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ANTIBACTERIAL ACTIVITY OF EXTRACT OBTAINED FROM *CATHARNTHUS ROSUES* AGAINST DIFFERENT PATHOGENIC BACTERIA

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ABSTRACT

Catharanthus roseus family Apocynaceae is highly reputed medicinal tree commonly known as the Sadabahar. Various tissues of *Catharanthus roseus* have demonstrated positive effects on heart diseases (root bark), dysentery and inflammation (leaves), diabetes (leaf extract), and ulcers. In the present study the antibacterial activity of the methanol, chloroform, ethanol, & aqueous extract from the leaves of *Catharanthus roseus* was studied using disc diffusion method & agar well method against pathogenic bacterial strains *of Achromobacter, Bacillus, Klebsiella, CoNS (Coagulase negative staphylococcus), Enterobacter, Enterococci, Pseudomonas, Proteus, Staphylococcus aureus, E. coli.* Best result showed in all extract against *Pseudomonas, Enterococci, S. aureus and Bacillus* in a well diffusion method but *S. aureus* showed highest zone of inhibition in methanol extract. In paper disc method ethanol extract showed best result against Pseudomona *sp.*

KEYWORDS: *Catharanthus roseus;* Pathogenic bacteria; Agar well diffusion method; Disc diffusion method; Inhibition zone.

INTRODUCTION

According to natural plant products may offer a new source of antibacterial agents. In recent years antimicrobial properties of Indian medicinal plants have been increasingly reported (**Aswal et al., 1996; Ahmad et al., 1998**). *Catharanthus roseus (L.)* belongs to family Apocynaceae, commonly known as Madagascar rosy periwinkle and Sadhabhar (Hindi),

(**George Don, 1856**). It is an evergreen subshurb or herbaceous plant growing 1 m tall. It is found all over India, from sub-Himalayan forest, Bengal, central and south India. The different parts of this plant contain number of Secondary Metabolites like flavonids, alkaloids, Terpenoids, sterols and essential oils. Various tissues of *Catharanthus roseus* have demonstrated positive effects on heart diseases Cancers,dysentery and inflammation (leaves) (**Arul et al., 2005**), diabetes (leaf extract) (**Kumar et al., 2009**),

MATERIALS AND METHODS

COLLECTION & PREPARATION OF PLANT MATERIAL

The fresh leaves of *Catharanthus roseus* were collected from outside of the Bhopal (M.P.) India in the month of December.

1) **Extract preparation**-: For this practical five types of extract prepared they are.

   The leaf are taken and then rinsed in running tap water, few leaves are shade dry in room for 6-7 days and few in oven dry for 4-5 days and then crushed with the help of mortar-pistle and make powder form for different extract preparation which is used for the practical

a) **Aqueous and Ethanol Extract**: 100gm powder of fresh, shade dry and oven dry leaf was dipped in 400ml distilled water in a conical flask and left for 7 days with occasional shaking. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask. The extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test.[2]

b) **Methanol Extract**: 50gm powder of fresh, shade dry and oven dry leaves sequentially extracted by shaking for 2 hours on Wrist Action Shaker after overnight soaking in 150 ml of relevant solvent. After filtration, samples were rinsed with additional 3 x 60 ml portions of the solvent. Combined filtrates were dried at room temperature under electric fan. The extracts were stored in the refrigerator at 4°C until required.[8]

c) **Chloroform Extract**: 10gm powder of fresh, shade dry and oven dry leaf was dipped in 100ml distilled water in a conical flask and left for 5 days. Filtered off using sterile filter paper (Whattman no. 1) into a clean conical flask. The extracts obtained were then stored in a refrigerator at 4°C for antibacterial activity test.[5]

TEST ORGANISM

Ten Bacterial strain used in the present study *(Achromobacter, Bacillus, Klebsiella, CoNS (Coagulase negative staphylococcus), Enterobacter, Enterococci, Pseudomonas, Proteus,*

Staphylococcus aureus, E. coli.) were obtained from Gandhi medical College Bhopal(M.P.). The bacterial species were identified.

SCREENING OF ANTIBACTERIAL ACTIVITY

Screening of antibacterial activity was performed by standard disc diffusion method & agar well diffusion method.

Disc Diffusion Method: - The Mueller Hinton agar media pour into presterilized petriplates after solidification 1ml of bacterial strain was uniformly speeded on agar plates with the help of glass spreader the plates. The sterilized discs were dipped in the plant extract of concentration. The filter paper disc soaked in the plant extract was placed on the surface of the bacteria on agar plates.

Agar Well Diffusion Method: - Two milliliter of inoculums of each selected bacterium was inoculated into the 45 – 50 ^{o}C, cooled agar & plated. After 30 minutes three wells approximately 5-6 mm diameter was bored on the medium plate with the help of sterile cork borer. Using a micropipette, 25µl of each extracts were added onto each well on all plates. After antibacterial test, the plates were placed in incubator for 24hrs at 37 ^{o}C.

RESULT AND DISCUSSION

RESULT

In this study four types of plant extract prepared. All extract is used for antibacterial test which include two types of method i.e., paper disc method and well diffusion method and in this study the practical was done triplet.

PAPER DISC METHOD FOR LEAVES EXTRACT

Table 1: Antibacterial activity of Aqueous and Ethanol extract of *Catharanthus roseus* in paper disc method (Mean ± SD) (mm).

Bacteria	Aqueous extract			Ethanol extract		
	Fresh	Shade dry	Oven dry	Fresh	Shade dry	Oven dry
Bacillus	15 ± 0.3	13 ± 0.8	10 ± 0.6	18 ± 0.2	15 ± 0.1	13 ± 0.4
Proteus	20 ± 0.8	18 ± 0.3	16 ± 0.2	21 ± 0.1	19 ± 0.7	15 ± 0.7
Pseudomonas	25 ± 0.8	17 ± 0.2	14 ± 0.4	25 ± 0.1	20 ± 0.6	15 ± 0.2
Achromobacter	21 ± 0.7	17 ± 0.3	13 ± 0.3	23 ± 0.2	12 ± 0.1	11 ± 0.2
S.aureus	19 ± 0.1	0.0 ± 0.0	15 ± 0.5	20 ± 0.7	10 ± 0.1	0.0 ± 0.0
CoNS	20 ± 0.3	17 ± 0.1	16 ± 0.3	21 ± 0.2	17 ± 0.3	16 ± 0.6
Enterococci	23 ± 0.9	20 ± 0.1	16 ± 1.3	26 ± 0.4	24 ± 0.8	20 ± 0.4
Klebsiella	20 ± 0.7	13 ± 0.8	10 ± 1.5	22 ± 0.3	18 ± 0.8	15 ± 0.5

Enterobacter	21 ± 0.4	13 ± 0.7	15 ± 0.2	21 ± 0.1	16 ± 0.8	18 ± 0.1
E.coli	21 ± 0.14	19 ± 0.9	18 ± 0.3	18 ± 0.4	23 ± 0.7	16 ± 0.5

Table 2: Antibacterial activity of Methanol and Chloroform extract of *Catharanthus roseus* in paper disc method (Mean ± SD) (mm).

Bacteria	Methanol extract			Chloroform extract		
	Fresh	Shade dry	Oven dry	Fresh	Shade dry	Oven dry
Bacillus	21 ± 0.4	17 ± 0.8	13 ± 1.7	17 ± 0.6	13 ± 0.4	12 ± 0.8
Proteus	24 ± 0.2	16 ± 0.5	15 ± 0.9	18 ± 0.9	15 ± 0.1	0.0 ± 0.0
Pseudomonas	23 ± 0.8	17 ± 0.3	14 ± 0.4	20 ± 0.2	20 ± 0.7	17 ± 0.6
Achromobacter	19 ± 0.3	18 ± 0.8	15 ± 0.5	18 ± 1.2	15 ± 0.9	14 ± 0.4
S.aureus	23 ± 0.3	22 ± 0.6	14 ± 0.9	18 ± 0.2	22 ± 1.3	15 ± 0.1
CoNS	20 ± 0.8	17 ± 0.4	13 ± 0.3	19 ± 0.9	16 ± 0.4	14 ± 0.1
Enterococci	26 ± 0.9	24 ± 0.7	20 ± 0.3	23 ± 0.4	20 ± 0.7	19 ± 0.1
Klebsiella	22 ± 1.4	19 ± 0.1	13 ± 0.3	18 ± 1.1	13 ± 0.6	11 ± 0.5
Enterobacter	21 ± 0.3	17 ± 0.7	13 ± 0.5	20 ± 1.9	17 ± 0.5	15 ± 0.7
E.coli	20 ± 0.7	18 ± 0.5	20 ± 0.9	19 ± 0.5	16 ± 0.2	15 ± 0.8

The antibacterial activity of aqueous, chloroform, ethanol, and methanol extract of Catharanthus roseus leaf were studied by paper disc diffusion method.

Ethanol extract

The result clearly showed in ethanol leaf extract of *Catharanthus roseus*.. This extract showed highest zone of inhibition against *Pseudomonas, Enterococci, Achromobacter, Klebsiella, Enterobacter, Proteus*, In fresh leaf extract (25 ± 0.1), (26 ± 0.4), (23 ± 0.2), (22 ± 0.3), (21 ± 0.1), (21 ± 0.1) respectively. In shade dry leaf extract (20 ± 0.6), (24 ± 0.8), (12 ± 0.1), (18 ± 0.8), (16 ± 0.8), (19 ± 0.7) resp. In oven dry leaf extract (15 ± 0.2), (20 ± 0.4), (11 ± 0.2), (15 ± 0.5), (18 ± 0.1), (15 ± 0.7) resp.

Aqueous extract

The result clearly showed in ethanol leaf extract of *Catharanthus roseus*. This extract showed highest zone of inhibition against *Pseudomonas, Enterococci, Achromobacter, Klebsiella, Enterobacter, Proteus*, In fresh leaf extract (25 ± 0.8), (24 ± 0.9), (21 ± 0.7), (20 ± 0.7), (21 ± 0.4), (20 ± 0.8) respectively. In shade dry leaf extract (17 ± 0.2), (20 ± 0.1), (17 ± 0.3), (13 ± 0.8), (13 ± 0.7), (18 ± 0.3) resp. In oven dry leaf extract (14 ± 0.4), (16 ± 1.3), (13 ± 0.3), (10 ± 1.5), (15 ± 0.2), (16 ± 0.2) resp.

Methanol extract

The result clearly showed in ethanol leaf extract of *Catharanthus roseus*. This extract showed highest zone of inhibition against *Pseudomonas, Enterococci, Achromobacter, Klebsiella,*

Enterobacter, *Proteus*, In fresh leaf extract (23 ± 0.8), (26 ± 0.9), (19 ± 0.3), (22 ± 1.4), (21 ± 0.3), (24 ± 0.2) respectively. In shade dry leaf extract (17 ± 0.3), (24 ± 0.7), (18 ± 0.8), (19 ± 0.1), (17 ± 0.7), (17 ± 0.5) resp. In oven dry leaf extract (14 ± 0.4), (20 ± 0.3), (15 ± 0.5), (13 ± 0.3), (13 ± 0.5), (15 ± 0.9) resp.

Chloroform extract

The result clearly showed in ethanol leaf extract of *Catharanthus roseus*. This extract showed highest zone of inhibition against *Pseudomonas*, *Enterococci*, *Achromobacter*, *Klebsiella*, *Enterobacter*, *Proteus*, In fresh leaf extract (20 ± 0.2), (23 ± 0.4), (18 ± 1.2), (18 ± 1.1), (20 ± 1.9), (18 ± 0.9) respectively. In shade dry leaf extract (20 ± 0.7), (20 ± 0.7), (15 ± 0.9), (13 ± 0.6), (17 ± 0.5), (15 ± 0.1) resp. In oven dry leaf extract (17 ± 0.6), (19 ± 0.1), (14 ± 0.4), (11 ± 0.5), (15 ± 0.7), (0.0 ± 0.0) resp.

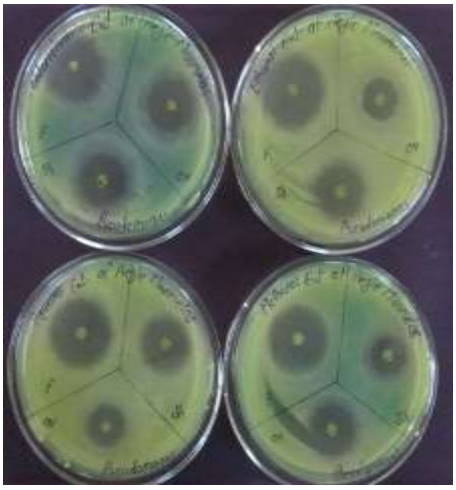

Fig:- 1 Zone of inhibition of *Pseudomonas*

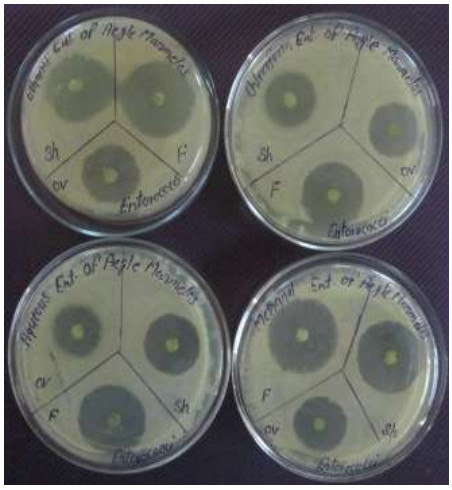

Fig:- 2 Zone of inhibition of *Enterococci*

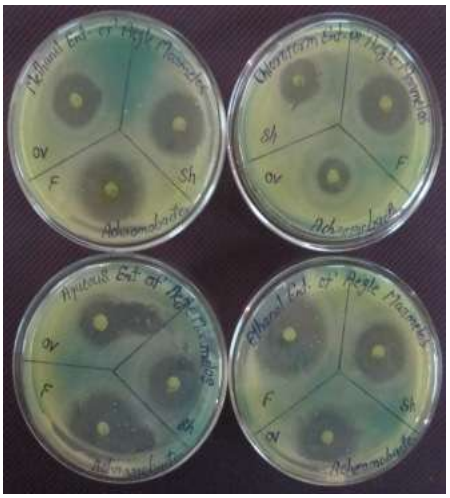

Fig:- 3 Zone of inhibition of *Achromobacter*

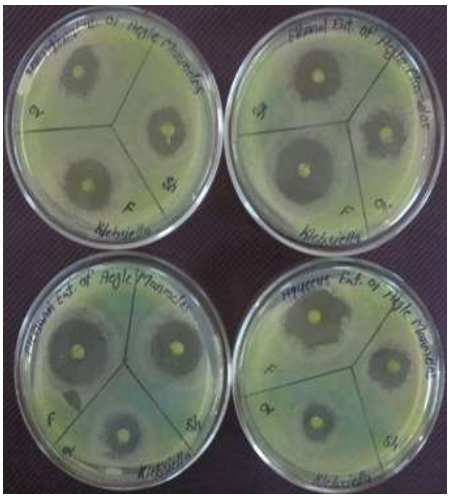

Fig:- 4 Zone of inhibition of *Klebsilla*

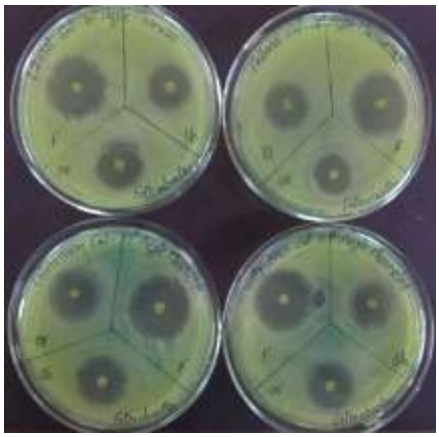

Fig:- 5 Zone of inhibition of *Enterobacter*

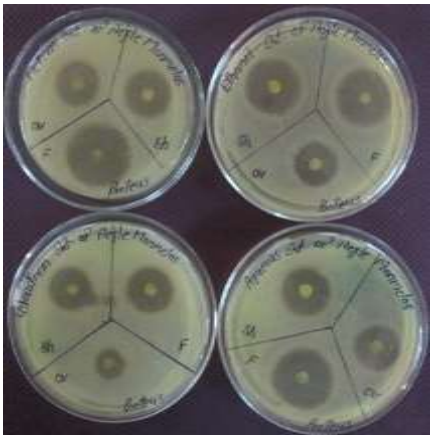

Fig:- 6 Zone of inhibition of *Proteus*

Fig- Zone of Inhibition of Leaf extract against human pathogenic bacteria

WELL DIFFUSION METHOD FOR LAEVES EXTRACT

Table 3: Antibacterial activity of Aqueous and Methanol extract of *Catharanthus roseus* in Agar well diffusion method (Mean ± SD) (mm).

Bacteria	Aqueous extract			Methanol extract		
	Fresh	Shade dry	Oven dry	Fresh	Shade dry	Oven dry
Bacillus	14 ± 0.3	15 ± 0.8	14 ± 0.1	21 ± 0.6	20 ± 0.9	14 ± 0.7
Proteus	17 ± 0.4	14 ± 0.7	0.0 ± 0.0	25 ± 0.1	19 ± 0.7	20 ± 0.5
Pseudomonas	15 ± 0.9	15 ± 0.4	13 ± 0.6	22 ± 0.3	15 ± 0.6	14 ± 0.7
Achromobacter	16 ± 1.1	13 ± 0.4	11 ± 0.7	17 ± 0.3	13 ± 0.8	0.0 ± 0.0
S.aureus	18 ± 0.7	15 ± 0.5	14 ± 0.7	24 ± 0.4	19 ± 0.9	20 ± 0.4
CoNS	13 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	14 ± 0.9	10 ± 0.5	0.0 ± 0.0
Enterococci	21 ± 0.9	16 ± 0.1	11 ± 1.3	21 ± 0.2	20 ± 0.3	13 ± 0.9
Klebsiella	13 ± 0.7	11 ± 0.8	0.0 ± 0.0	15 ± 0.3	13 ± 0.8	11 ± 0.5
Enterobacter	19 ± 0.7	12 ± 0.7	0.0 ± 0.0	21 ± 0.9	19 ± 0.5	17 ± 0.4
E.coli	17 ± 0.4	14 ± 0.9	0.0 ± 0.0	20 ± 0.9	15 ± 0.6	0.0 ± 0.0

Table 4: Antibacterial activity of Ethanol and Chloroform extract of *Catharanthus roseus* in Agar well diffusion method (Mean ± SD) (mm).

Bacteria	Chloroform			Ethanol extract		
	Fresh	Shade dry	Oven dry	Fresh	Shade dry	Oven dry
Bacillus	20 ± 0.7	18 ± 0.3	9 ± 0.3	23 ± 0.1	18 ± 0.7	16 ± 0.8
Proteus	18 ± 0.3	14 ± 0.7	0.0 ± 0.0	18 ± 0.9	16 ± 0.4	0.0 ± 0.0
Pseudomonas	15 ± 0.1	14 ± 0.5	11 ± 0.8	18 ± 0.8	14 ± 0.4	12 ± 0.9
Achromobacter	14 ± 0.3	12 ± 0.9	11 ± 0.7	19 ± 0.3	17 ± 0.6	10 ± 0.9
S.aureus	15 ± 0.9	15 ± 0.2	14 ± 0.4	20 ± 0.5	18 ± 0.5	20 ± 0.1
CoNS	9 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	14 ± 0.2	12 ± 0.7	10 ± 0.4
Enterococci	22 ± 0.7	20 ± 0.1	0.0 ± 0.0	23 ± 0.9	21 ± 0.4	15 ± 0.4
Klebsiella	11 ± 0.8	9 ± 0.4	9 ± 0.8	13 ± 0.3	10 ± 0.6	9 ± 0.7
Enterobacter	17 ± 0.7	20 ± 0.2	6 ± 0.3	18 ± 0.8	17 ± 0.2	8 ± 0.3
E.coli	19 ± 0.4	19 ± 0.9	0.0 ± 0.0	21 ± 0.6	19 ± 0.5	20 ± 0.9

The antibacterial activity of aqueous, chloroform, ethanol, and methanol extract of *Catharanthus roseus* leaf were studied by agar well diffusion method.

Aqueous extract

The result clearly showed in ethanol leaf extract of *Catharanthus roseus*. This extract showed highest zone of inhibition against *Enterococci, Pseudomonas, S. aureus, Bacillus*, in fresh leaf extract (21 ± 0.9), (15 ± 0.9), (18 ± 0.7), (14 ± 0.3) respectively. In shade dry leaf extract (16 ± 0.1), (15 ± 0.4), (15 ± 0.5), (15 ± 0.8) resp. In oven dry leaf extract (11 ± 1.3), (13 ± 0.6), (14 ± 0.7), (14 ± 0.1) resp.

Methanol extract

The result clearly showed in ethanol leaf extract of *Catharanthus roseus*. This extract showed highest zone of inhibition against Enterococci, *Pseudomonas, S. aureus, Bacillus*, In fresh leaf extract (21 ± 0.2), (22 ± 0.3), (24 ± 0.4), (21 ± 0.6) respectively. In shade dry leaf extract (20 ± 0.3), (15 ± 0.6), (19 ± 0.9), (20 ± 0.9)resp. In oven dry leaf extract (13 ± 0.9), (14 ± 0.7), (20 ± 0.4), (14 ± 0.7) resp.

Chloroform extract

The result clearly showed in ethanol leaf extract of *Catharanthus roseus*. This extract showed highest zone of inhibition against *Enterococci, Pseudomonas, S. aureus, Bacillus.* In fresh leaf extract (22 ± 0.7), (15 ± 0.1), (15 ± 0.9), (20 ± 0.7) respectively. In shade dry leaf extract (20 ± 0.1), (14 ± 0.5), (15 ± 0.2), (18 ± 0.3) resp. In oven dry leaf extract (0.0 ± 0.0), (11 ± 0.8), (14 ± 0.4), (9 ± 0.3) resp.

Ethanol extract

The result clearly showed in ethanol leaf extract of *Catharanthus roseus*. This extract showed highest zone of inhibition against *Enterococci, Pseudomonas, S. aureus, Bacillus.* In fresh leaf extract (23 ± 0.9), (18 ± 0.8), (20 ± 0.5), (23 ± 0.1), respectively. In shade dry leaf extract (21 ± 0.4), (14 ± 0.4), (18 ± 0.5), (18 ± 0.7) resp. In oven dry leaf extract (15 ± 0.4), (12 ± 0.9), (20 ± 0.1), (16 ± 0.8) resp.

Fig:- 7 Zone of inhibition of *Enterococci*

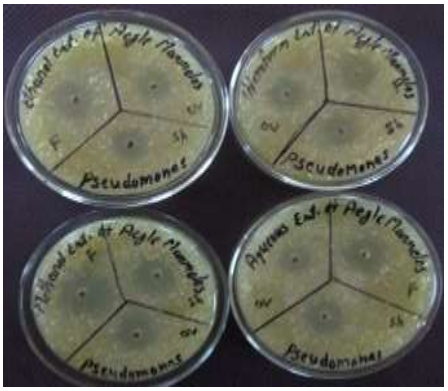

Fig:- 8 Zone of inhibition of *Pseudomonas*

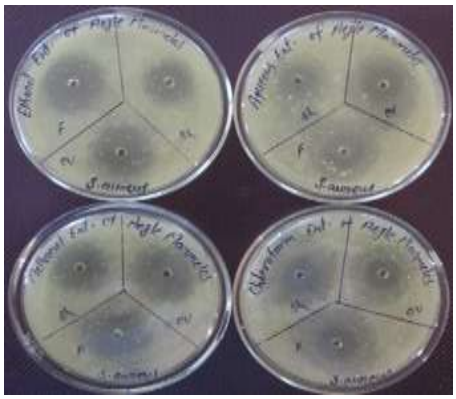

Fig:- 9 Zone of inhibition of *S.aureus*

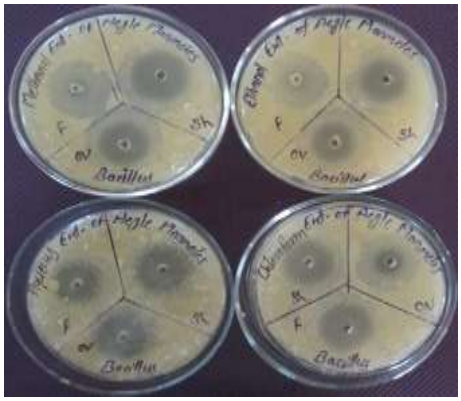

Fig:- 10 Zone of inhibition of *Bacillus*

Fig- Zone of Inhibition of Leaf extract against human pathogenic bacteria

DISCUSSION

According to (D. Venhatsan, and M. Karunakaran, 2009) the antibacterial activity of *Catharanthus roseus* extract of both solvents Aqueous and ethanolic against different pathogenic bacteria by paper disc method. The ethanolic extract showed considerably more activity compare than the aqueous extract. Maximum antibacterial activity was shown against *Pseudomonas sp.* compare than other bacteria. In this present study ethanolic & aqueous leaf extract of *Catharanthus roseus* against different pathogenic bacteria. The result clearly showed that ethanol extracts of these plants were certainly much better and powerful against *Pseudomonas* (in fig 1). According to (Saroj Kothari, and Savita Bharat , 2011) *Catharanthus roseus* leaf extracts of both solvents chloroform & methanol showed good result in antimicrobial activities against *Pseudomonas, Klebsiella sp.* by paper disc method. The chloroform leaf extract showed considerably more activity than the methanolic extract. But in present study methanol leaf extract of *Catharanthus roseus* shows best result against *Pseudomonas, and Klebsiella sp.* compare than chloroform extract. According to (M. Poonkothai, M. Saravanan, 2007), the best result showed in aqueous extract against *Bacillus*

sp. campare than other extract and other bacteria in well diffusion method. In present study the best result showed in all extract against *Pseudomonas, Enterococci, S. aureus and Bacillus* but *S. aureus* (in fig 9) showed highest zone of inhibition in methanol extract. The leaf extracts of *Catharanthus roseus*to inhibit growth of bacteria is an indication of its broad spectrum antibacterial activity, which may be employed as a source to develop new antimicrobial agents.

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