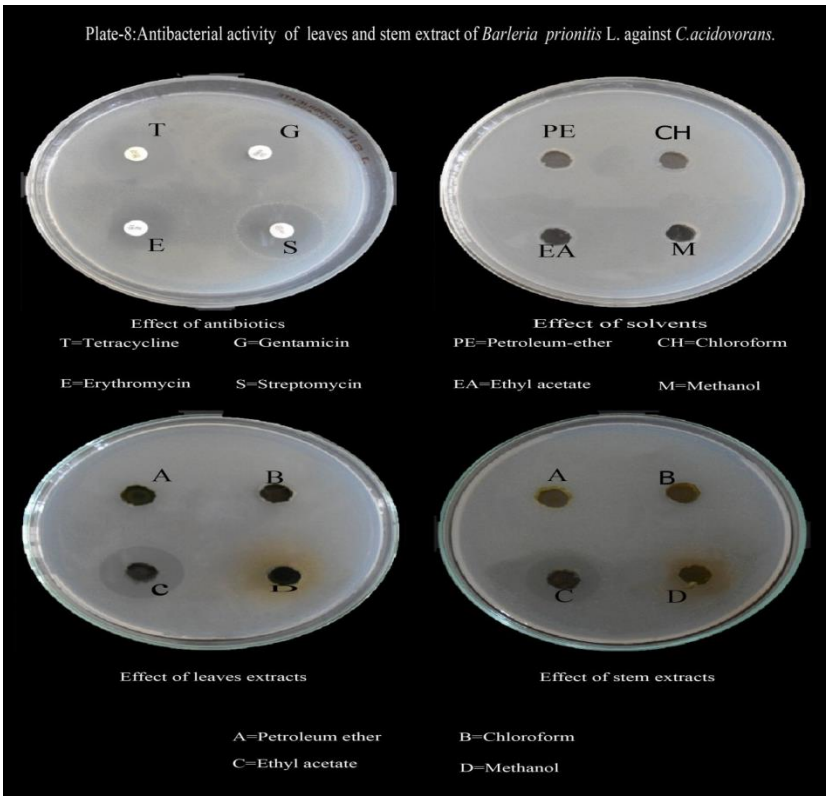

Figure 8. Antibacterial activity of leaves and stem extract of *Barleria prionitis L*. against *Comomonas acidovorans.*

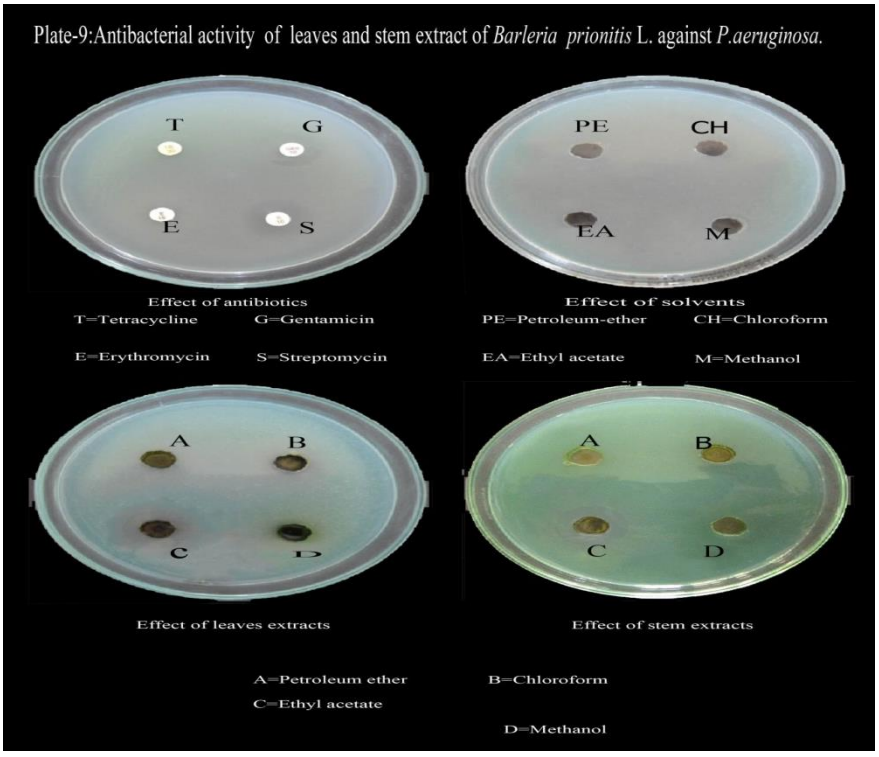

Figure 9. Antibacterial activity of leaves and stem extract of *Barleria prionitis L*. against *Pseudomonas aeruginosa.*

Table 1. Antibacterial activity of leaves and stem extracts of Barleria *prionitis* L. and antimicrobial agents.

Tested microorganisms		Diameter of inhibition zone (mm)											
		Petroleum ether extract		Chloroform extract		Ethyl acetate extract		Methanol extract		Microbial agent			
		Leaves	Stem	Leaves	Stem	Leaves	Stem	Leaves	Stem	TE^{30}	GEN^{10}	E^{15}	S^{10}
Gram positive bacteria	*Bacillus pumilus*	-	0.46	1.83	1.16	9.83	8.08	1.16	1.33	10.0	4.50	8.10	4.50
	Bacillus subtilis	-	-	1.00	0.91	6.25	6.00	0.50	1.00	8.00	5.50	6.00	6.00
	Streptococcus pyogenes	-	-	-	1.16	7.00	5.16	-	0.33	7.83	4.50	7.33	6.00
	Bacillus cereus	-	-	0.665	1.50	6.16	6.50	0.83	-	7.00	4.50	7.00	5.66
	Escherichia coli	-	-	-	0.50	3.00	3.66	-	0.16	1.41	-	-	-
Gram negative bacteria	*Serratia marcescens*	-	-	1.33	1.00	5.16	4.91	-	-	1.50	4.00	-	4.66
	Comomonas acidovorans	-	-	1.00	1.50	6.00	5.00	-	1.08	8.25	6.00	4.00	5.00
	Pseudomonas aeruginosa	-	-	1.33	3.08	4.75	5.58	-	2.33	-	5.00	-	3.08

Key: (-) = No inhibition zone, TE^{30} = Tetracycline 30 mcg/disc, E^{15}= Erythromycin 15 mcg/disc, GEN^{10} = Gentamicin10 mcg/disc, S^{10} = Streptomycin 10 mcg/disc.

plant against bacteria found in oral cavity is also important. Various plants have been evaluated for their efficacy in oral hygiene. Deshpande et al. (2011) evaluated antibacterial activity of different extracts of *J. regia* against oral micro flora and found that acetone extract was more effective against oral microflora. Similar results were observed in the present investigation, where chloroform extract of *Barleria prionitis* L**.** leaves was more effective. Ogundiya et al. (2009) carried out antimicrobial activity of acetone and ethanol extract of stem and root of Terminalia glaucescens and reported that ethanol extract had significantly higher effect. Antibacterial potential of aqueous decoction of *Piper nigrum L.*, *Laurus nobilis L*., *Pimpinella anisum L.* and *Coriandum sativum L*. against 176 bacteria isolated from oral cavity of 200 individuals was carried out by Nazia and Perween (2006). More et al. (2008) studied ethanol extract of eight plant species used traditionally in South Africa against oral pathogens such as *Actinomyces* and *Candida* species and got resistance activity.

Antibacterial activity against six bacteria *(B. cereus* (MTTT 430), *B. licheniformis* (MTCC 1483), and *S. aureus (E. coli, S. typhi, A. faecalis*) was carried out. Methanol extract of leaf showed highest antibacterial activity against *B. cereus (*22.66 mm in diameter) followed by pet ether leaf extract against *E. coli* (21.66 mm in diameter). Various extracts of *B. prionitis* were comparable to control antibacterial agents, Ampicillin, Tetracycline. Maximum inhibition was shown by tetracycline against *S. aureus* (28.20) followed by Ampicillin against *B. cereus* (28.40). Resazurin 96 well assay was used to assess minimum inhibition concentration (MIC)**;** petroleum ether of leaf demonstrated the least MIC value against *B. cereus* (0.05 mg/ml) and *E. coli (0.2* mg/ml)*,* while the methanol extract of bark and leaf demonstrated 0.2 mg/ml against B. cereus (Kumar et al., 2013).

In this study, leaves extract of ethyl acetate showed maximum potential of antibacterial activity against *B. pumilus* (9.83 mm zone diameter of inhibition) while minimum activity was observed against *Bacillus subtilis* (0.50 mm zone diameter of inhibition)in (Table 1).

Stem extract of ethyl acetate showed maximum potential of antibacterial activity against *B. pumilus* (8.80 mm zone diameter of inhibition) and lowest activity was observed in methanol extract against *Streptococcus pyogenes* (0.33 mm zone diameter of inhibition) in (Table 1).

Leaves extract of ethyl acetate showed maximum potential of antibacterial activity against *Comomonas acidovoran* (6.00 mm zone diameter of inhibition) and chloroform extract showed minimum inhibitory activity against *Comomonas acidovorans* (1.00 mm zone diameter of inhibition) in (Table 1) .

Ethyl acetate extract of stem showed maximum potential of antibacterial activity against *Pseudomonas aeruginosa* (5.58 mm zone diameter of inhibition) and methanol extract showed minimum activity against *E. coli* (0.16 mm zone diameter of inhibition) in (Table 1).

Minimum inhibitory concentration (MIC) of ethyl acetate extract

In the present finding, leaves and stem part of *Barleria prionitis* L. (BPL & BPS) showed MIC activity against selected strains of Gram positive bacteria (*Bacillus*

Table 2. Minimum inhibitory concentration (MIC) of *Barleria prionitis* L.

Tested microorganisms		Minimum inhibitory concentration (MIC) mg/ml	
		Ethyl acetate extract	
		Leaves	Stem
Gram positive bacteria	*Bacillus pumilus*	1.0	4.0
	Bacillus subtilis	4.0	4.0
	Streptococcus pyogenes	2.0	4.0
	Bacillus cereus	2.0	4.0
Gram negative bacteria	*Escherichia coli*	-	-
	Serratia marcescens	2.0	4.0
	Comomonas acidovorans	2.0	4.0
	Pseudomonas aeruginosa	1.0	2.0

pumilus, Bacillus subtilis, Streptococcus pyogenes and *Bacillus cereus*). The result in Table 2 shows that the ethyl acetate extract of leaves showed maximum MIC against *B. pumilus*. It required minimum concentration of 1.0 mg/ml which was sufficient to inhibit the growth activity. However, this was followed by *Streptococcus pyogenes* and *B. cereus,* where both required MIC of 2.00 mg/ml. wt of leaves extract to inhibit growth activity. Minimum MIC value was shown by *Bacillus subtilis,* where 4.0 mg/ml. wt leaf extract was required to inhibit the growth activity.

For stem extract, the result shows that all the tested Gram positive bacteria had same value of MIC value (4.0 mg/ml) which was sufficient to inhibit the growth activity.

In the present investigation, leaves and stem part of *Barleria prionitis* L. (BPL and BPS) showed MIC activity against selected strains of Gram negative bacteria (*Serratia marcescens*, *Comomonas acidovorans* and *Pseudomonas aeruginosa*). Result revealed that the MIC value of leaves extract was found in the range of 1.00 mg/ml to 2.00 mg/ml. However, maximum result was found against *Pseudomonas aeruginosa,* where it is required the minimum concentration of 1.00 mg/ml of leaves extract, which was sufficient to inhibit the growth activity. However, Serratia *marcescens* and *Comomonas acidovorans* required same minimum concentration of 2.00 mg/ml of leaves extract, which was sufficient to inhibit the growth activity (Table 2).

In case of stem extract, the result reported 2.00 mg/ml to 4.00 mg/ml. Maximum result was noted against *Pseudomonas aeruginosa,* where it is required the minimum concentration of 2.00 mg/ml of stem extract which was sufficient to inhibit the growth activity. However, *Serratia marcescens* and *Comomonas acidovorans* required the same amount of minimum value of 4.00 mg/ml of stem extract to inhibit the growth activity (Table 2).

Conclusion

We concluded from the present study that leaves and stem extract of *Barleria prionitis* L. showed difference in antibacterial activity

Conflict of interests

The authors did not declare any conflict of interest.

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