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ANTIMICROBIAL ACTIVITIES OF SOME RARE AERIAL HEMI PARASITIC TAXA OF SOUTH WEST BENGAL, INDIA

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ABSTRACT

Scurrula atropurpurea, *Macrosolon cochichinensis* and *Viscum album* are hemiparasites found in the forests of South West Bengal, showed antimicrobial activities against four bacterial strains *(Bacillus subtilis, Klebsella pneumoniae, Vibrio cholerae* and *Escherichia coli*). Among them, *Macrosolon cochichinensis* showed maximum antimicrobial activity with the inhibition zone ranging from 6– 8 mm followed by *Scurrula atropurpurea* with inhibition zone 5- 6 mm and *Viscum album* with inhibition zone ranging from 2- 4 mm. A review of literature revealed that so far no body has reported about the antimicrobial activities of the species, *S. atropurpurea* and *M. cochichinensis*. With regard to their natural distribution in the forests of southern parts of west Bengal it was observed that the *Scurrula atropurpurea* and *Viscum album* are now very scant and difficult to locate many times in the field.

Key words: Hemiparasitic taxa, Antimicrobial acitivies, Inhibition zone, South West Bengal.

INTRODUCTION

Various plants and their parts are used for medicinal purpose from ancient age. Now a day it is observed that the African gorillas also use various plants and their parts to cure themselves (Tripathi and Mondal, 2012). The medicinal plants have been used by Hakims and in folklore medicines as 80% of the population lives in rural areas that mostly depend on Unani system of medicines (Soomro *et al*., 1997). Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular all over the world. Tribal people have frequently used plants to treat common infectious diseases, and some of these traditional medicines are still part of the habitual treatment of various diseases. The plant extracts have been developed and proposed for use as antimicrobial substances (Mann, Banso and Clifford, 2008). Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rate than the modern medicines (Del Campo *et al*., 2000). Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential (Lindequist and Mothana, 2005; Bajpai *et al*., 2005; Wojdylo *et al*., 2007). Antimicrobial activities of many plants have been reported by the researchers (Reddy *et al*., 2001; Ateb and ErdoUrul , 2003; Gunalan *et al*., 2011; Doughari, 2006; Dogruoz *et al*., 2008). Antimicrobial effects of some hemi parasites also reported (Hussainn *et al*., 2011). Hemi parasites are the type of parasitic plants, which carry on some photosynthesis but obtain a portion of food, water or minerals from the host plant. The tribal people of south West Bengal use some hemi parasites to cure their health problems. They use *Viscum album* as a protection against sorcery and witchcraft. It was thought that if it was hung around the neck it repelled witchcraft. They also use the plant to normalize cardiovascular system as it lowers the heart rate and blood pressure. The leaf paste of *Macrosolon cochichinensis* is taken as a remedy for severe jaundice. *Scurrula atropurpurea* is traditionally used as a reducing agent in tumor treatment. But in our

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survey it was found that these plants are very rare in the forests of South West Bengal and also very difficult to recognize being hemi parasitic in nature. The camouflaging nature of these species makes it further strenuous to locate in the forest. They grow on the stem of their host but they look like a branch of the tree.

MATERIALS AND METHODS

The preliminary survey was made in different rural areas of the lateritic zone of South West Bengal during the period of March 2012 to April 2012. The places under the zone of survey included Gar Panchkot (23°37'24" N latitude and 86°45'53" E longitude) and Ajodhya Hills (23°13'54"N latitude and 86°5'36" E longitudes). Plants or plant parts [*Viscum album (*Fig. 3), *Macrosolon cochichinensis* (Fig. 1) and *Scurrula atropurpurea* (*Fig. 2*)] were collected from the different areas. Field information regarding their habit, habitat, dominance, local uses and their ethno-medicinal uses are based on personal observation and detailed discussions with the tribes inhabiting these areas, particularly the aged people and the ojhas (quack doctors) during the regular field surveys.

Antimicrobial activity determination

Collection and preparation of plant material for extraction

Plant parts were washed with 70% alcohol and then rinsed with sterilized distilled water and air dried. Clean dry plant samples were stored in cotton bags. The materials were homogenized to a fine powder with the help of a mixer grinder.

Preparation of methanolic extracts

10 g of powdered material of each sample was soaked in 30 ml of 70% methanol and kept at 37°C for 24 h on a rotary shaker. After 24 h the previous portion of added methanol was evaporated and the same volume of methanol was again added and placed on a rotary shaker for another 24 h at 37°C. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant was then collected and allowed to evaporate until it was completely dry. The extracts were kept in sterile air tight bottles at 4°C until further use. Before use 30 mg of dry extract was re-suspended in 1 ml of 70% methanol so that the final concentration of the extract was 30 mg/ml (Ushimaru *et al*., 2007).

Preparation of aqueous extracts

2 g of powdered material of each sample was soaked in 20 ml of distilled water and kept at 37°C for 24 h on a rotary shaker. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 min. The supernatant was then collected and allowed to evaporate until it was completely dry. The extracts were kept in sterile air tight bottles at 4°C until further use (Das *et al*., 2011).

Bacterial strains

Pure cultures of four bacterial strains *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Vibrio cholerae* were used for the study.

Agar well diffusion

Antimicrobial activity was determined by the agar-well diffusion method. Mueller Hinton Agar was used as media. To standardize the inoculum density for sensitivity test, a Barium Sulphate (BaSO4) turbidity standard, equivalent to 0.5 Mac Farland standard was used and was cultivated on agar medium. Thereafter 6 mm diameter wells were punched in the agar plates. Aqueous and methanolic extracts (100 µl) of the different plant extracts were added to the wells. The plates were then incubated at 37°C for 24 h. After incubation the antimicrobial activity was evaluated by measuring the inhibition zone diameter observed. Each test was performed twice and the average of the results was taken (Ulusoylu *et al*., 2001).

RESULTS

The result of screening plant extracts for antimicrobial activity was summarized in the Table No. 1 & 2. It was found that all the plant extracts had antimicrobial activity of different degrees. Antimicrobial activities are produced to different extent by the aqueous extracts of *Macrosolon cochichinensis* and *Viscum album*. The aqueous extracts of *Macrosolon cochichinensis* and *Viscum album* inhibited the growth of *Bacillus subtilis* (Fig. 5) . The inhibition zone of *Bacillus subtilis* by *Macrosolon cochichinensis* was 2 – 2.5 mm. and *Viscum album* was 2 – 3 mm. The aqueous extracts of plants showed no inhibition against the other bacterial strains. The aqueous extracts of *Scurrula atropurpurea* did not play any role against these bacterial strains.

The methanolic extracts of these plants showed a different result. The methanolic extracts of *Scurrula atropurpurea, Macrosolon cochichinensis* and *Viscum album* indicated antimicrobial activity.

After 24 hrs incubation it was seen that *Scurrula atropurpurea* produced inhibition zone against *Bacillus subtilis* (5 mm), *Klebsella pneumoniae* (6 mm), *Vibrio cholereae* (7 mm) and *Escherichia coli* (5 mm). *Macrosolon cochichinensis* produced inhibition zone against *Bacillus subtilis* (4 mm), *Klebsella pneumoniae* (7 mm) and *Vibrio cholerae* (6 mm) but they showed no inhibition zone against *Escherichia coli* (Fig. 4). But in case of *Viscum album* no inhibition zone was produced against these bacterial strains except *Klebsella*

pneumoniae (Fig. 6). The inhibition zone of *Klebsella pneumoniae* by *Viscum album* was 3 mm.

After 48 hrs incubation it was seen that no new inhibition zone was formed against new bacterial strains but the previously formed inhibition zones were increased minutely (0.5 – 1 mm).

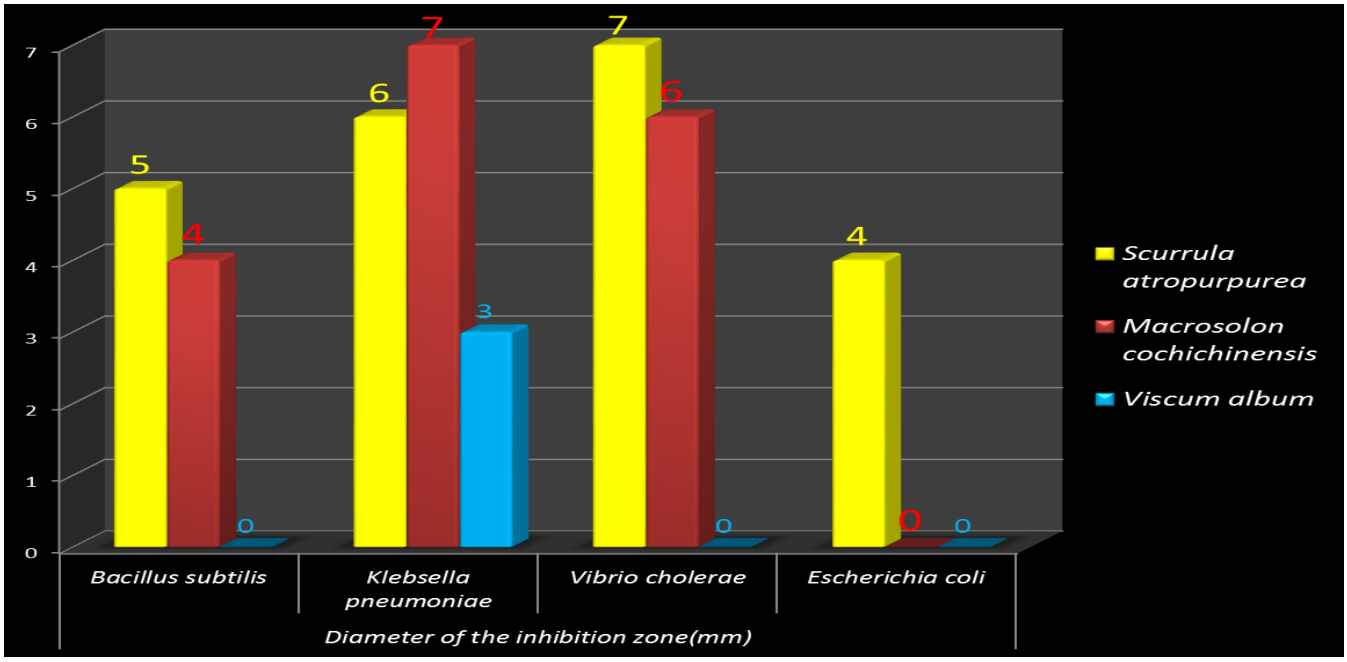
Table 1. Antimicrobial effect of the methanolic extracts of the three plants.

Plant	Diameter of the inhibition zone(mm)							
	Bacillus subtilis		*Klebsiella pneumoniae*		*Vibrio cholerae*		*Escherichia coli*	
	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs
***Scurrula atropurpurea* (Blume) Danser.**	5	6	6	7.5	7	8	4	5.5
***Macrosolen cochinchinensis* (Lour.) Tiegh.**	4	4.5	7	8	6	6.5	0	0
***Viscum album* L.**	0	0	3	3.5	0	0	0	0

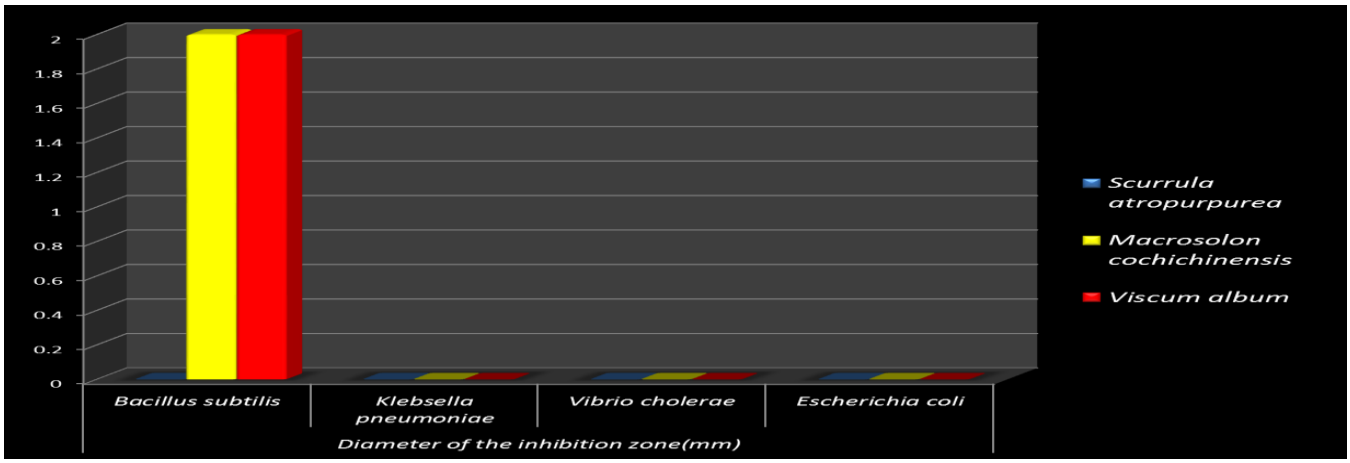
Table 2. Antimicrobial effect of the aqueous extracts of the three plants

Plant	Diameter of the inhibition zone(mm)							
	Bacillus subtilis		*Klebsiella pneumoniae*		*Vibrio cholerae*		*Escherichia coli*	
	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs	after 24 hrs	after 48 hrs
***Scurrula atropurpurea* (Blume) Danser.**	0	0	0	0	0	0	0	0
***Macrosolen cochinchinensis* (Lour.) Tiegh.**	2	2.5	0	0	0	0	0	0
***Viscum album* L.**	2	3	0	0	0	0	0	0

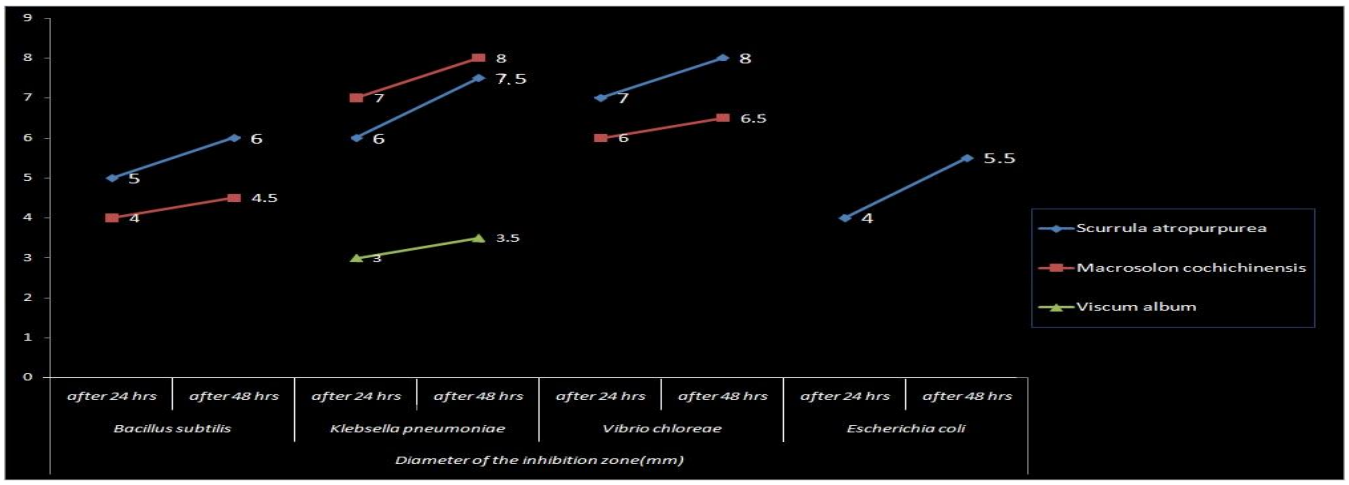
Graph 1. Antimicrobial effect of the methanolic extracts of the three plants



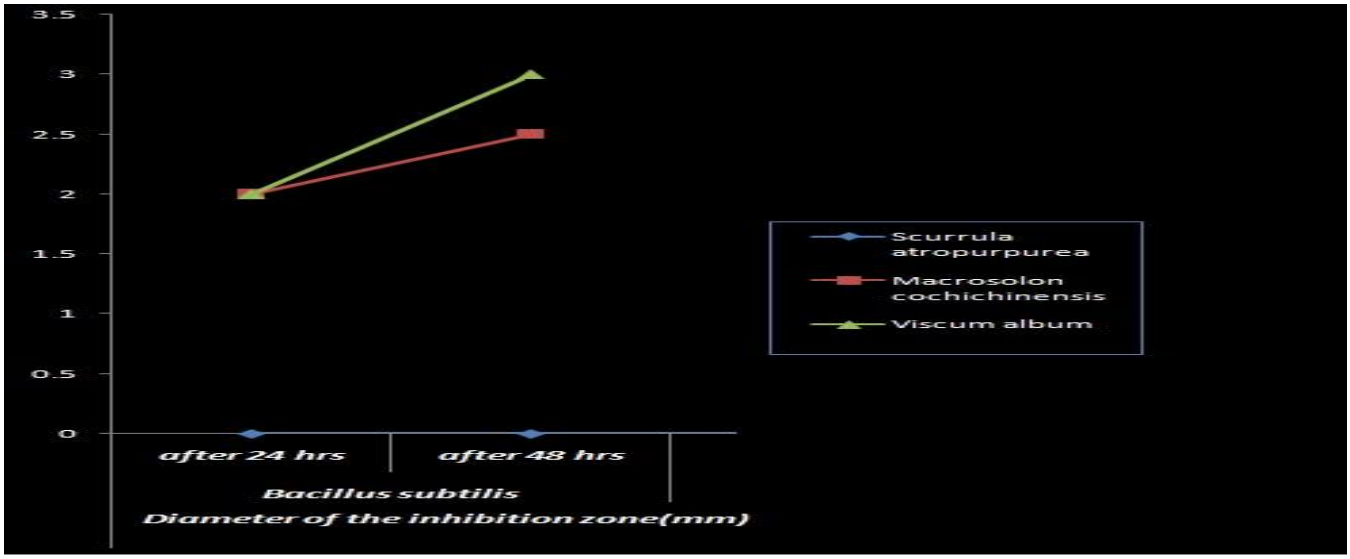
Graph 2. Antimicrobial effect of the aqueous extracts of the three plants.



Graph : 3. Increase of inhibition zone of bactetia in methanolic extraction from 24 hrs to 48 hrs



Graph 4. Increase of inhibition zone of bactetia in aqueous extraction



Figure

Fig. 1. *Macrosolen cochinchinensis* and the junction to its host

Fig. 2. *Scurrula atropurpurea* and the junction to its host.

Fig. 3. *Viscum album* and the junction to its host.

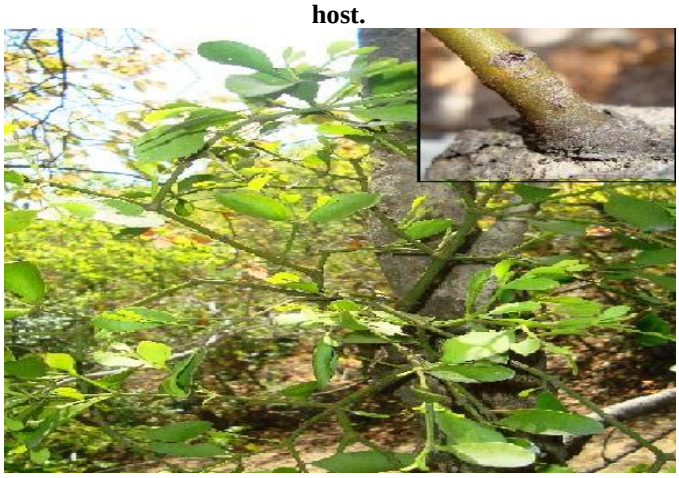


Fig. 4. Anti microbial activity of the three hemi parasites against *Escherichia coli.*

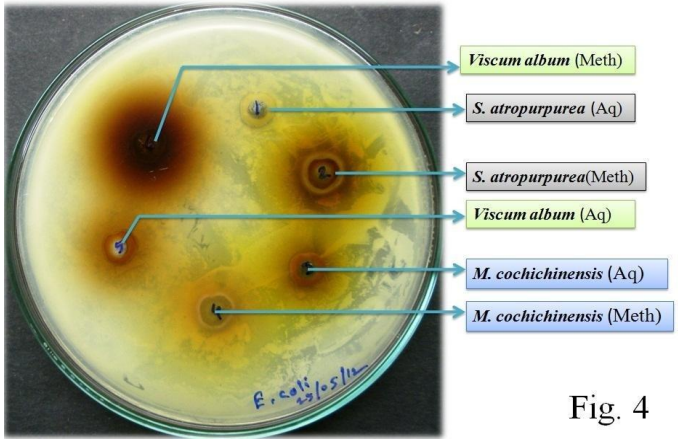


Fig. 5. Anti microbial activity of the three hemi parasites against *Bacillus subtilis*.

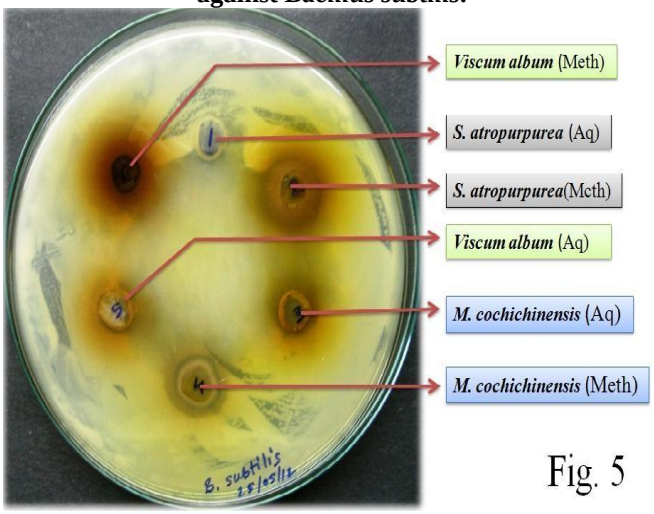


Fig. 6. Anti microbial activity of the three hemi parasites against lebsiellapneumonia.

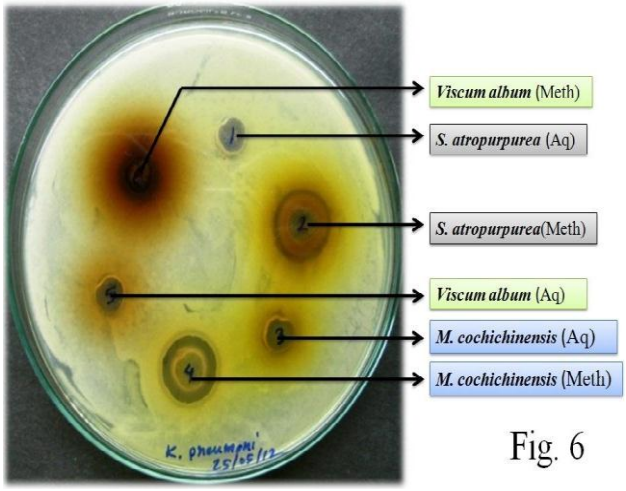


Fig. 7. Anti microbial activity of the three hemi parasites against *Vibrio cholerae.*

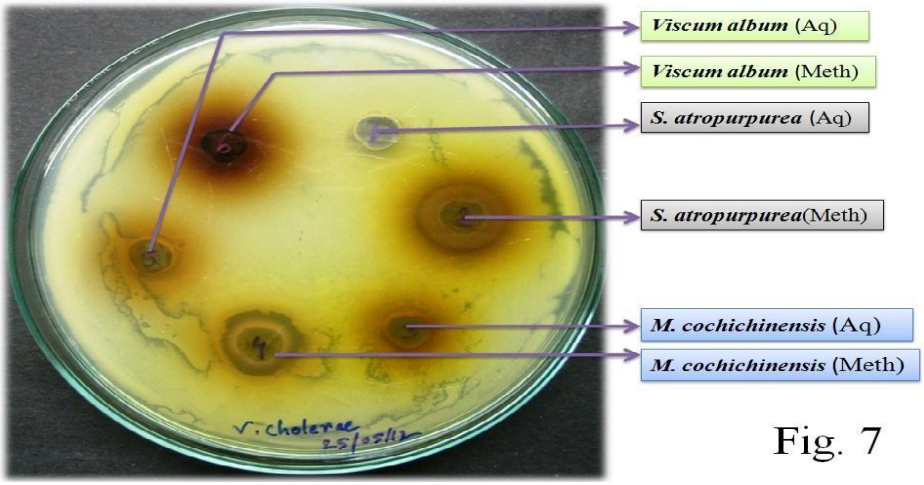


Fig. 7

DISCUSSION

The result of screening plant extracts for antimicrobial activity is summarized in Table No. 1 & 2. The noncytotoxic concentrations of plant extracts were used for antimicrobial activity tests. This experiment suggests that the antimicrobial activity is not due to the cytotoxic activity of extracts. Antibacterial activities were produced to different extents by the aqueous and methanolic extracts of *Scurrula atropurpurea*, *Macrosolon cochichinensis* and *Viscum album.* In this study, it was found that the methanolic extract of *Scurrula atropurpurea* showed antimicrobial activity against *Bacillus subtilis*, *Klebsella pneumoniae*, *Vibrio cholerae* and *Escherichia coli.* In aqueous extract it did not show any antimicrobial activity. *Macrosolon cochichinensis* showed antimicrobial activity against *Bacillus subtilis*, *Klebsella pneumoniae* and *Vibrio cholerae* (Fig. 7) but in aqueous extract showed antimicrobial activity only against *Bacillus subtilis. Viscum album* in methanolic extract show antimicrobial activity only against *Klebsella pneumoniae* but in aqueous extract it showed a different result i.e. it showed no antimicrobial activity against except *Bacillus subtilis.* Here it was clearly observed that the plant extracts showed variation in activity due to different solvent i.e. methanolic extracts of plants showed more antimicrobial activity than the aqueous extracts (Graph-1, 2). *Scurrula atropurpurea* showed maximum antimicrobial activity against *Vibrio cholerae. Macrosolon cochichinensis* showed maximum antimicrobial activity against *Klebsella pneumoniae* (Graph-1).

In this investigation the antimicrobial effect was observed maximum within 24 hrs. After 48 hrs the inhibition zone increased minutely (Table 1, 2).

This investigation revealed that the bacterial inhibition can vary with the plant extract, the solvent used for extraction, and the organisms tested.

CONCLUSION

The demonstration of broad spectrum of antibacterial activity by *Scurrula atropurpurea*, *Macrosolon cochichinensis* and *Viscum album* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of gastrointestinal, respiratory tract and dysentery. The effect of these plants on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

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