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COMPARATIVE ANALYSIS OF ANTIMICROBIAL ACTIVITY OF AQUEOUS ETHANOLIC AND CHLOROFORM EXTRACTS OF *POLYGONATUM CIRRHIFOLIUM*

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ABSTRACT

The aim of this study is to determine antimicrobial activity of aqueous ethanol (5:5) and chloroform extracts of rhizomes of *Polygonatum cirrhifolium* by agar well diffusion method. Organisms used for testing antimicrobial activity are *Salmonella typhi, Kocuria rhizophila, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Psedomonas aerugenosa, Escherichia coli, Candida albicans, Saccromyces cervisiae.* Agar well diffusion method was used to determine antimicrobial investigation of rhizomes of *Polygonatum cirrhifolium*. Aqueous ethanol extract of *P.cirrhifolium* displays significant zone of inhibition of *Staphylococcus aureus* (23.2mm), *Bacillus subtilis* (26.1mm), *Kocuria rhizophila* (17.5mm), *Salmonella typhi* (25.7mm), *Klebsiella pneumonia* (9.2mm), *Psedomonas aerugenosa* (34.8mm) whereas chloroform extract zone of inhibition against *Kocuria rhizophila* (16.1 mm), *Staphylococcus aureus* (18.3 mm), *Bacillus subtilis* (20.9mm),

Salmonella typhi (22.4 mm), *Klebsiella pneumonia* (8.4 mm), *Psedomonas aerugenosa* (40.6 mm) at 5 µg/ml. Maximum inhibition in aqueous ethanol as well as chloroform extract were shown in the case of *Psedomonas aerugenosa*, microbe which is a gram negative bacteria. However, inhibition zone was nil in case of fungi *Candida albicans* and *Saccromyces cervisiae*. Results from the study suggest that aqueous ethanol of *P.cirrhifolium* shows better antibacterial action. This can be used as the alternative to currently available antibacterial drugs and can be used for burn and wound infection.

KEYWORDS: Extracts, *Polygonatum cirrhifolium*, rhizome, well diffusion method, antimicrobial activity DMSO (Dimethylsulphoxide).

INTRODUCTION

Natural products like plant have unlimited match of chemical diversity thus provide opportunities for new drugs. According to WHO, for the primary healthcare needs, more than 80% population depend on traditional medicines.[1] From the ancient times, we are using herbal plants and they served as basis of several effective medicines. Traditional uses of plants have led to its bioactive compound investigation and then help in detecting its therapeutic activity. Multi drug resistant bacteria are increasing thus having lesser susceptibility to currently available antibiotics. This leads to search for new effective therapeutic agents.[2] Men had turned to ethanopharmacognosy due to side effects of currently available drugs.

Polygonatum cirrhifolium is a herb of height 0.6 -1.2 cm. Greenish white or pinkish purple flowers are available in the month of June-July, leaves in the whorl of 3-6 sessile and linear with glaucous lower surface. Berries are round and black. It has creamy rhizome of 0.1-0.2 m long, having brittle and short fracture and non-odorous. The phytochemical studies on this plant have revealed the presence of steroidal saponins, polysaccride.[3] Other species of the Polygonatum predominantly contain phyto-hormones, glycosides, polysaccharides, alkaloids, lignins, flavonoid, homoisoflavanones, phenethylcinnamide, lectins, saponins (steroidal and triterpenoid) and flavonoids.[4] Rhizomes of P. cirrhifolium are used as tonic for weakness and aphrodisiac. The rhizomes of *Polygonatum verticillatum* have already shown remarkable antimicrobial activity against bacteria Escherichia *coli*, *Shigella flexeneri*, *Salmonella typhi*, *Staphylococcus aureus* and fungus *Fusarium* solani and *Microspoum canis*.[5] Activity against leishmania and insect has also been reported and excellent activity is present against *Lemna acquinoctialis* in both crude extract and its fractions. This plant also show some

activity against Rhyzopertha *dominica* but no activity is seen against leishmania.[6] Of these fungal strain which include *Candida albicans*, *Trichophyton* longifusus, *Candida* glaberata, *Microsporum* canis, *Fusarium solani*, *Aspergillus flavus*, *P.veticillatum* shows antifungal activity against *Microsporum canis* only.[7]

MATERIALS AND METHODS

Plant Collection and identification

The rhizomes of *P. cirrhifolium* were collected in fresh conditions from the hills of Sunder van forest, Dehradun during the month of July- August. Identification of plant P. cirrhifolium by recognized Botanist and voucher having ref no- NISCAIR/RHMD/Consult/2018/3249-50 provided. This work was done at the Dept. of Pharmacy, KIET Group of Institution, Ghaziabad and IPC, Ghaziabad.

Phytochemical Studies

The powdered materials was used to determine preliminary phytochemicals[8] and give information about the plant constituents like alkaloids, steroids, terpenoids, tannins, gums and mucilage, flavonoids, fixed oils, glycosides, and fats and saponins.

Phytochemical extraction

The rhizome portion were dried under shade and then powdered. Preliminary phytochemical screening was done after the authentication of plant. For this, take 1 gm powdered drug and 10 ml different solvent are taken in different volumetric flask of 10 ml; cover it with aluminium foil for 48 hours. Then filter it and filtrate was subjected to various phytochemical tests and result of this is shown in table 1. Based on the result obtained, we have prepared two extracts that are chloroform extract and aqueous ethanol extract. For the chloroform extract preparation, cold maceration is used. Take 50 gm powdered drug +200 ml of chloroform for 5 days. Then filter and concentrate the sample to get solid mass. For the aqueous ethanol extract, hot percolation method is used and 100 g coarsely powdered drug was packed and 250 ml aqueous ethanol[5] was kept in round bottom flask (RBF). This process is continued for 2-3 days.

Determination of antimicrobial activity

Antimicrobial activity of chloroform and hydro-alcoholic extract of plant Polygonatum cirrhifolium was assayed by Agar well diffusion method. Cultures of (24 hours old) of bacteria cultured on Nutrient Agar Media (Hi-Media GM561A-500EG) were used. Take 2 ml

of bacterial culture and mix it into 250 ml of nutrient media and mix it properly. Take sterilized petridish and pour 25 ml of nutrient media into each petri plate. Then it is allowed to cool to solidify. After that bore holes with a diameter of 6 to 8 mm with sterilized borer and remove the well by sterilized forceps. Now pour sample (100 µL) into these wells and keep these plates into incubator at 32.5° C for 24 hours. DMSO was used for preparation of dilutions of drugs and it does not have any antimicrobial activity. Diameter of inhibition zone was measured by vernier caliper.[9]

Table 1: Results of Preliminary Phytochemical Tests for the Presence Of Active Constituents In Rhizomes Extract Of *Polygonatum Cirrhifolium*.

Solvents	Alkaloids	Glycosides	Terpenoids	Flavanoids	Saponin	Carbohydrate	Protein	Lipids	Volatile Oils	Steroids	Phenol & Tannin	Gums & Mucilages
DMSO (Dimethylsulphoxide)	+	-	-	-	-	-	-	+	+	-	-	+
n-heaxane	-	-	-	-	-	-	-	+	+	-	-	-
Petroleum ether	-	-	-	_	-	-	-	+	++	-	-	+
Choloroform	+	_	-	+	+	-	-	+	+	-	+	+
Dichloromethane	-	+	-	-	-	-	-	+	++	-	-	+
Acetone	_	+	-	-	-	-	-	+	+	-	-	+
Ethyl Acetate	_	+	-	_	-	-	-	+	+	-	-	+
Methanol	-	-	+	-	-	-	-	+	+	-	-	-
Ethanol	-	++	-	_	-	-	-	+	+	-	-	+
Water	+	+	-	-	-	-	-	+	+	-	-	+
Methanol:Water(7:3)	+	+	_	-	-	-	-	+	+	-	-	+
BAW (n-butanol:aceticacid: water)(4:1:5)	+	+	-	-	-	-	-	+	+	-	-	+
Ethylmethylketone	_	_	_	_	_	_	_	+	+			+

Table 2: Antimicrobial Activity of rhizomes of *Polygonatum cirrhifolium* against various organisms.

S.no	Organism used	Sample loaded /well (µL)	Inhibition zone (mm)									
			Aqueous ethanol extract					Chloroform extract				
			5 µg/mL	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL	5 µg/mL	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL
1.	Staphylococcus aureus	1oo	23.2	24.6	26	28.1	29.4	18.3	26.0	29.2	30.1	32.6
2.	Bacillus subtilis	1oo	26.1	31.1	_	_	_	20.9	23.5	_	_	_
3.	Kocuria rhizophila	1oo	17.5	24.6	_	_	_	16.1	18.4		_	_
4.	Salmonella typhi	1oo	25.7	27.6	_	_	_	22.4	24.8	_	_	_
5.	Escherichia coli	1oo	_	7.7	8.9	9.6	11.5	_	9.0	11.1	12	13.7
6.	Klebsiella pneumonia	1oo	9.2	10.5	_	_	_	8.4	9.4	_	_	_
7.	Psedomonas aeruginosa	1oo	34.8	36.9	_	_	_	40.6	43.6	_	_	_
8.	Candida albicans	1oo	NS	NS	_	_	_	NS	NS	_	_	_
9.	Saccromyces cervisiae	1oo	NS	NS	_	_	_	NS	NS	_	_	_

NS: No zone of inhibition

RESULT AND DISCUSSION

Nowadays multi drug resistant pathogens are increasing which is the main cause of mortality and morbidity.[10] Natural antimicrobial are effective against microorganism which are resistant to antibiotic so these natural substance are getting popularity.[11] Earlier research papers reports show that other species of Polygonatum has antimicrobial (fungal and bacterial) activity. MICs of P. verticillatum extracts against E. coli, S. aureus, S. typhi, and S. flexeneri were concentration of µg/ml. P. verticillatum also shows activity against Microspoum canis and Fusarium solani. This result has shown marked antimicrobial activity.

Comparatively, P. cirrhifolium shows that lower zone of inhibition in chloroform extract as compared to aqueous ethanol of P.cirrhifolium. Aqueous ethanol extract used against E. coli, S. aureus, Klebsiella pneumonia, Bacillus subtilis, Kocuria rhizophila, S. typhimurium were showed better activity than chloroform extract whereas Chloroform extract showed better activity against Psedomonas aeruginosa. But no significant activity was observed in both the extracts against C.albicans and S.cervisiae. Aqueous ethanol extract of P.cirrhifolium showed inhibition zone for Staphylococcus aureus (23.2 mm), Bacillus subtilis (26.1 mm), Kocuria rhizophila (17.5 mm), Salmonella typhi (25.7 mm), Klebsiella pneumonia (9.2 mm), Psedomonas aeruginosa (34.8mm) whereas chloroform extract show inhibition zone for B. subtilis (20.9mm), Staphylococcus aureus (18.3 mm), , Kocuria rhizophila (16.1 mm), S. typhi (22.4 mm), K. pneumonia (8.4 mm), Psedomonas aeruginosa (40.6 mm). So, this plant can be used for the treatment of serious inflammation in inner ear, meninges and endocardium.

No toxicity was observed for Rhizomes of P. cirrhifolium. This plant could be used for more study so that it can be used as antimicrobial agent. Phytochemical studies can be done to separate the main bioactive compounds, which are responsible for activity.

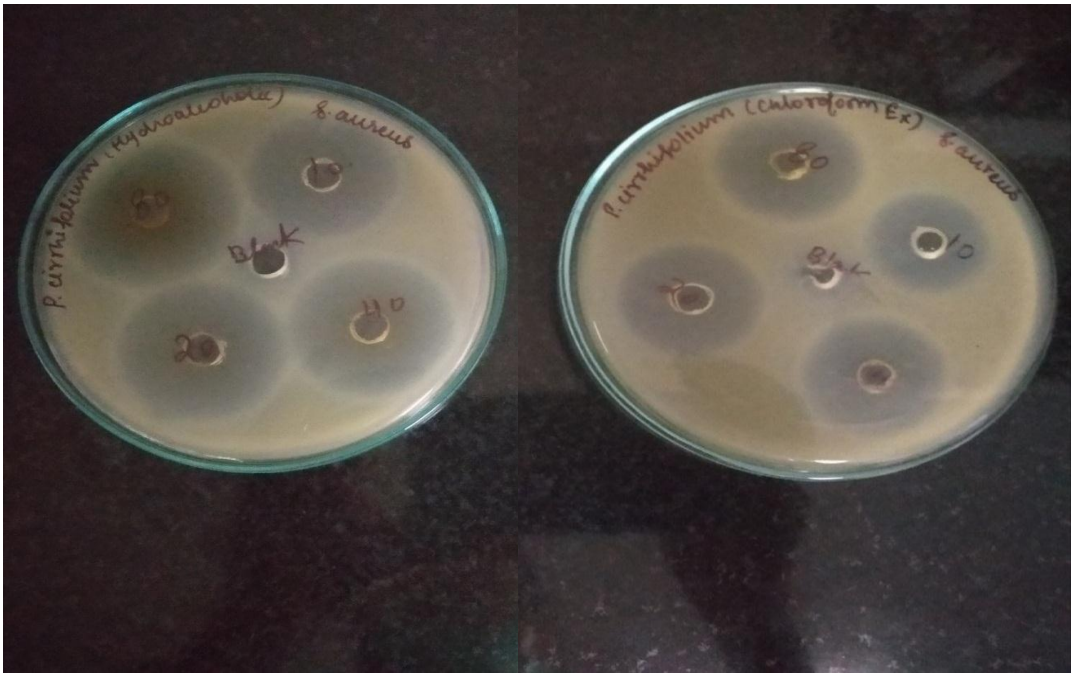

Fig 1: Antibacterial Activity of P. cirrhifolium rhizomes extracts on S. aureus by well diffusion method.

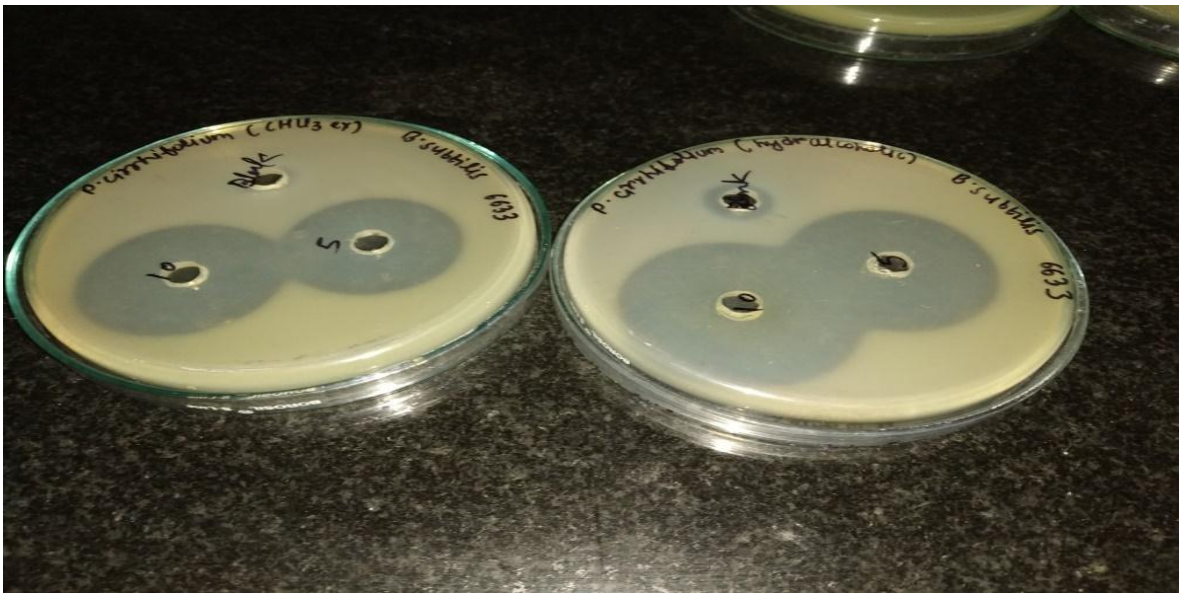

Fig 2: Antibacterial Activity of P. cirrhifolium rhizomes extracts on B. subtilis by well diffusion method.

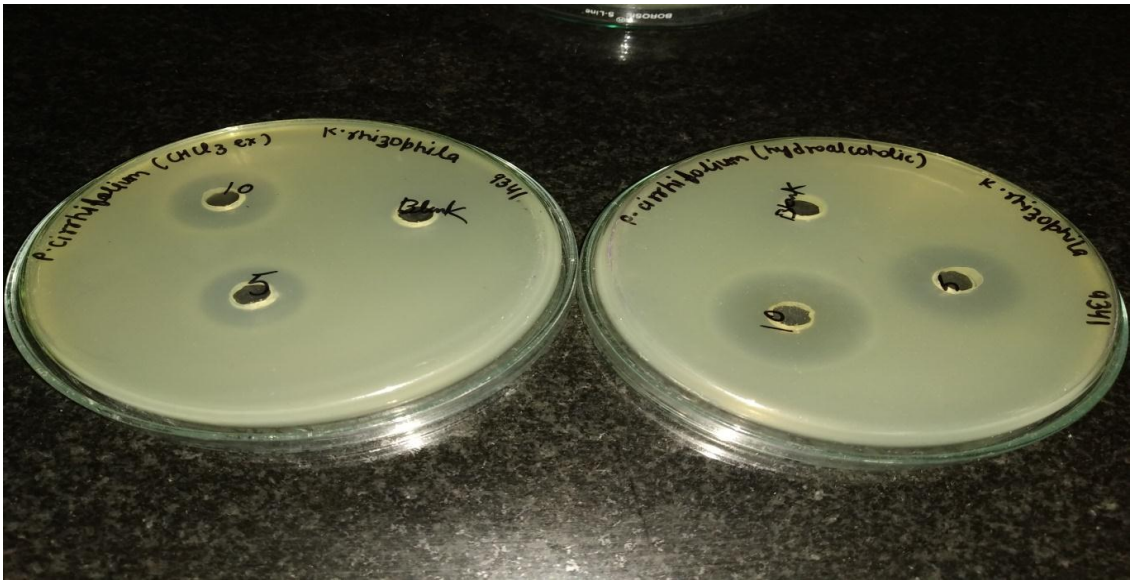

Fig 3: Antibacterial Activity of P. cirrhifolium rhizomes extracts on K. rhizophila by well diffusion method.

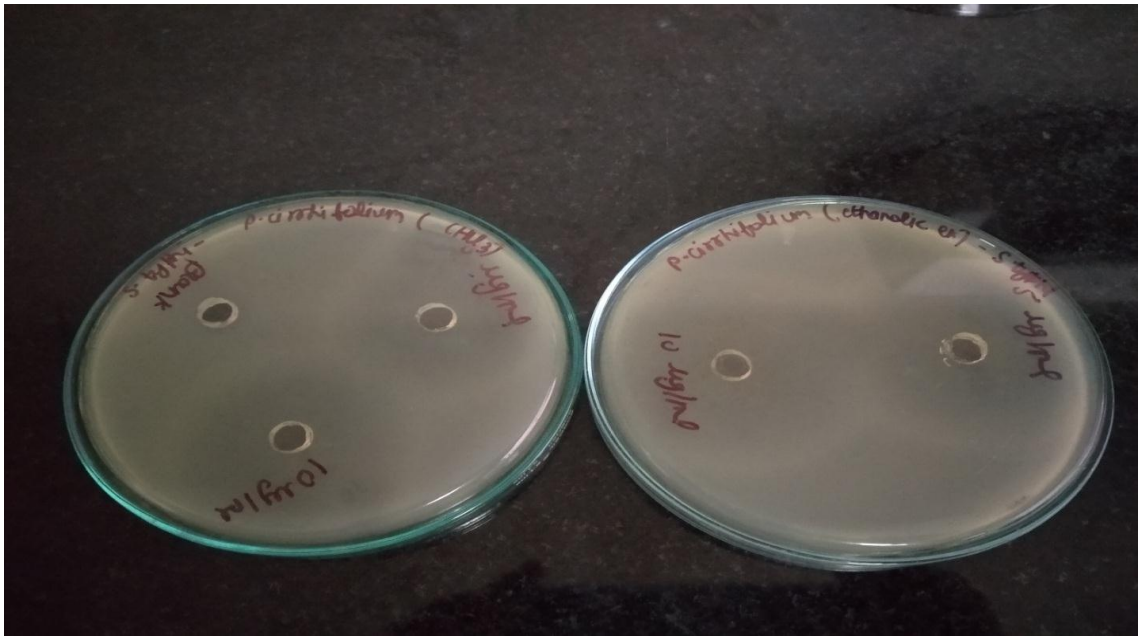

Fig 4: Antibacterial Activity of P. cirrhifolium rhizomes extracts on S. typhi by well diffusion method.

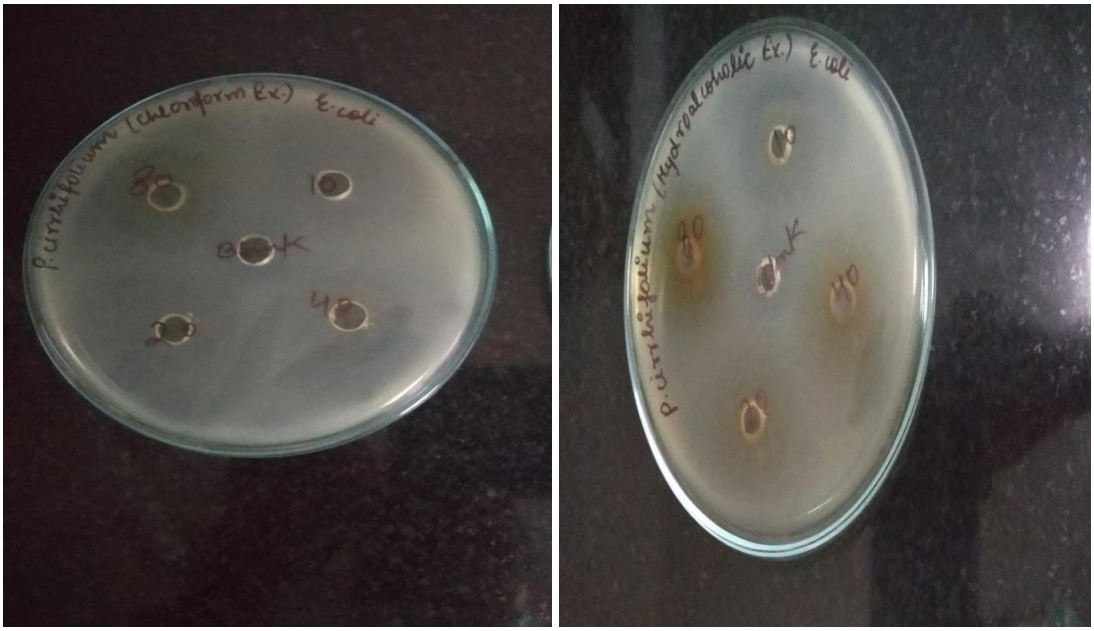

Fig 5: Antibacterial Activity of P. cirrhifolium rhizomes extracts on E. coli by well diffusion method.

Fig 6: Antibacterial Activity of P. cirrhifolium rhizomes extracts on K. Pneumonia by well diffusion method.

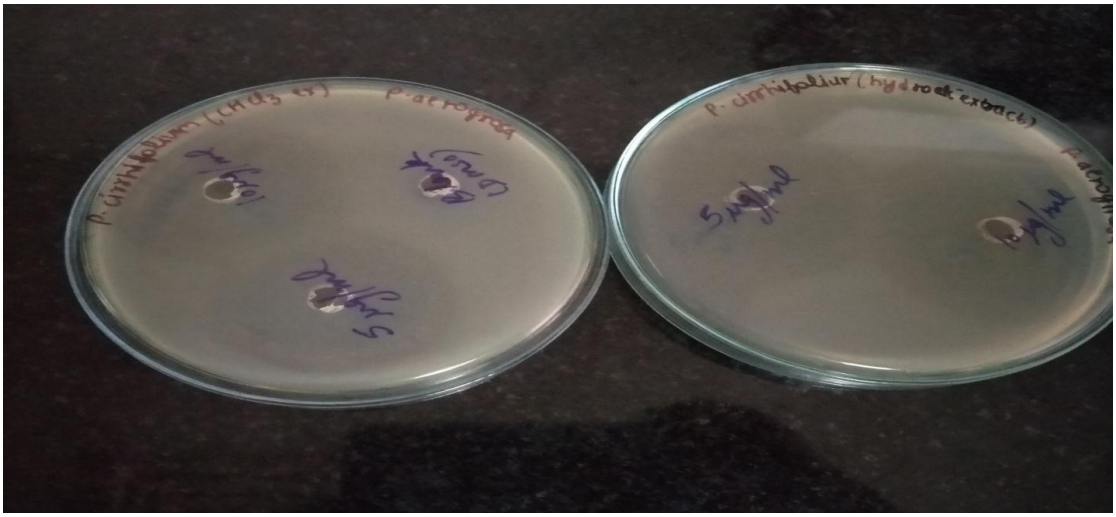

Fig 7: Antibacterial Activity of P. cirrhifolium rhizomes extracts on P. aeruginosa by well diffusion method.

Fig 8: Antifungal Activity of P. cirrhifolium rhizomes extracts on S. cervisiae by well diffusion method.

Fig 9: Antifungal Activity of P. cirrhifolium rhizomes extracts on C. albican by well diffusion method.

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