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Antibacterial Activity of Boerhaavia diffusa L. (Punarnava) On certain Bacteria

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Abstract: - Plants have been a valuable source of natural products since the time immemorial. The use of plant compounds for pharmaceutical purposes has gradually increased in India. The use of plant extract with antimicrobial properties can be of great significance in therapeutic treatments. The present work is carried out on different parts of Boerhaavia diffusa (punarnava) on the basis of the literature obtained from the ethno medicinal documentation. Keeping in view the tremendous ethno medicinal use of Boerhaavia diffusa the study was aimed to scientifically validate antibacterial property of different plant components (leaf, stem & root) of Boerhaavia diffusa.

Keywords*: Antibacterial study, Boerhaavia diffusa, Minimum Inhibitory Concentration, Punarnava*

I. INTRODUCTION

The effect of plant extracts on bacteria and fungi have been studied by a very large number of researchers in different parts of the world as well as in India. Punarnava is an important rejuvenating drug used in Ayurveda. The whole plants viz root, leaves, flowers, seed etc. are used for medicinal purposes. Plant is used in Ayurvedic, Unani, Siddha and Homoeopathy Systems (Guhabakshi *et al.,* 1999). Apart from above, *Boerhaavia diffusa* also possess marked antimicrobial properties viz- antibacterial properties (Olukoya *et al.,* 1993; Aladesanmi *et al.,* 2007) and antifungal properties (Agrawal *et. al*., 2004). All the properties have made this plant very important in the treatment of human and plant diseases.Thus the main objective of this study was to investigate the antibacterial activity of different plant components (leaf, stem and root) of *Boerhaavia diffusa,* which are used in traditional medicinal system of Kumaun Himalaya for the treatment of diarrhoea, urinary tract infection, typhoid etc.

II. MATERIALS AND METHODS

2.1: Collection of Plant material and pre-extraction preparation The plant *Boerhaavia diffusa* was collected during the month of January–February from subtropical to temperate regions of Kumaun Himalaya. The botanical identity of plant was established and authenticated from the herbarium of the Department of Botany, Kumaun University, S.S.J. Campus Almora, Uttarakhand The different parts of the plant viz- leaf, stem and root were separated cut and air dried in shade at laboratory temperature.

2.2: Preparation of aqueous extract of *Boerhaavia diffusa*: The plant extract of *Boerhaavia diffusa* was prepared by standard methodology **(Pharmacopoeia of India, 1985).**

2.2 (a): Extraction of plant component- leaf: The plant leaf extract was prepared by soaking 50 g shade dried powdered leaf material of *Boerhaavia diffusa* in 50% ethyl alcohol for 24 hr with intermittent stirring at 40^0C with the help of magnetic stirrers. The infusions were filtered through muslin cloth to get the supernatant. The filtrate was dried under reduced pressure with the help of rotary vacuum pump evaporator to get the final extract. The percent yield of the leaf extract was recorded approximately 10 percent.

2.2 (b): Extraction of plant component– stem: The plant stem extract was prepared by soaking 50 g shade dried stem powdered material in 50% of ethyl alcohol for 24 hr with intermittent stirring at 40 ^{0}C with the help of magnetic stirrers. The infusions were filtered through muslin cloth to get the supernatant. The filtrate was dried under reduced pressure with the help of rotary vacuum pump evaporator to get the final extract. The percent yield of the stem extract was recorded approximately 6 % percent.

2.2 (c):Extraction of plant component- root: The plant root extract was prepared by soaking 50 g shade dried root powdered material in 50% of ethyl alcohol for 24 hr with intermittent stirring at 40^0C with the help of magnetic stirrers. The infusions were filtered through muslin cloth to get the supernatant. The filtrate was dried under reduced pressure with the help of rotary vacuum pump evaporator to get the final extract. The percent yield of the root extract was approximately 5% percent. The shade dried powdered aqueous extract of leaf, stem & root of the plant was used throughout the study. This undiluted plant extract was diluted, wherever required,

in N.S.S. (Normal Saline Solution). The standard procedure suggested by **Cruiskshank *et al.* (1975)** was followed to make serial two fold dilution-

1. Five clear sterile cotton plugged test tubes of medium size were taken in duplicates and marked as 1-5.
2. Using aseptic technique, 5 ml of NSS was taken into all the five tubes.
3. To the first tube, 5 ml solution (200 mg/ml) of the plant leaf extract was added; contents of the tubes were mixed properly by pipetting.
4. From first tube, 5 ml of suspension was transferred to second tube, mixed well and again 5 ml suspension was transferred from second tube to the third tube.
5. This process was continued up to tube number five and 5 ml solution from the last tube was discarded. In this way, two series of serial two fold dilutions was prepared, one served as the test series and another one as parallels control, Same process was followed for making serial two fold dilution of the other plant component (stem and root extract) and dilutions obtained were as 1:2, 1:4, 1:8, 1:16 ,and 1:32.

III. PREPARATION OF MICROBIAL STRAINS

3 (a): Text- Bacteria

The antibacterial activity of different plant component (leaf, stem and root) was assessed against three bacterial species *Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* and they were procured from the Department of Veterinary Microbiology, G.B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar. The Inoculums for the study were prepared by growing these bacterial species in nutrient agar (Hi media Bombay Pvt. Ltd.) at 37^0C for 18 hr & purity of culture was checked after 8 h of incubation, kept at 4 ^{0}C until used. The bacterial cultures were diluted in sterile N.N.S. for further study via serial two fold dilution (Cruiskshank *et al.*, 1975).

Table3.1: Bacterial dilutions used in the study

Bacterial Strains	**Dilutions**				
Escherichia Coli	1:10	1:100	1:1000	1:10000	1:100000
Staphylococus aureus	1:10	1:100	1:1000	1:10000	1:100000
Salmonella typhi	1:10	1:100	1:1000	1:10000	1:100000

I. Antibiogram study: Agar Disk diffusion Techniques

Filter paper disc impregnated with different plant component viz- leaf, stem and root of *Boerhaavia diffusa* used to study the antibacterial activity (reduction in colony diameter).

4 (a) ANTIBACTERIAL ASSAY:

Antibacterial activity of different plant component (Leaf, stem and root) of different concentration was determined by the method of **Murray *et al***. (1995). Efficacy of the extract was determined by comparing the zone of inhibition around the sensitivity disc. The bacterial suspension (each organism) of different concentrations (1:10,1:100,1:1000,1:10,000, 1:10,0000) were spread over the plates containing nutrient agar (Hi-Media) using a sterile cotton swab in order to get uniform microbial growth on test plates. Under aseptic conditions, empty sterilized filter paper disc (Hi-Media, 10 diameter.) were impregnated with different plant component extracts (leaf, stem, and root) of different concentrations (1:2, 1:4, 1:16, and 1:32) and dried at room temperature Sterile forceps were used to place each of the discs (loaded with different plant component of different concentration) on agar surface. Paper disc moistened with N.S.S. were placed on a seeded Petri dishes as a control. All the Petri dishes were left for 30 minutes at room temperature to allow the diffusion of plant extract and then they were incubated at 37 ^{0}C for 24 hours. The zone of inhibition was measured in mm with a "Hi Antibiotic Zone Scale". Studies were performed in triplicate and mean value was calculated. The results were expressed as mean ± SEM (standard error of mean).

4 (b): MINIMUM INHIBITORY CONCERTRATION (MIC Assay):

Based on the previous study, Minimum Inhibitory Concentration (MIC) of the positive extracts were determined by the method (test with different gradient concentration) suggested by Scott (1989). By this method, the test organisms were seeded uniformly over an agar surface and exposed to decreasing concentrations (from 200 mg/ml to 12.5 mg/ml) of different plant component extracts (leaf, stem and root) diffusing from a paper disc (Disc Diffusion Test). The plates were then incubated at 37 ^{0}C for 18 hours. The bacteria which were sensitive to the plant component they were inhibited from growing in a circular zone around the paper disc. Zone of inhibition was measured in mm as above to determine the MIC (table -7.10).

IV. RESULTS AND DISCUSSION

The antibacterial activity of different plant components (leaf, stem and root) of *Boerhaavia diffusa* aqueous extract against *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli* were summarized in table- 5.1, 5.2 and 5.3. The result of this investigation revealed that the different plant components of *Boerhaavia diffusa* showed antibacterial activity with varying magnitudes. The zone of inhibition (reduction in colony diameter) above 10 mm in diameter was taken as positive result. Generally, most of the test organisms were sensitive to different plant components (leaf, stem and root) extracts of *Boerhaavia diffusa*. Sensitivity of bacterial species against active extract was observed in the decreasing order of *Salmonella typhi>Staphylococcus aureus >Escherichia coli*. It is also evident from the data that all the plant part marked inhibitory effect against two test organism except *Escherichia coli.* The plant part which exhibited highest bacterial activity was Root> Leaf> Stem. There was no zone of inhibition with the control (NSS disc).

Table-5.1: Mean inhibition zone diameter (ZD) by loaded disc (leaf) with 5 mg extract dissolved in NSS against different bacterial strains

Different concentration of plant component leaf (mg/ml)	Bacterial strains											
	Salmonella typhi			*Staphylococcus aureus*			*Escherichia coli*			Control (NSS)		
	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM
200	A	24 + 0.571		A	23 + 0.471		N	-		N	-	
100	A	23 + 2.41		A	21 + 0.816		N	-		N	-	
50	A	17 + 0.471		A	16 + 0.943		N	-		N	-	
25	A	16 + 1.247		A	12 + 0.943		N	-		N	-	
12.5	A	12 + 0.472		N	10 + 0.471		N	-		N	-	

Table-5.2: Mean inhibition zone diameter (ZD) by loaded disc (stem) with 5 mg extract dissolved in NSS against different bacterial strains

Different concentration of plant component leaf (mg/ml)	*Bacterial strains*											
	Salmonella typhi			*Staphylococcus aureus*			*Escherichia coli*			*Control (NSS)*		
	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM
200	A	23 + 0.942		A	22 +0.431		N	-		N	-	
100	A	21 + 1.247		A	19 +0.471		N	-		N	-	
50	A	14 + 0.943		A	14 +2.49		N	-		N	-	
25	A	13 + 1.41		A	12 +0.943		N	-		N	-	
12.5	A	11 +1.633		N	9 +0.472		N	-		N	-	

Table-5.3: Mean inhibition zone diameter (ZD) by loaded disc (root) with 5 mg extract dissolved in NSS against different bacterial strains.

Different concentration of plant component leaf (mg/ml)	Bacterial strains											
	Salmonella typhi			*Staphylococcus aureus*			*Escherichia coli*			*Control (NSS)*		
	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM	Status	ZD (mm)	SEM
200	A	27 + 0		A	25 + 0.431		N	-		N	-	
100	A	25 + 0.816		A	20 + 0.471		N	-		N	-	
50	A	19 + 1.632		A	19 + 2.49		N	-		N	-	
25	A	15 + 1.414		A	15 + 0.943		N	-		N	-	
12.5	A	13 + 0.816		N	10 + 0.472		N	-		N	-	

Key to tables – 5.1, 5.2 and 5.3

Symbol Meaning
A - Active
N - Not Active
SEM - Standard error of the mean
ZD - Zone diameter (mm)
(-) - No effect

Studies have been carried out to test antibacterial activity of *B. diffusa* (Abo and Ashide, 1999). Similarly Kumar *et al. (1997)* have also reported antibacterial activity in the Seeds of *B. diffusa* against *Bacillus Subtilis* (zone diameter 3.30mm/mg) *Pseudomonas cichorii* (Zone diameter 6.60 mm/mg) except *Escherichia coli.* Nair and Chanda (2006) reported antibacterial activity of *B. diffusa* leaves against *Pseudomonas aeruginos,* Aladesanmi *et al.* (2007) also reported antibacterial activity of plant *B. difJusa* against *Bacillus subtilis* (Inhibition zone 2.0 mm (40mg/ml) and 6.0 mm (225 mg/ml), *Pseudomonas aeruginosa* (inhibition zone 3 mm (225mg/ml), *Staphylococcus aureus* (inhibition zone 1 mm, 1 mm, 2 mm, 4 mm / 10, 20, 40, 225 mg/ml respectively) except *Escherichia coli.*

However, in the present investigation result obtained showed that two fold dilutions of different plant components viz leaf, stem, and root extracts of *B. diffusa* gives antibacterial activity against *Salmonella typhi, Staphylococcus aureus* except *Escherichia coli* (Fig 5.1.A, B, C). The Bacteria S. *typhi* causes Typhoid fever, Neonatal meningitis infection via intestinal route (Banavandi 2005, Todar 2005). The root component of *B. diffusa* showed maximum antibacterial activity against S. *typhi* (inhibition zone 27-13 mm) (Plate 5.1 A, B, C, D and E) followed by leaf extract (inhibition zone 24-12 mm) (Plate 5.2 A, B, C, D and E) and least in case of stem extract (23 - 11 mm) (Plate 5.3 A, B, C, D and E), while the bacteria *Staphylococcus aureus* causes urinary trouble, middle ear infection, scarlet fever, food poisoning, toxic shock syndrome, as well as Gram -ve cocci causing gonorrhoea (Todar 2005). According to result given above root component of *B. diffusa* had maximum antibacterial activity against *S. aureus* (inhibition zone 25-10 mm) (Plate 5.4 A, B, C, D and E) followed by leaf component (inhibition zone 23-10 mm) (Plate 5.5 A, B, C, D and E) and least in case of stem component (Inhibition zone 22-9 mm) (Plate 5.6 A, B, C, D and E).

The result further showed that the root extract possess maximum antibacterial activity against *Salmonella typhi* (Inhibition zone 27 mm) and *Staphylococcus aureus* (Inhibition zone 25 mm) while the stem extract marked least antibacterial activity against *Salmonella typhi* (inhibition zone 23 mm) and *Staphylococcus aureus* (inhibition zone 22 mm) and activity of leaf extract reported against *Salmonella typhi* (inhibition zone 24 mm) and *Staphylococcus aureus* (inhibition zone 23 mm) was intermediate between these two extract (Root and leaf). This appears to be the first study that actually investigated antibacterial activity of different plant component (leaf, Stem and Root) of *B. diffusa* against *S. typhi* and *S. aureus.* However, there have been extensive study on *B. diffusa* seeds and leaf on different bacteria was done by Nair and Chanda (2006); Aladescmi *et al.* (2007) have reported that *B. diffusa* extracts have little activity against bacteria and no antifungal activity against fungi in Nigeria. However, the investigation is much similar to Nair and chanda (2006) because in both investigations the plant *B. diffusa* fail to inhibit *E. coli* but as result expressed that plant *B. diffusa* have shown the potential to inhibit the *S. typhi* and *S. aureus.*

Thus result expressed that *B. diffusa* can be used in the treatment of typhoid fever, urinary tract infection, ear infection, fever, food poisoning caused by bacteria *S. typhi* and *S. aureus* respectively. This finding also confirmed that folkloric claims of locally consumed *B. diffusa* extract to cures typhoid fever, ear infection, fever, urinary trouble in local Kumaun Hills. \Minimum Inhibitory Concentration (MIC) for different plant component (leaf, stem and root) extract ranged from 25 mg/ml to 50 mg/ml (table-5.4) as can be seen in the table 5.4 the MIC zone increased with increasing concentration of different plant components. This study also revealed that the root and leaf extract showed maximum activity with MIC value being 25 mg/ml followed by stem extract with MIC value 50 mg/ml. In the present study minimum inhibitory concentration (MIC) for different plant component (leaf, stem and root) extract ranged from 25-50 mg/ml (Fig 5.2) it is clear from the figure, the zone diameter increased with increasing concentration of different plant components. The results also revealed that (Plate5.7 A, B, and C) the different plant components (leaves, stem and root) of *B. diffusa* were able to inhibit *S. typhi*. Whereas (Plate 5.8 A, C) the root and leaf extract was effective against *S. aureus*, while stem extract fail to inhibit said bacterium (Plate 5.8 B).

Table 5.4: Minimum Inhibitory Concentration of the active compound against tested bacteria

Plant component	Extract 200 mg/ml	*Salmonella typhi*		*Staphylococcus aureus*		*Escherichia coli*	
		MIC mg/ml	ZD (mm)	MIC mg/ml	ZD (mm)	MIC mg/ml	ZD (mm)
Leaf	NSS	25	11	25	11	-	-
Stem	NSS	50	10	-	-	-	-
Root	NSS	25	12	25	10	-	-

Table 5.5: Relationship among various concentration of different plant component (leaf, stem and root) extracts with zone of inhibition on against *Salmonella typhi* in determination of MIC

Concentration of different plant extract (mg/ml)	Leaf	Stem	Root
	ZD (mm)	ZD (mm)	ZD (mm)
200	21	16	23
100	18	14	18
50	14	10	16
25	11	-	12
12.5	-	-	-

Table-5.6: Relationship among various concentration of different plant component (leaf, stem and root) extracts with zone of inhibition on against *Staphylococcus aureus* in determination of MIC

Concentration of different plant extract (mg/ml)	Leaf	Stem	Root
	ZD (mm)	ZD (mm)	ZD (mm)
200	15	-	16
100	14	-	14
50	13	-	12
25	11*	-	10
12.5	-	-	-

In this study, different bacteria marked different zone inhibition diameter against different plant components Fig 5.3 showed that root extract have always shown higher zone of inhibition diameter against *S. typhi* with 25 mg/ml (inhibition zone 23 to 12 mm) then stem and leaves extracts. Leaves extract revealed next higher zone of inhibition diameter (inhibition zone 21 to 11 mm) against S. *typhi* where as stem extract marked minimum zone inhibitory diameter (inhibition zone 16 to l0 mm) against the said bacterium.

Present study also revealed zone of inhibition diameter versus plant extracts (Fig. 5.4) against *S. aureus.* Root extract have always marked maximum zone diameter (inhibition zone 16 to 12 mm) at higher concentration (200 and 100 mg/ml) where as at lower concentration (50 and 25 mg/ml) leaves revealed higher zone of inhibition (inhibition zone 13 to 11 mm) then stem and root extracts. From above figures it is here by suggested that root extract could be used against diseases caused by *Salmonella typhi* where as root extract and leaf extracts may be also used against diseases caused by *S. aureus* at higher and lower concentration respectively. *B. diffusa* extract is not effective against *Escherichia coli* bacteria.

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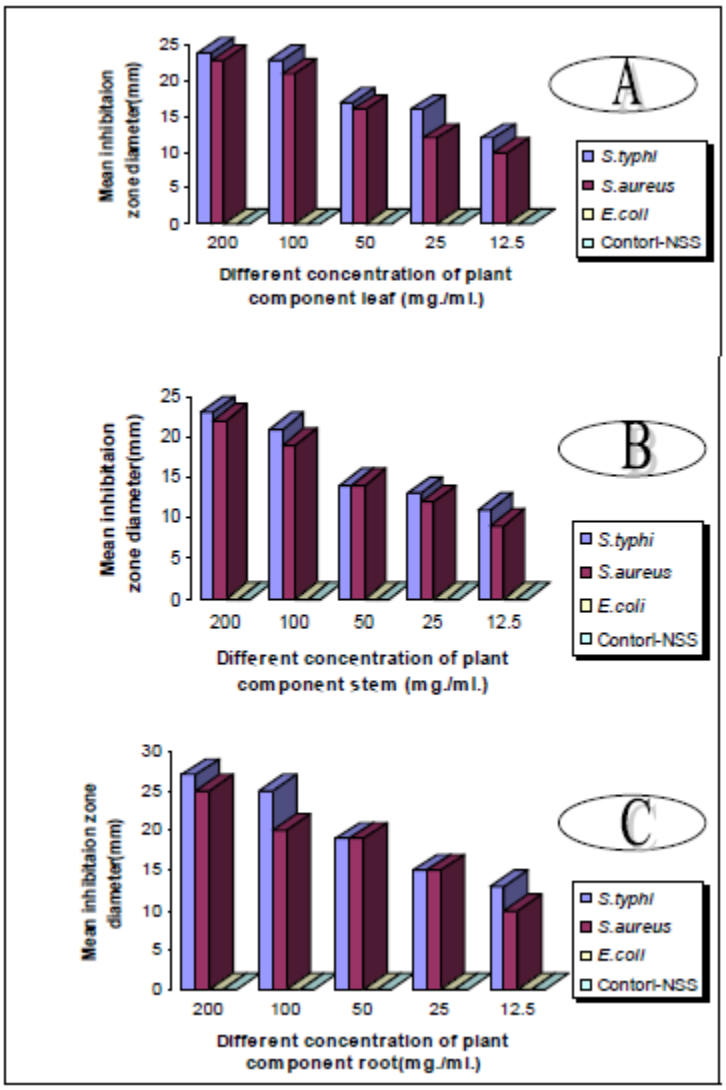

Fig. 5.1- Mean inhibition zone diameter (ZD) by loaded disc (A) leaf, (B) stem and (C) root with 5 mg extract dissolved in NSS.

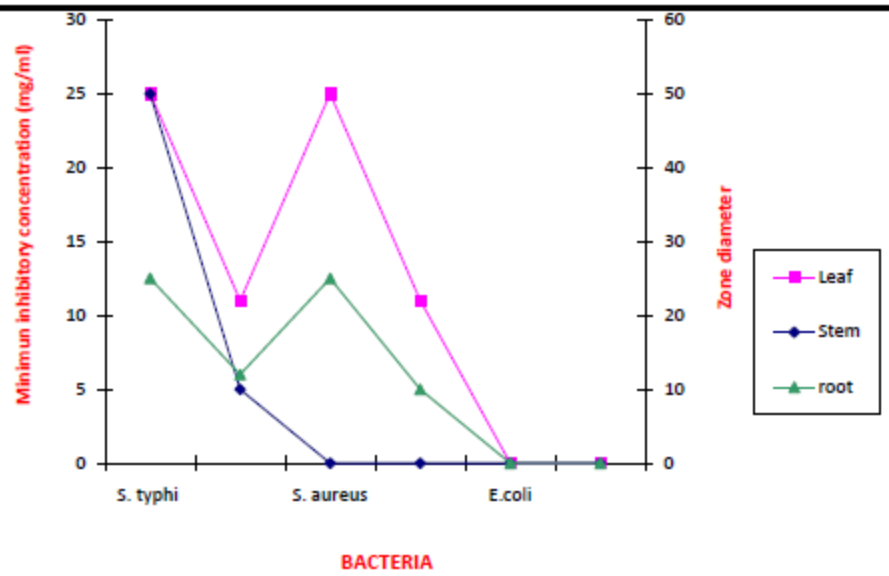

Fig. 5.2 Minimum inhibitory concentration (MIC) of the active compound tested against bacteria

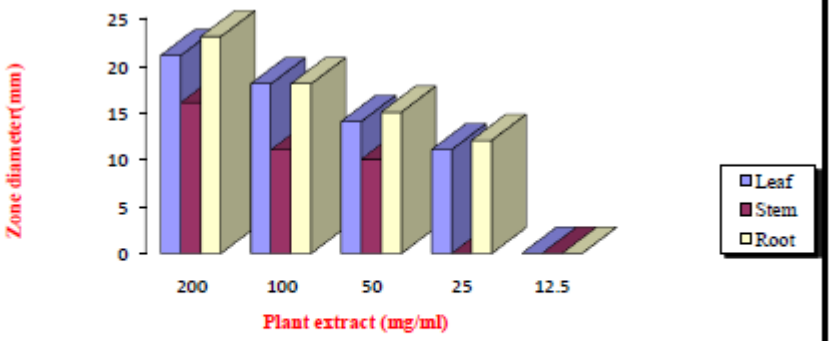

Fig 5.3 Relationship among various concentration of different plant component(leaf, stem and root) extract with zone of inhibition against *Salmonella typhi*

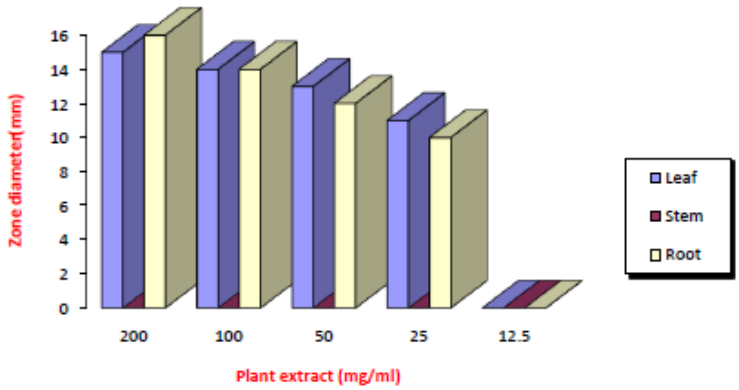

Fig. 5.4 Relationship among various concentration of different plant component (leaf, stem and root) extract with zone of inhibition against Staphylococcus aureus

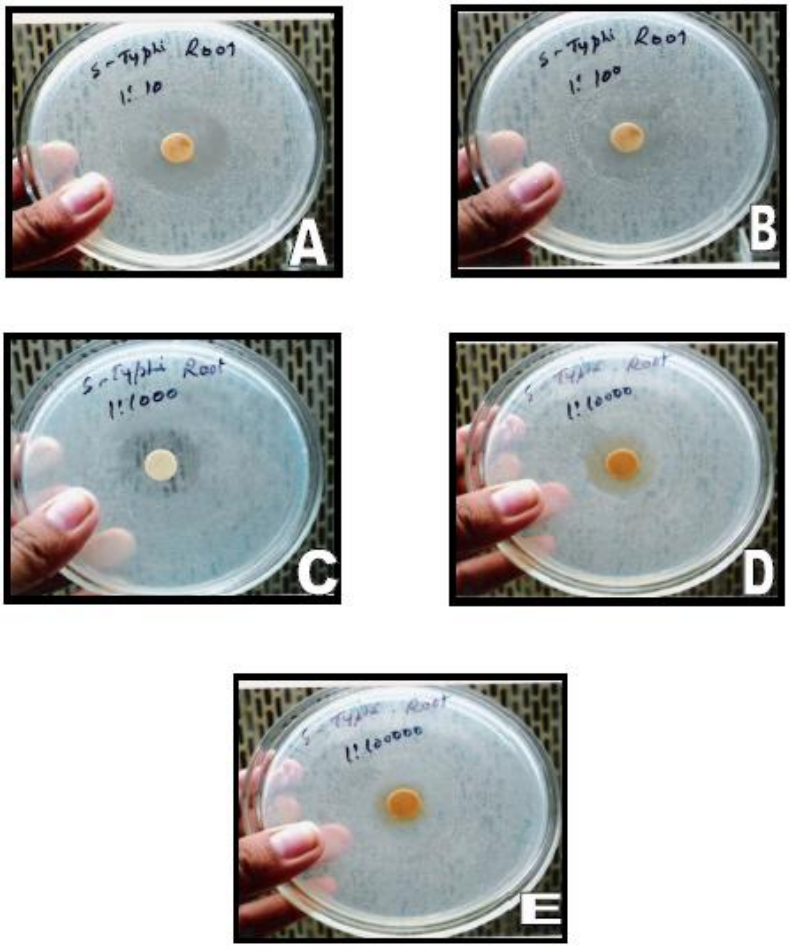

Plate 5.1: A, B, C, D and E: The root component of *B. diffusa* showed maximum antibacterial activity against *S. typhi* with inhibition zone 27 – 13 mm at different concentration (200 – 12.5 mg/ml) of root component

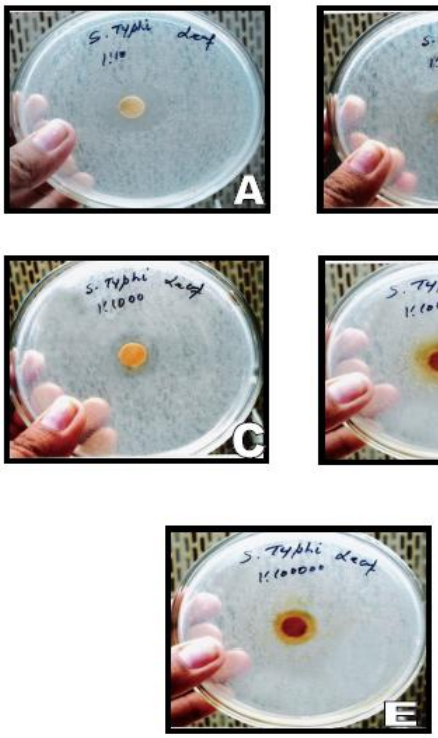

Plate 5.2: A, B, C, D and E: The leaf component of *B. diffusa* showed maximum antibacterial activity against *S. typhi* with inhibition zone 24 – 12 mm at different concentration (200 – 12.5 mg/ml) of leaf component

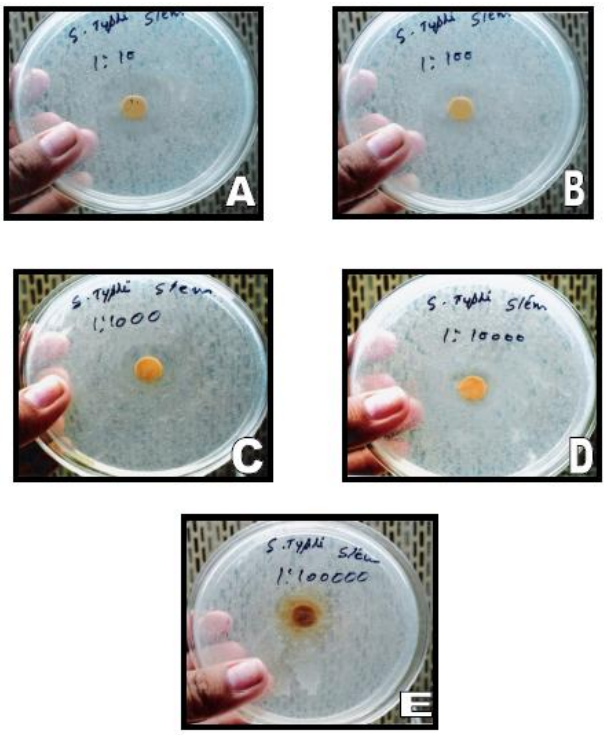

Plate 5.3: A, B, C, D and E: The stem component of *B. diffusa* showed maximum antibacterial activity against *S. typhi* with inhibition zone 23 – 11 mm at different concentration (200 – 12.5 mg/ml) of stem component

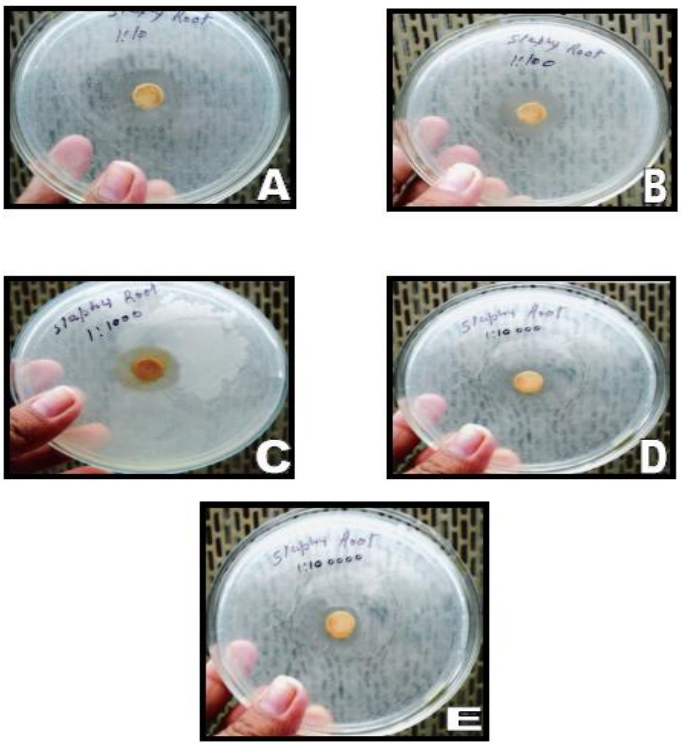

Plate 5.4: A, B, C, D and E: The root component of *B. diffusa* showed maximum antibacterial activity against *S. aureus* with inhibition zone 25 – 10 mm at different concentration (200 – 12.5 mg/ml) of root component

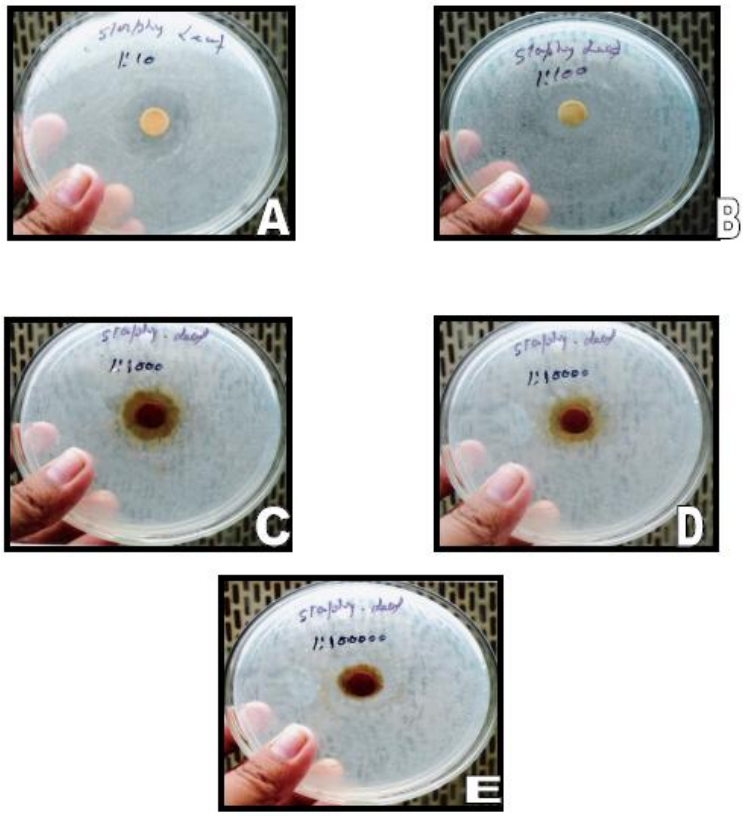

Plate 5.5: A, B, C, D and E: The leaf component of *B. diffusa* showed maximum antibacterial activity against *S. aureus* with inhibition zone 23 – 10 mm at different concentration (200 – 12.5 mg/ml) of leaf component

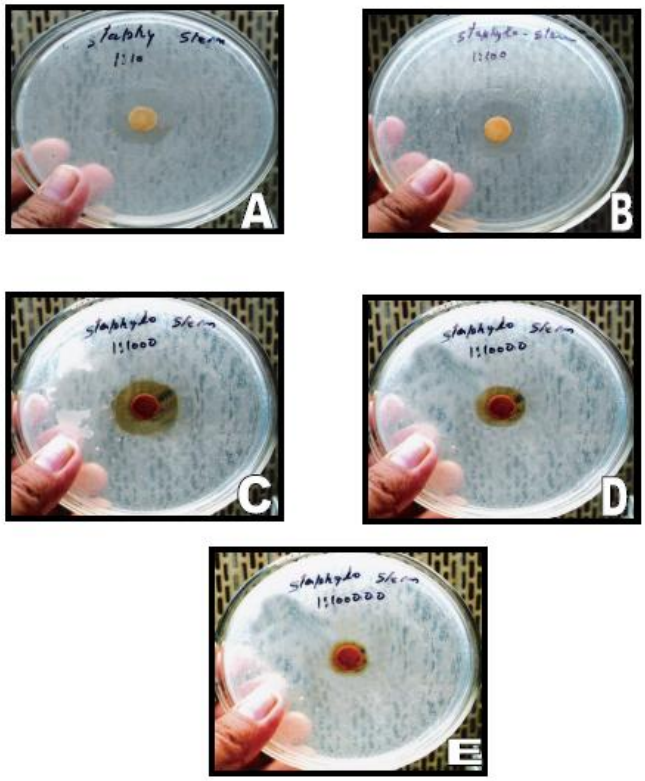

Plate 5.6: A, B, C, D and E: The stem component of *B. diffusa* showed maximum antibacterial activity against *S. aureus* with inhibition zone 22 – 9 mm at different concentration (200 – 12.5 mg/ml) of stem component

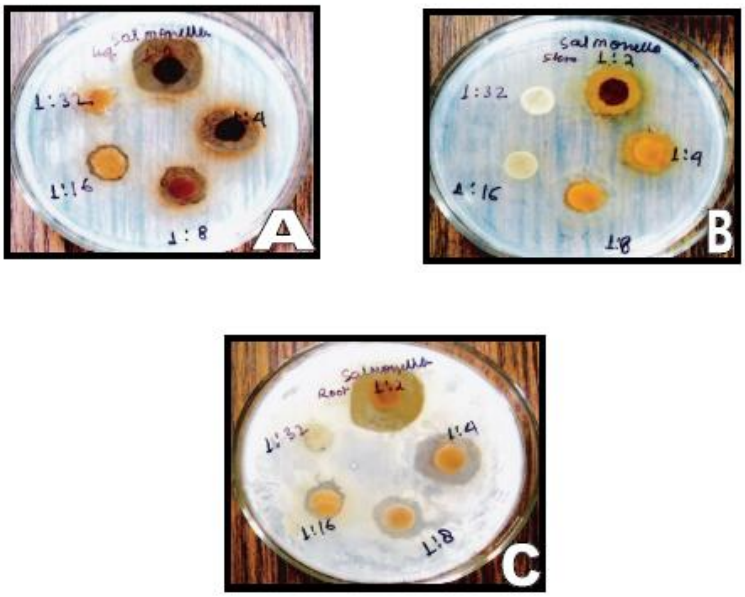

Plate 5.7: A, B, and C: Different components (leaf, stem and root) of *B. diffusa* were able to inhibit *S. typhi* at different concentrations

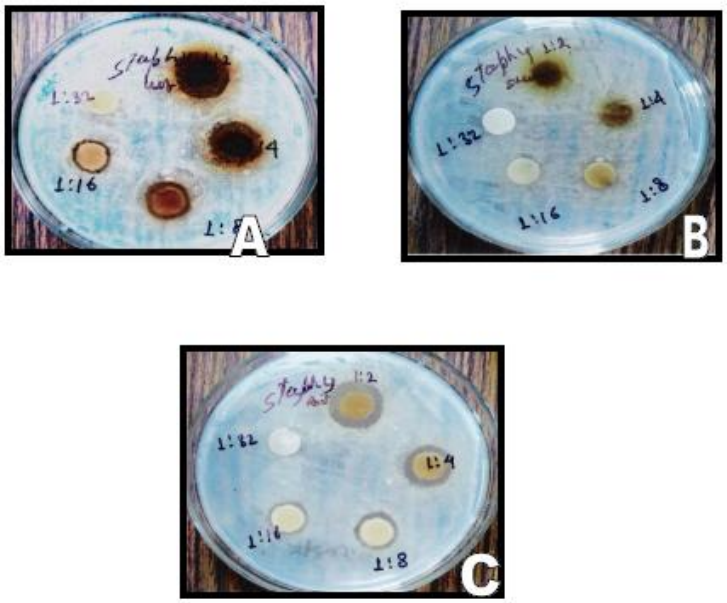

Plate 5.8: A, B, and C: Different components (leaf, stem and root) of *B. diffusa* were able to inhibit *S. aureus* at different concentrations